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The Influences of Ethanol and Other Factors on the Excretion of Urinary Salsolinol in Social Drinkers

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HIRST, M., D. R. EVANS, C. W. GOWDEY AND M. A. ADAMS. *The influences of ethanol and other factors on the excretion of urinary salsolinol in social drinkers.* PHARMACOL BIOCHEM BEHAV 22(6) 993–1000, 1985.—Salsolinol, a substance that may participate in the development of alcoholism, has been identified in urine and other biological samples from alcoholics. Differentials have been observed between alcoholics and controls. Salsolinol forms when dopamine reacts with acetaldehyde, which may exist in higher concentrations in the blood of alcoholics after alcohol ingestion. Hence, it was postulated that there is a relationship between level of social drinking and the elaboration of salsolinol. Salsolinol is also found in certain food and beverage products. Eighty volunteers, balanced for gender, social drinking level, ethanol dose administered and experimental diet provided urine samples 90 minutes and three hours after ethanol was consumed. Salsolinol levels were analysed in urine using high performance liquid chromatography. A 24 hour carryover effect was observed. Diet, ethanol dose and social drinking level had main and interactive effects on excreted quantities of salsolinol. Gender, situational stress and cigarette smoking had minor if any influence on salsolinol excretion. While there was no evident difference in amounts of salsolinol excreted by light and heavy drinkers in the absence of external sources of salsolinol, heavy social drinkers excreted less salsolinol than did light drinkers after consuming a "salsolinol-enriched" diet, suggesting that they differ in some aspect of absorption, distribution, or metabolism of salsolinol after drinking ethanol. Accordingly, studies that attempt to determine whether salsolinol has any relationship to drinking behaviour in humans should be particularly concerned with salsolinol that occurs in exogenous sources.

Social drinking Urinary salsolinol Ethanol dose Diet Gender Dopamine

IT has been suggested that the tetrahydroisoquinoline, salsolinol, contributes to the development of alcoholism [5]. Studies in rats have shown that intracerebral injections of the substance may enhance preference for ethanol in free-choice situations [12, 25, 26], animals will select to self-administer salsolinol [2], and rats trained to discriminate ethanol from other drugs respond as if they had received the alcohol when given salsolinol [1]. These experiments have provided support for earlier hypotheses that linked aspects of alcoholism to formation of tetrahydroisoquinolines in vivo following the consumption of ethanol [8,10].

Salsolinol is formed rapidly in vitro when dopamine reacts irreversibly with acetaldehyde [21,34]. As acetaldehyde is the primary oxidation product of ethanol, and dopamine is widely distributed in tissue [4,7], salsolinol should be present after alcohol has been administered. Investigations have, however, demonstrated that the formation of salsolinol in animal tissue does not occur readily after animals have been exposed to ethanol [18, 29, 37]. Nonetheless, analyses of urine samples from alcoholics have been found to contain higher concentrations of salsolinol than occur in samples from control subjects [9, 38, 39]. This latter dichotomy may well be based on the increased quantities of alcohol that are processed by alcoholics. In addition, it can be hypothesized that heavy drinkers generate differential quantities of salsolinol after drinking. Several workers have suggested that alcoholics and their male descendants have higher blood acetaldehyde levels following consumption of alcohol than do controls [22, 36, 42], but a recent study has questioned this finding [15]. It was considered that measurements of salsolinol might provide indirect information on this issue.

For these reasons a study was undertaken to examine the quantities of salsolinol that appeared in urine after volunteers had consumed fixed quantities of ethanol. The participants were heavy or light social drinkers of both genders. As salsolinol has been identified in certain foodstuffs and beverages [13, 32, 33], it was thought that the urinary determinations might be confounded by exogenous material from this source. This issue was addressed by incorporating diets likely to contain differential quantities of salsolinol into the experiment. Equally, salsolinol has been found in adrenal tissue of rats [19], raising the possibility that it might exist in releasable form in chromaffin tissue in humans. The study was extended to consider this point by evaluating also the

impact of measures that might result in such release, namely situational stress within the experimental setting as well as the influence of nicotine arising from cigarette smoking.

METHOD

Subjects

Participants were 80 paid volunteers solicited by advertisements. Equal numbers of male (N=40) and female (N=40), and light (N=40) and heavy (N=40) social drinkers were assigned at random to the four possible alcohol dose (2) by order of diet (2) conditions in the experiment. Hence, there were 5 volunteers in each cell. The mean age for males was 21.95 years and for females, 22.58 years.

