Abstract

The route of alcohol administration used in the experimental study of the role of alcohol in carcinogenesis should mimic the human condition: the p.o. route should, therefore, have major attention. This route appears in concord with epidemiological evidence of alcohol involvement in cancers of the upper digestive tract. The relative advantages of schedule-induced polydipsia, an alcohol solution as the sole fluid source, and an alcohol-containing fluid diet are assessed, as well as the intake of alcohol via the respired air. These routes allow a wide range of daily alcohol doses to be ingested, the largest resulting in continuous intoxication and the development of physical dependence. The equivocal results from various already published typical experiments are discussed, indicating the necessity of using a variety of appropriate dosages, dosage routes, and dosage forms in appropriate controls, as well as indicating the necessity of investigating the role of concentration and kind of alcoholic beverage in a variety of strains and species in both sexes and at various ages.

The epidemiological evidence in many complicated ways indicates that any action of alcoholic beverages as carcinogenic, cocarcinogenic, initiating, or promoting arises from the frequent consumption of alcohol and that such action is concentration, dose, and beverage related. Any anticarcinogenic or inhibitory action of alcohol might arise from the infrequent, but timely, ingestion of alcoholic beverages taken in low concentrations. Alcohol intake not only provides a metabolism-reducing atmosphere by raising the NADH:NAD⁺ ratio, but its chronic intake also leads to an increase in the activity of various enzyme systems (13). Contrariwise, even in small amounts, alcohol acts as an inhibitor of various metabolic transformations (2). Thus, conceptually, the duration and time at which alcohol is circulating in the organism in relation to the presence of carcinophilic agents may be a factor of some importance in the actions of alcohol. In addition, some beverages might be free of other nonethanolic constituents or contain constituents which are themselves inhibitory or anticarcinogenic (27, 28).

Considering these assumptions, how should alcohol be administered to animals so as best to test the effects of alcohol vis-à-vis carcinogenesis? How appropriate are the various modes of alcohol administration, both those which result in the maintenance of alcohol intoxication and the development of dependence and those which have been used in assessing alcohol involvement in cancer genesis?

The variety of schemes which have been used for the production of intoxication and dependence (21) are, of course, also applicable in low-dosage regimens not yielding intoxication and dependence; they are not all applicable, however, to long-term chronic treatment regimens because many (e.g., Ref. 18) produce substantial mortality after a few days of alcohol treatment. One should, to the extent possible, mimic the human condition: I shall not deal with these methods, assuming that the usual route of alcohol ingestion in humans is oral, although it is sometimes breathed and sometimes taken by more extraordinary routes. It is probably not a coincidence that cancers of the human upper digestive tract are of major significance because of the common oral ingestion of alcoholic beverages. For experimental studies in animals, it seems irrelevant to discuss intubation, gastric infusion, i.v. administration, or i.p. injection, although it may well be that the latter route can serve to illuminate the role of systemic as opposed to more local effects of alcohol. Despite the fact that few humans are exposed to alcohol via the inspired air [except those few purposely exposed more than 25 years ago (12)], this route may prove useful in animals for other reasons.

Experimental Requirements. Although it is common to expect a monotonic relationship between dose and effect, any experiment conducted on this assumption is at hazard. With ethanol, especially with a maximum possible dosage regimen, substantial caloric substitution, equaling or exceeding the basal metabolic requirement of the organism, is an important factor. A prudent protocol calls for at least 3 levels of alcohol intake, each level controlled by pair feeding and by an additional group of ad libitum-fed control animals, plus whatever other combination of other materials (carcinogens or other agents) may also be used. The highest daily dosage should be the maximally tolerable ethanol dose not producing mortality in a short-term experiment.

