Notice to Contributors

Science is taking drastic steps to reduce its backlog of accepted reports and thus to attain faster publication in future. This can only be achieved by a sharp temporary reduction in the rate of acceptance of manuscripts. The problem will be overcome by 1 October. Financial limitations prevent diminishing the backlog by substantially increasing the pages devoted to reports. Because of our large domestic and international circulation, the cost of delivering a two-page report to all our readers is about $1600. We need to reduce the backlog by 100 reports.

Until 1 October, the acceptance rate for reports will be about 10 percent. Unfortunately, many excellent manuscripts will be rejected. To save authors' time and to avoid asking conscientious reviewers to devote efforts to examining manuscripts that must ultimately be declined, some reports will be returned to authors without review. We regret any inconvenience that these temporary measures may entail.

Reports

Inhibition by Alcohols of the Localization of Radioactive Nitrosonornicotine in Sites of Tumor Formation

Abstract. *Oral administration of ethanol,* n-*butanol, or* t-*butanol to mice 20 minutes before injection of carbon-14–labeled nitrosonornicotine inhibited the localization of radioactivity in bronchial and salivary duct epithelium and in the liver. Localization of radioactivity in the nasal epithelium and esophagus was not significantly reduced. These alcohols therefore may selectively inhibit tumor formation in three of the five sites where this carcinogen typically acts.*

When nitrosonornicotine, the most abundant carcinogen in tobacco smoke, is labeled with ^{14}C and administered intravenously to mice, radioactivity accumulates in nasal, salivary duct, esophageal, and bronchial epithelium and in the liver (*1*). These are the same sites where tumors appear in rodents after the administration of nitrosonornicotine (*2*). The radioactivity retained is probably in the portion of the molecule that is the proximal carcinogen. Since this selective, intense localization resembles a chemical-receptor interaction, we hypothesized that some noncarcinogenic compounds might exist that can block this accumulation and hence reduce the incidence of tumors in these tissues. During a systematic investigation of potential blockers we discovered that several common alcohols inhibit localization of the radioactivity from [^{14}C]nitrosonornicotine in some of these sites.

Adult male C57BL/6J mice were injected intravenously with [2′-^{14}C]nitrosonornicotine (0.12 to 0.19 μCi per gram of body weight, which corresponds to a dose of 0.4 to 1.9 mg/kg) (New England Nuclear; specific activity, 18.4 or 51.7 mCi/mmole). One hour later the mice were lightly anesthetized with ether and frozen by immersion in dry ice and hexane. Whole-body sagittal sections of the frozen mice were placed on Scotch tape and processed for autoradiography (*3*). Photometric density in areas of the developed autoradiographs was measured with an ADG Instruments photometer and a photocell (aperture, 3 mm) lying on the easel of a photographic enlarger. The x-ray film was placed in the enlarger and raised to produce a magnification of ×35 on the easel.

Aqueous solutions of ethanol, *n*-butanol, and *t*-butanol were administered by oral intubation to some of the mice 20 minutes before the injections of [^{14}C]nitrosonornicotine. Ethanol (1 or 5 g/kg) and *n*- and *t*-butanol (0.2 or 1 g/kg) solutions were prepared so that each mouse received 0.02 ml per gram of body weight.

Localization of radioactivity in salivary duct and bronchial epithelium and in both the periportal and central areas of the liver was reduced by prior treatment with ethanol and to a greater extent with *n*-butanol; *t*-butanol at the higher dose almost completely prevented the localization of radioactivity in bronchial epithelium (Fig. 1). Furthermore, the reduction in photometric density was dose-related (Fig. 2). The greatest inhibition was seen in bronchial epithelium with *t*-butanol; the reductions were similar in both areas of the liver for all three alcohols at the doses used. There was no significant difference between the control and treated groups in the absorbances in nasal and esophageal epithelium.