Measures

Medical Questionnaire. In consultation with a physician a "Checklist of Contraindications of Consumption of Alcohol for Research Purposes" was developed. Prior to participation in the experiment each volunteer completed the checklist, which was then reviewed and permission to continue into the study given by the physician.

Alcohol Consumption Questionnaire. The questions used by Cahalan, Cisin and Crossley [6] to establish level of social drinking were adapted to a questionnaire format. Using the method described by Cahalan, Cisin and Crossley [6] volunteers were classified as either light or heavy social drinkers. Heavy consumers were those who fell into their heavy and moderate drinker categories and light consumers were those who fell into the light drinker category.

Subjective Stress Scale. The Subjective Stress Scale developed by Neufeld and Davison [28] was administered to each participant at the time of ingesting the ethanol dose and at 90 minutes and three hours postingestion. The adjectives in the scale were presented in a different random order at each administration.

Cigarette consumption. For those participants who smoked cigarettes, the number and type of cigarettes smoked in each experimental session was recorded. On the basis of this data, the total nicotine available to each participant was calculated for each session.

Blood alcohol level. An instrument that measured breath alcohol (Lion Alcolmeter S-D2) was used at regular times throughout each session until the release criterion of 30 mg/dl alcohol in blood was met.

Urinary salsolinol level. Urine samples were collected from each participant during each session. Samples were collected in separate plastic containers (1 l) just prior to ethanol ingestion, and 90 minutes and three hours following ingestion. Their volumes were recorded. Samples were adjusted to pH 1.8–2.0 with concentrated hydrochloric acid. Aliquots (4×10.0 ml) were stored in polypropylene tubes (12.5 ml) and frozen (−75°). The urine samples were analysed by a high-performance liquid chromatography procedure [19]. Urine samples (10 ml) were thawed and then heated at 85° for 40 min. 3,4-Dihydroxybenzylamine was added after cooling. TRIS (1 ml, 0.2 M, pH 6.5) was then added prior to alumina (300 mg), and the pH adjusted to 7.8–8.0. After shaking vigorously for 15 min the supernatant was aspirated and the alumina washed with water (10 ml). Hydrochloric acid (5 ml, 0.1 N) was pipetted onto the alumina residue left after aspiration of the water wash. After a 10 min period of vigorous shaking the aqueous phase was adjusted to pH 3, then percolated through Bio-Rex 70 (200–400 mesh, sodium form). After washing with water (20 ml), the amines were eluted into hydrochloric acid (1.5 ml, 1 N). Samples were mixed with saturated phosphate buffer, pH 4.8, before injection (50 μl) into the chromatograph. Catecholamines and related isoquinolines were recovered in the 60–70% range. Analysis by high performance liquid chromatography used a Varian 5060 pump, an Ultrasphere-ODS column (5 μm) and an electrochemical detector (Bio Analytical Systems, LC-2A) set at +0.8 volts against a silver-silver chloride reference cell. The mobile phase, delivered at 2 ml/min, consisted of a methanol:water (10% v/v) solution of potassium dihydrogen phosphate (1% w/v) and sodium octyl sulphate (0.0125% w/v), adjusted to pH 4.5. Of the 320 operational analyses performed, 12 samples provided data that were not included in analyses. The excluded samples were obtained from three participants who had extremely high values that exceeded their specific cell mean by a factor of ten or more. When this occurred all four values for that subject were replaced by specific cell means. (As noted later, one individual in the female, heavy social drinker class, given the high dose of ethanol and the "salsolinol-lean" diet, excreted atypical quantities of salsolinol, but these did not reach exclusion criteria and remained included in analyses.)

Urinary Dopamine Levels

The quantities of dopamine excreted in the urine of volunteers were established from the chromatograms obtained for salsolinol analyses. As for salsolinol, the quantities of dopamine were derived by area ratios to an internal standard peak, calibration curves being corrected for incomplete recovery [19]. Of 320 operational analyses performed, nine samples obtained from three participants provided chromatographic data that could not be considered to represent dopamine alone. The data for these samples were replaced by specific cell means.

Conditions

Ethanol dose. Participants were randomly assigned in equal numbers to consume either 0.4 g of ethanol/kg of body weight (low dose) or 0.8 g of ethanol/kg of body weight (high dose). The alcohol was diluted by an equal volume of a drink prepared from fruit flavored crystals (Tang) and consumed within 10 minutes.