Oral Intake. Polydipsic intake of alcohol solutions (3, 10) can be at levels which maintain intoxication with eventual production of dependence. Since fluid intake follows the intermittent provision of food, alcohol intake is easily modulated by the schedule of food presentation. Essentially, any concentration of alcohol [at least up to 32% (19, 20)] and probably any kind of alcoholic beverage can be given by this means; even highly aversive quinine solutions will be licked by the rat, although these may not necessarily be swallowed! As in any experiments involving alcohol, the actual intake should be monitored for assurance that spillage, if any, is taken into account; it is incumbent on the investigator to determine whether the apparent consumption of substantial volumes does in fact take place: can alcohol be found in the blood, for example? The importance of polydipsic intake for present purposes is that animals, certainly rats, will drink more than trivial quantities of relatively high concentrations of alcohol, concentrations not ordinarily drunk in such amounts even when alcohol is the sole fluid source. Since the drinking of alcohol at varying concentrations is under relatively simple experimental...
control, polydipsic consumption would appear to be a method of choice for alcohol administration in the study of carcinogenesis. Other ingested nutrients are also amenable to the same kind of control. The major disadvantage of this route is that animals require maintenance at about 80% of their free-feeding weight and individual housing, at least during ingestive periods, and each cage requires a means for delivery of food pellets at intermittent intervals.

Alcohol in large amounts can be ingested when it is incorporated in a liquid diet which provides all other nutrients (15); a large number of such diets, with variable ratios of protein, fat, and carbohydate and variable vitamin and mineral contents, have been described (1, 4, 11, 14–17, 22, 26). By and large, the calories provided by alcohol approach the basal metabolic rate of the animal. If such calories are continuously ingested over any lengthy period, the difference between being essentially sober and being drunk amounts to only an additional 2 to 3 g of ethanol per kg. In none of these diets, however, which are the sole source of food and fluid for the animals, does the concentration of alcohol usually exceed 6%. Thus, although the liquid diet offers a great deal of flexibility in its makeup and administration, including the advantages of simplified pair feeding, it may not be amenable to the incorporation of higher concentrations of alcohol, and the fact that other substances are also present together with the alcohol may ameliorate any effects that alcohol might otherwise have.

Giving alcohol as the sole fluid source, with solid food available ad libitum, can also produce intoxication and dependence. Alcohol in concentrations of at least 25% will be ingested. Such a noninstrumental technique, the acme of simplicity, has an apparent advantage over polydipsic ingestion. Polydipsic ingestion, however, allows a greater degree of experimental control so that ingestion may proceed more or less uniformly throughout the 24 h, rather than at the “whim” of the animal, and at probably higher alcohol concentrations than animals would otherwise ingest from an alcohol solution as the sole fluid source.

Inhalation. Small experimental animals can inhale large amounts of alcohol by virtue of their higher ratios (than humans) of ventilation to body weight and produce intoxication and dependence (5). No measures of the concentration of alcohol that results in the mouth and other parts of the upper digestive tract have been published. At levels above 20 mg of ethanol per liter of inspired air, eye irritation becomes prominent. About 62% of the respired alcohol (in humans) is absorbed from the respired air (12). Obviously, some alcohol dissolves in the mouth; what concentration it reaches as it passes to the esophagus and stomach is not known. What effects it might have, together with tobacco smoke, on lung tissue is also not known. Inhalation of alcohol, with and without tobacco smoke, appears, perhaps too simplistically, to offer a means of assessing the role of alcohol in the production of cancer. It offers, too, a more attractive and realistic alternative than those routes which, like that of Stenback (25), assess the effects of alcohol by applying 99.5% alcohol topically with and without a carcinogen (9,10-dimethyl-1,2-benzanthracene). Relatively few experimental investigations of the possible interaction of alcohol and other possible carcinogens have been carried out. The available results have been equivocal, and the criticism of some varied, but not untypical, reports which follows indicates some of the possible reasons for the necessary equivocation.