Diet. Participants consumed a chocolate enriched meal ("salsolinol-enriched") in one session and a nonchocolate meal ("salsolinol-lean") in the other session. They were randomly assigned in equal numbers to receive their meals in each of the two possible orders. The respective meal was consumed prior to ingesting the dose of alcohol. The chocolate enriched meal consisted of two slices of white toast spread with peanut butter and jam, chocolate milk and a chocolate bar. The nonchocolate meal included two slices of white toast spread with peanut butter and jam, white milk, and two vanilla wafer biscuits.

Procedure

Once a prospective participant had responded to the advertisement an appointment was made for preexperimental assessment. During this session prospective participants read the "Instructions For Participants," completed the Medical Questionnaire and the Alcohol Consumption Questionnaire. Those who met the medical criteria were then assigned to either the high or low alcohol consumption con-

dition on the basis of their Alcohol Consumption Questionnaire responses. This accomplished, they were then randomly assigned to receive either a high or low dose of ethanol, and the chocolate enriched diet on either day one or day two of the experiment. Participants were asked to abstain from drinking alcoholic beverages for 24 hours prior to day one and not to consume alcohol between sessions. They were requested to not consume food for three hours prior to each session.

On the first day participants read and signed the required consent forms and completed a brief demographic questionnaire. They then provided a urine sample, a breath sample, completed the Subjective Stress Scale and were then fed the prescribed meal. Next they consumed the assigned dose of ethanol. For the next 90 minutes they were permitted to watch television, read, or otherwise entertain themselves. At the end of this time, they gave a further urine sample and completed the Subjective Stress Scale. Another 90 minute activity period was engaged in. They then provided a three hour urine sample and completed the Subjective Stress Scale. Once the release blood-alcohol level was met participants were sent home in a taxi. The second day was identical to the first. At the end of each session the number and type of cigarettes consumed by those participants who smoked was recorded.

RESULTS

The results of the experiment are presented as follows: there is a description of factors associated with the chromatographic procedures; the potential relationship between urinary salsolinol and dopamine is examined; the levels of salsolinol pertinent to the diets is identified; the adequacy of the assignments to the social drinking levels within gender was clarified. The results of the statistical analyses are then given. The preliminary analysis involved a complex design with the assignment of subjects on the basis of four, fully crossed variables and a repeated measure. The four variables were gender, social drinking level, ethanol dose and order of diet. Each subject participated in the experiment on two occasions in which the experimental diet was interchanged. The secondary analysis involved a between groups analysis of day one data. Four fully crossed variables were examined. They were gender, social drinking level, ethanol dose and diet. Further analyses were conducted for each diet condition. Following each analysis a covariate analysis was conducted to evaluate the influence of smoking and situational stress. The sample size for which significant results were found varied between 10 to 80 for the repeated measures analysis and between 10 and 40 in the various between groups analyses.

Analyses of Urinary Salsolinol and Dopamine

Preliminary chromatographic analyses using differing mobile phases and electrochemical cell voltages showed consistently that the peaks suspected of being dopamine and salsolinol had the retention times and sensitivities of standard samples. Under the stated chromatographic conditions tracings showed that salsolinol was well resolved from other constituents, noradrenaline and dopamine were well differentiated, whereas adrenaline was not separated from another substance [19].

In a separate study, adulteration of urine samples (N=6) with ethanol (to 100 mg/dl) was performed prior to hydrolysis of urine and extraction of catecholamines and salsolinol. This was undertaken to see if oxidation of ethanol might occur after samples had been collected and processed for analysis, there being a readily available source of dopamine in urine that might react with any derived acetaldehyde. Giles and Meggiorini [17] have observed artifactual production of acetaldehyde in urine samples, although this generation was at higher pH than used in the present study. There were no differences in salsolinol levels from before (mean=2.37, s.d.=0.54 μg/dl) and after (mean=2.28, s.d.=0.44 μg/dl) addition of ethanol.

Relationship Between Urinary Salsolinol and Dopamine

The chromatographic procedure used to derive urinary salsolinol levels afforded also measures of dopamine content. As salsolinol is a product of the condensation of dopamine with acetaldehyde, then a relationship might exist between the two substances. Following each analysis reported below, parallel analyses of covariance were performed with the respective dopamine level as the covariate. In no instance was there a significant covariate effect of dopamine upon the quantity of salsolinol excreted. In order to evaluate the general proposition that excretion of the two substances is related, a series of Pearson product moment correlations were conducted under specific experimental conditions. For the 40 participants receiving the nonchocolate diet on day one, the Pearson produce moment correlation between 90 minute urinary salsolinol and dopamine was significant, $r(38)=.29$, $p<0.04$, as was the correlation between the three hour values, $r(38)=.36$, $p<0.01$. For the 40 participants under the chocolate diet condition during session one the correlation for 90 minute data was nonsignificant, $r(38)=.25$, $p=0.06$ and also the correlation between the three hour values was nonsignificant, $r(38)=.07$, $p=0.33$.

Analyses of Dietary Salsolinol

The following were common to both diets: two slices of white toast spread with peanut butter and jam and milk. The distinctive dietary components were then that one was enriched with cocoa-based products, chocolate and a chocolate drink in the milk, and the second included milk taken with vanilla wafer biscuits. As it was intended that one diet be enriched with salsolinol to serve as a confounding contributor to urinary salsolinol, the chocolate, chocolate syrup and wafer biscuits were analysed. The results indicated that appreciable quantities of salsolinol were present in the chocolate (about 0.3 mg per bar, 50 g) and the chocolate syrup (1.3 μg/ml). Salsolinol was not present in detectable concentrations in the wafer biscuits.

Social Drinking Level Assignment Check

It is often assumed that males have a higher alcohol consumption level than females. Based upon this assumption it might have been possible, by forming two groups having low and high social drinking levels, that the males would be at the high end and the females at the low end of each group. In order to evaluate this possibility, the data obtained on the Alcohol Consumption Questionnaire was transformed into an alcohol consumption score. This score was calculated for each participant according to a formula that was based upon the criteria provided by Cahalan, Cisin and Crossley [6]. A gender (2) by social drinking level (2) analysis of variance was performed on the alcohol consumption scores. The main effect of gender, $F(1,76)=0.57$, $p=0.45$, was not significant.

TABLE 1

MEANS AND STANDARD DEVIATIONS FOR THE MAIN EFFECTS OF SOCIAL DRINKING LEVEL, GENDER, DIET AND ETHANOL DOSE ON SALSOLINOL* IN THE 90 MINUTE URINE SAMPLE

	Light Social Drinkers				Heavy Social Drinkers			
	Males		Females		Males		Females	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
"Salsolinol-lean Diet"								
Low Ethanol Dose	1.29	1.65	1.70	2.67	0.66	1.40	0.55	0.46
High Ethanol Dose	0.47	0.26	0.08	0.12	1.13	1.31	8.13†	12.18
"Salsolinol-enriched" Diet								
Low Ethanol Dose	4.89	6.22	7.23	7.78	4.11	4.46	1.54	0.72
High Ethanol Dose	9.32	6.35	16.94	7.73	5.16	6.16	4.90	2.14

*Units in μg.
†This cell contained one subject with atypical values, but these did not reach exclusion criteria.
N per cell equals 10.

As expected, the main effect of level was significant, $F(1,76)=43.43, p<0.01$. The interaction effect between gender and level was not significant, $F(1,76)=3.81, p<0.06$. That this effect approached significance is probably due to the sizable main effect of level, rather than any potential interaction effect given the minimal main effect of gender.

Repeated Measures Analyses of Urinary Salsolinol

A social drinking level (2) by gender (2) by ethanol dose (2) by order of diet (2) by diet (2) analysis of variance with repeated measures over diet was performed on the amount of salsolinol produced in the 90 minute urine sample. The main effect of diet on salsolinol produced in the 90 minute urine sample was significant, $F(1,64)=44.74, p<0.01$. The following interactive effects on the 90 minute urinary salsolinol produced were also significant: diet by social drinking level, $F(1,64)=8.04, p<0.01$; diet by ethanol dose by order of diet, $F(1,64)=6.82, p<0.02$; and social drinking level by ethanol dose by order of diet, $F(1,64)=5.18, p<0.03$. All other main effects and interactive effects on the 90 minute urinary salsolinol produced were nonsignificant.