There has been one experiment, that of Reddy et al. (23), which exposed mice to cigar smoke and alcohol vapors, but the negative conclusions (absence of cancer in all treated groups, but “benign dysplasia and squamous metaplasia of the bronchial epithelium with occasional precancerous pleomorphism and cell disarray”) drawn by these authors are vitiated by the absence of knowledge of the concentrations of either the tobacco smoke or of the alcohol to which the mice were exposed. It is not at all clear that the exposure daily for 1 year lasted more than 30 min a day. Given the length of the experiment, the investigator’s courage in using only 10 to 15 mice/group (smoke = 15, smoke plus alcohol = 15, alcohol = 10, and control = 10) is astonishing.

Two cases of esophageal carcinoma in mice have been reported by Horie et al. (6). Of some 193 C F1 mice, one-third were treated with benzopyrene and one-third with 4-nitroquinoline-1-oxide, with the carcinogens dissolved in 70% alcohol; one case of esophageal carcinoma appeared in each of these groups. The alcohol solution of carcinogens was the sole source of fluid for 4.7 days/week. The remaining one-third of the mice, a so-called control group, received 70% alcohol without an added carcinogen for the same time period. There was no group paired to drink either of the carcinogens in the volume consumed by the alcohol groups, the authors assuming that previous experiments in which mice were treated with carcinogens in oil and where no cancer resulted, served as appropriate controls. The mortality was 100% (in 9 months) in the benzopyrene:alcohol group and 72% (in 12 months) in the nitroquinoline:alcohol and alcohol-only groups. Despite the substantial investment of effort, the results appear to have little meaning because of the absence of appropriate control animals, the extremely high alcohol concentrations available to the mice, the high early mortality, and the absence of any firm knowledge as to intake.

The work of Kuratsune et al. (9), in which the effect of daily oral ingestion by C F1 mice of whiskey, sherry, sake, and purified alcohol in concentrations as high as 43% (as sole fluid source) was studied, gives no support for the activity of alcohol in cancer production. However, this experiment did not assess (see, for example, Ref. 24) the effect of p.o.-ingested alcohol with a known carcinogen, mimicking the apparent human condition. On the other hand, Ketcham et al. (8) found alcohol, as the sole fluid source, at a level of 20% (for up to 1 year) not to promote or stimulate cancer spread in female mice treated thereafter with a tumor inoculum, nor did the intake of alcohol alone at this concentration for 15 months produce tumor development. How much alcohol was actually ingested in either of these experiments is not known.

The findings of Kahn (7) indicate that both 0.5 and 5% alcohol as a drinking fluid by pregnant mothers throughout gestation or during the first 2 weeks postpartum lead to a significant 67% decrement in hepatomas in offspring of treated mothers at 15 months of age. Again, the defects in this work are (a) the lack of pair-feeding controls (animals had unlimited access to their solid food) and (b) the anomaly that a 0.5% alcohol solution had as profound an effect as a 5% solution. Because of the apparent substantial anticarcinogenic effects found, this experiment deserves redosing with changes to overcome these perceived deficiencies.

Similarly, Yamamoto et al. (29) found that alcohol at levels of 10 and 20% in the drinking fluid of male Fischer rats reduced...
nodule sizes of hepatomas resulting from concurrent ingestion of a solid diet containing N-hydroxy-N-2-fluorenylacetamide. In female Fischer rats, however, 10% alcohol appeared to increase the incidence and size of nodules, while in NIH black rats there appeared to be no effect. Unfortunately, the distinct decrement in nodule size may be related to the fact that ethanol-treated rats ate less of their solid diet, thereby reducing their concomitant intake of N-hydroxy-N-2-fluorenylacetamide. Had there been a group of pair-fed rats in whom nodule size was not decreased, these results might have provided strong evidence for the anticarcinogenic activity of ethanol.

In the complexity of factors leading to cancer production, alcohol plays no obviously simple role. Experiments designed to elucidate the role of alcohol will have to deal with its concentration, with the varying composition of its many beverage forms, with the strains and species of experimental animals used and their sexes and ages, with the alterations perforce introduced into the diet by the calories provided by alcohol, and with the temporal relations between alcohol and associated carcinogen ingestion.

References

Modes of Alcohol Administration Appropriate for the Study of the Role of Alcohol in Carcinogenesis

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