A social drinking level (2) by gender (2) by ethanol dose (2) by order of diet (2) by diet (2) analysis of variance with repeated measures over diet was performed on the amount of salsolinol produced in the three hour urine sample. The main effects of social drinking level, $F(1,64)=6.54, p<0.02$; gender, $F(1,64)=4.29, p<0.05$; ethanol dose, $F(1,64)=7.14, p<0.01$; and diet, $F(1,64)=96.66, p<0.01$ were all significant. The following interactive effects on the three hour urinary salsolinol produced were also significant: gender by ethanol dose, $F(1,64)=6.09, p<0.02$; diet by social drinking level, $F(1,64)=10.11, p<0.01$; level by gender by order of diet, $F(1,64)=5.38, p<0.03$; social drinking level by alcohol dose by order of diet, $F(1,64)=4.39, p<0.05$; diet by social drinking level by ethanol dose, $F(1,64)=5.25, p<0.03$; and diet by ethanol dose by order of diet, $F(1,64)=5.28, p<0.03$. All other main effects and interactive effects on the three hour urinary salsolinol produced were nonsignificant.

As can be seen from the above analyses, order of diet was a component in a number of significant interactions affecting the salsolinol content of both urine samples. This would suggest a 24 hour carryover effect of salsolinol and perhaps ethanol acquired on day one to salsolinol excreted on day two. Thus, a conservative approach was followed in which day one data was examined under the between groups design.

Between Groups Analyses of Urinary Salsolinol

A social drinking level (2) by gender (2) by ethanol dose (2) by diet (2) analysis of variance was performed on the salsolinol produced in the 90 minute urine sample during day one. The means and standard deviations of salsolinol for these samples are shown in Table 1. The following main effects were significant: ethanol dose, $F(1,64)=6.85, p<0.02$; and diet, $F(1,64)=18.90, p<0.01$. In addition, the following interactive effects were significant: social drinking level by diet, $F(1,64)=10.35, p<0.01$; gender by social drinking level by diet, $F(1,64)=4.58, p<0.04$; and ethanol dose by social drinking level by diet, $F(1,64)=4.85, p<0.04$. An analysis of covariance with change in situational stress and total nicotine available as covariates was also performed. None of the regression effects for covariates were significant.

A social drinking level (2) by gender (2) by ethanol dose (2) by diet (2) analysis of variance was performed on the salsolinol produced in the three hour urine sample during day one. The means and standard deviations of salsolinol for these samples are shown in Table 2. The following main effects were significant: gender, $F(1,64)=5.80, p<0.02$; ethanol dose, $F(1,64)=10.40, p<0.01$; and diet, $F(1,64)=34.48, p<0.01$. In addition, the following interactive effects were significant: social drinking level by diet, $F(1,64)=9.20, p<0.01$; gender by social drinking level by diet, $F(1,64)=5.33, p<0.03$; and ethanol dose by social drinking level by diet, $f(1,64)=8.06, p<0.01$. An analysis of covariance with change in stress and total nicotine available over the three hour period as covariates was also performed. None of the regression effects for covariates were significant.

The repeated measures analyses and the between group analyses of day one data showed a particularly strong influence of diet. To examine this effect in more detail, separate between groups analyses were performed on the day one data for each diet condition.

Social drinking level (2) by gender (2) by ethanol dose (2) analyses of variance were performed on the salsolinol produced in the 90 minute and three hour urine samples dur-

TABLE 2

MEANS AND STANDARD DEVIATIONS FOR THE MAIN EFFECTS OF SOCIAL DRINKING LEVEL, GENDER, DIET AND ETHANOL DOSE ON SALSOLINOL* IN THE THREE HOUR URINE SAMPLE

	Light Social Drinkers				Heavy Social Drinkers			
	Males		Females		Males		Females	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
"Salsolinol-lean Diet"								
Low Ethanol Dose	0.71	1.28	0.64	0.83	0.27	0.52	0.38	0.39
High Ethanol Dose	0.62	0.60	0.43	0.59	0.67	0.96	6.39†	10.01
"Salsolinol-enriched" Diet								
Low Ethanol Dose	3.79	3.16	6.17	4.97	3.95	2.69	4.55	3.33
High Ethanol Dose	8.51	5.20	18.79	9.65	5.13	3.50	5.18	4.18

*Units in μg.
†This cell contained one subject with atypical values, but these did not reach exclusion criteria.
N per cell equals 10.

TABLE 3

MEANS AND STANDARD DEVIATIONS FOR THE MAIN AND INTERACTIVE EFFECTS OF SOCIAL DRINKING LEVEL AND ETHANOL DOSE ON SALSOLINOL* IN THE THREE HOUR URINE SAMPLE UNDER THE "SALSOLINOL-ENRICHED" DIET CONDITION

	Ethanol Dose					
	Low		High		Total Group	
Social Drinking Level	Mean	s.d.	Mean	s.d.	Mean	s.d.
Light	4.98	4.12	13.65	9.10	9.32	8.19
Heavy	4.25	2.87	5.16	3.63	4.70	3.22
Total Group	4.61	3.48	9.40	8.03		

*Units in μg.

ing day one under the "salsolinol-lean" diet condition. No significant main or interaction effects were found in these analyses. Analyses of covariance with change in situational stress and total nicotine available as covariate were also performed. None of the regression effects were significant. The overall mean amount of salsolinol excreted in the 90 minute sample was 1.75 μg (s.d.=4.78), that in the three hour sample was 1.26 μg (s.d.=3.82).

Social drinking level (2) by gender (2) by ethanol dose (2) analyses of variance were performed on the salsolinol produced in the 90 minute and three hour urine samples collected after consumption of the "salsolinol-enriched" diet on day one. The main effects of ethanol dose, $F(1,32)=6.52$, $p<0.02$ and social drinking level, $F(1,32)=9.84$, $p<0.01$ upon salsolinol excreted in the 90 minute sample were significant. Participants given the low dose of ethanol excreted a mean of 4.44 μg (s.d.=5.43) of salsolinol, whereas those receiving the high dose of ethanol had a mean of 9.07 μg (s.d.=7.42). The means for light and heavy social drinkers were 9.60 μg (s.d.=7.97) and 3.92 μg (s.d.=3.92), respectively. The impact of the covariates, change in stress and total nicotine available, on the above data was evaluated and found to be nonsignificant.

For the three hour urinary salsolinol data the main effects of gender, $F(1,32)=4.37$, $p<0.05$; ethanol dose, $F(1,32)=9.05$, $p<0.01$; and social drinking level, $F(1,32)=8.40$, $p<0.01$ were all significant. The interactive effect of ethanol dose and social drinking level was also significant, $F(1,32)=5.95$, $p<0.02$. Males and females excreted mean levels of 5.35 μg (s.d.=3.96) and 8.67 μg (s.d.=8.19), respectively. The means and standard deviations for the ethanol dose and social drinking level effects are shown in Table 3. As in previous analyses, the impact of the covariates, change in stress and total nicotine available, were nonsignificant.

DISCUSSION

Several investigators have shown that alcoholics may have higher blood acetaldehyde levels than nonalcoholics after consumption of ethanol [22,42], a discovery that has been extended to their nonalcoholic scions [36]. Acetaldehyde condenses readily and irreversibly with dopamine under pseudo-physiological conditions, to form the tetrahydroisoquinoline, salsolinol. Alcoholics in the post-intoxication phase have been found to excrete greater quantities of salsolinol than do controls, suggesting that this substance has formed, *in vivo*, as a consequence of drinking ethanol [9, 38, 39]. For these reasons, it was hypothesized that social drinkers would excrete differential quantities of salsolinol, depending on their drinking proclivities, with high

level social drinkers excreting greater amounts of the tetrahydroisoquinoline than those who drink less. Many, albeit controversial studies in animals have indicated that dopamine derived tetrahydroisoquinolines may influence ethanol drinking behaviour [12, 25, 26]. Accordingly, the formation of salsolinol, *in vivo*, could have relevance to the development of alcohol abuse in humans.

The present study was undertaken to examine the hypothesis by examining urinary excretion of salsolinol in volunteers given ethanol. Beyond this, however, the study was expanded to determine the impact of other variables on salsolinol excretion. These were gender, to extend previous research that has been confined, in the main, to males; diet, as dietary sources of salsolinol might confound that resulting from the condensation reaction; ethanol dose, those given being equivalent to quantities consumed in other studies [15, 20, 36, 39]; situational stress and cigarette smoking during the experiment, to see if bodily stores of salsolinol in chromaffin cells would be released by these variables and contaminate urinary salsolinol originating elsewhere. The influences of these variables and their interactions were pursued by primary and secondary statistical analyses, as mentioned in the Results section. The former were conducted by repeated measures analysis of variance, the latter by considering day one data alone. As noted, the sequence of secondary analyses led to a separate examination of the role of dietary salsolinol on the urinary excretion of the tetrahydroisoquinoline. In addition, a number of supportive analyses were undertaken to consolidate the results of the study. Thus, chemical analyses were performed on the differential food materials used in the study; the possible oxidation of excreted ethanol with subsequent, post-sampling formation of salsolinol was evaluated; the consistency of volunteer assignment into high and low level social drinkers, with gender; and a possible relationship between the appearance of salsolinol and dopamine in urine was examined.

The data revealed that the social drinking level assignment between males and females who participated in the study did not differ. Chemical analyses pursuing the possible post-sampling synthesis of salsolinol after oxidation of ethanol during processing for analysis were negative in that concentrations of salsolinol before and after adulteration of samples with ethanol and processing them did not differ significantly. This evidence for a lack of post-sampling formation of salsolinol, while not eliminating the possibility that minor quantities of salsolinol may form after collection—which might be pursued using radioactive precursors—implies that such synthesis had little influence on the data from this study. It is worthy of note that acetaldehyde has been detected in warmed urine samples that contained large quantities of ethanol and were at higher pHs than in the present study [17]. The lack of support for post-sampling synthesis found here suggests that acetaldehyde generation may not occur under more acidic conditions, or that these conditions suppress the condensation of dopamine and acetaldehyde to yield salsolinol. In this regard, this finding extends the results of others who were unable to induce condensation of acetaldehyde with dopamine in urine held under acidic conditions, or treated with sulfatase [9,38]. Salsolinol was present in significant quantities in the chocolate and chocolate syrup that contributed to the "salsolinol-enriched" diet, with negligible amounts being contained in the control, wafer biscuit component of the "salsolinol-lean" diet. Covariate analyses with situational stress and nicotine from cigarettes smoked during the experiment, performed after primary and secondary analyses of variance, demonstrated that these factors were not contributing to salsolinol excretion. While this finding does not address the issue of whether or not salsolinol is present in chromaffin tissue in humans, initial concerns that these variables might cause the release of endogenous stores in chromaffin cells were not substantiated.

Repeated measures analyses revealed that the order in which individuals ate the respective diets, in conjunction with taking a dose of ethanol, moderated the impact of other variables on salsolinol excretion. This suggests that the salsolinol acquired on day one was still influencing the appearance of salsolinol in urine on the second day. This raises the possibility that salsolinol is not excreted rapidly in humans, it may enter tissue stores with a subsequent delay in elimination into urine. This is consistent with evidence that simple tetrahydroisoquinolines can be stored in sympathetic neurons [23], but the distribution of salsolinol *in vivo* has not been investigated. In support of a slow excretion of salsolinol in humans, Sjoquist *et al.* [38] observed continuingly elevated levels of this compound in urine of alcoholics after several days of abstinence. The carryover effect revealed here suggests that both diet and ethanol consumption be controlled for at least a day when evaluating the appearance of salsolinol in urine in response to a dose of ethanol.

Both the repeated measures and the between groups analyses showed that salsolinol present in the diet had a powerful effect on the elaboration of urinary salsolinol that was evident within 90 minutes of ingestion of the diet. Significant amounts were present also in the three hour samples. This suggests that salsolinol can absorb from the gastrointestinal tract of humans, with some component of this being excreted by the renal system. This finding extends the observations of Riggin and Kissinger [32] who detected the presence of salsolinol in cocoa-based products, and of Duncan and Smythe [13] who found salsolinol as an ingredient in some alcoholic drinks, by showing that ingested salsolinol can enter the body. Needless to say, the presence of exogenous salsolinol and its facile absorption has the potential to complicate studies investigating relationships between salsolinol and drinking behaviour. As noted earlier, salsolinol was identified in adrenal glands of alcohol-naive rats [19], and it has been found in newborn rat pups [27]. It is possible that dietary sources contributed to these determinations.

The major objective of the study was to see if the level of social drinking had an influence on the elaboration and excretion of salsolinol. Analysis of day one data collected under the "salsolinol-lean" diet condition showed that the excretion of salsolinol was similar regardless of the experimental dose of ethanol consumed, gender, or social drinking level. The research of Truitt [42] and Korsten *et al.* [22] would suggest that heavy drinkers could synthesize more salsolinol than light drinkers, based upon the former group having higher blood levels of acetaldehyde. The present results, however, found no support for there being quantitative differences in urinary salsolinol in heavy and light drinkers. The results from this study would be more consistent with data reported by Eriksson and Peachey [15], who were unable to detect differences in blood acetaldehyde taken from alcoholics and controls. The finding that gender had no impact on salsolinol excretion after consumption of ethanol in this diet condition suggests indirectly that females may not differ from males in having gender-based levels of blood acetaldehyde after drinking alcohol. It is worthy of note that current studies of blood acetaldehyde levels reveal that min-

imal quantities of acetaldehyde are present *in vivo* after alcohol consumption [11, 14, 20].

In contrast, gender, quantity of ethanol consumed and level of social drinking had significant influences on the excretion of salsolinol occurring in the three hour period after ingestion of the "salsolinol-enriched" diet. The gender effect was seen only in the three hour sample, where females excreted more than males. This may have arisen because all subjects received a constant quantity of exogenous salsolinol, that present in the chocolate bar and syrup, irrespective of body mass. There was a two-fold increase in the amount of salsolinol excreted by individuals given the high dose of ethanol. In the absence of evidence supporting an influence of the experimental doses used on salsolinol excreted in the "salsolinol-lean" situation, this suggests that the higher ethanol dose enhanced the absorption and/or the excretion of ingested salsolinol. Subjects given the high dose of ethanol produced greater volumes of urine in both urine samples than those drinking the low dose. Thus, the diuretic effect of ethanol may have contributed to this finding. Compared with light drinkers, heavy drinkers excreted notably less salsolinol. This effect was seen in both urine samples. It is possible that heavier social drinkers are less able to absorb salsolinol, they retain salsolinol more effectively than light drinkers, and/or they metabolize salsolinol more efficiently than do light drinkers. While little is known concerning the absorption or distribution of salsolinol, in the absence or presence of ethanol, it is known to undergo metabolic O-methylation [3, 24, 30] and may be oxidised by hepatic enzymes [16]. The possibility of differential degrees of conversion between heavy drinkers and others remains to be determined. In this regard, Collins *et al.* [9] found that alcoholics excreted greater amounts of methylated salsolinol in the post-intoxication phase than of salsolinol, although this was not seen by Sjöquist *et al.* [38].

The presence of salsolinol in human urine was first reported by Sandler *et al.* [35]. Samples obtained from Parkinsonian patients receiving L-dopa contained large amounts of the tetrahydroisoquinoline, which increased if alcohol was consumed. Trace quantities of salsolinol appeared to be present in urine of two normal individuals [35]. While Sjöquist *et al.* [39] could find no evidence for enhanced excretion of salsolinol in moderate drinkers given ethanol, urine samples from all subjects contained measurable quantities of salsolinol, these investigators did obtain a significant correlation between dopamine and higher quantities of salsolinol in urine from alcoholics [38]. Significant correlations were found between dopamine and salsolinol in urine samples from social drinkers taking the "salsolinol-lean" diet with single doses of ethanol in the present study, but not in those given the enriched diet, where the excretion of salsolinol was modified by other factors. As all volunteers in the present study were given alcohol, the issue of the impact of alcohol itself on salsolinol formation cannot be addressed directly. Nonetheless, trace levels of salsolinol were seen in urine samples from some volunteers who participated in a preliminary, less controlled survey that led to the present study, prior to them being given ethanol, and increased amounts were identified in urine collected thereafter [19]. Although this evidence is suggestive, the results of the fuller investigation indicate that salsolinol excretion can be modulated in a complex fashion by several variables. Thus, changes in salsolinol excretion occurring in association with alcohol ingestion in humans should be interpreted cautiously in the absence of controlled conditions.

In summary, the present results show urinary excretion of salsolinol was similar in low and high level social drinkers given ethanol experimentally; when exogenous salsolinol was restricted, this excretion correlated to urine content of dopamine. Salsolinol present in food materials can be readily absorbed, at least when alcohol is ingested also. High and low level social drinkers differ in their ability to excrete salsolinol, which may denote underlying differences in salsolinol pharmacokinetics in these groups. As evidence for the penetration of extracerebral salsolinol into the central nervous system is controversial [31,41], it is equivocal whether salsolinol from exogenous sources could have contributed to that found in human brain tissue and cerebrospinal fluid [38, 39, 40].

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