The results suggest that prior treatment with simple alcohols inhibits localization of the proximal carcinogen in bronchial and salivary duct epithelium and in the liver but not in nasal and esophageal epithelium in male C57BL/6J

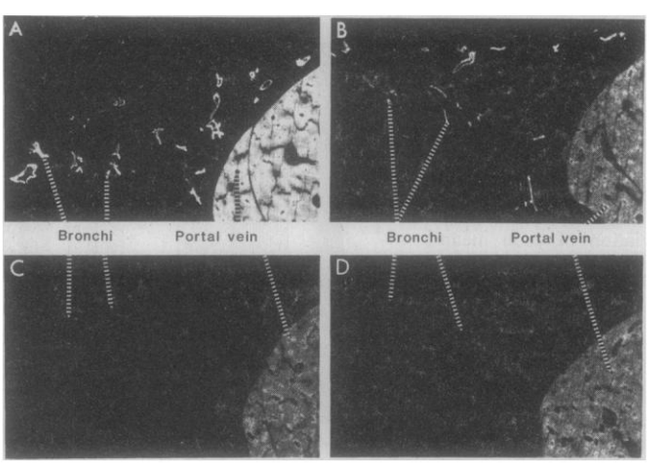

Fig. 1. Enlarged autoradiographs of lung and liver areas from male C57BL/6J mice that received [^{14}C]nitrosonornicotine only (A), advance treatment with ethanol (5 g/kg, orally) (B), advance treatment with *n*-butanol (1 g/kg, orally) (C), or advance treatment with *t*-butanol (1 g/kg, orally) (D). White areas correspond to radioactivity.

mice. Since, at a molar dose, *t*-butanol has approximately 50 times the potency of ethanol in inhibiting the localization in bronchial epithelium, there may be other compounds that are even more potent. Propyl, butyl, and amyl alcohols are present in fermented beverages in different proportions depending on the raw materials used for fermentation and on other factors (*4*). In addition, the presence of alcohols in certain types of cigarettes and foods should not be overlooked.

The specificity of the inhibition suggests that more than one mechanism, or at least a different level of interaction, is operative. Further studies to define the biological molecule that directs the specificity at each site should allow an even more refined selection of blocking agents. Although the molecular mechanism by which alcohols inhibit this localization in bronchial and salivary duct epithelium and in the liver is still not known, our consideration of the available information suggests two enzymes that may be involved. One is the "secondary alcohol dehydrogenase" described by Hardonk (*5*), which is found in very high concentrations in the bronchial epithelium of mice (*6*). The other is P-450$_{LM3a}$, which has a higher catalytic activity toward alcohols (*7*) and nitrosamines (*8*) than other P-450 isozymes. The initial reaction of nitrosonornicotine metabolism in vivo is thought to be α-hydroxylation (*9*), which creates a tertiary alcohol.

When these experiments were initiated, *t*-butanol was thought not to be metabolized; the compound was selected in order to compare metabolizable alcohols (ethanol, *n*-butanol) and nonmetabolizable alcohols (*t*-butanol). However, Baker *et al.* (*10*) recently demonstrated the formation of acetone and CO_2 from *t*-butanol in rats. Consequently, we are uncertain as to whether metabolic processes or purely solvent effects inhibit the localization. Furthermore, an indirect, secondary action or other mechanisms could be involved. The site specificity and marked differences in potency of the alcohols do strongly favor metabolic inhibition.

Inhibition of the localization of this fragment of nitrosonornicotine in the three organs only suggests that tumor incidence will be reduced at these sites. Experiments involving long-term exposure must be conducted to determine whether this is in fact the case. It is, of course, possible that inhibiting the localization in these sites may increase the incidence of tumor formation in other sites. In a study by McCoy *et al.* (*11*), the carcinogenicity of nitrosonornicotine was not altered by feeding hamsters a liquid diet containing 6 percent ethanol. However, considerations of the dose and potency of the alcohol used and species differences preclude a direct comparison with our results.

Although most epidemiological studies of cigarette smokers have not separated the effects of different levels of alcohol consumption on the incidence of cancer with a defined level of smoking, several reports do contain some pertinent information. In their table 4, Schottenfeld *et al.* (*12*) presented data from which one can calculate a reduction in standardized mortality ratios from cancer (from 12.6 to 5.3; $P = .047$) in persons who drink heavily and smoke lightly. With high tobacco exposure and high alcohol consumption, the reduction in standardized mortality ratios from cancer was even more significant (from 26.7 to 17.5; $P = .004$). These estimates are pooled over all ages and were not calculated by Schottenfeld *et al.* Other epidemiological studies (*13*) suggest that the interaction between smoking and drinking may include inhibition of tumors at some sites, but data now available do not permit a conclusion as to the effect of alcohol on lung cancer in smokers. Extrapolation from the animal data in this report to tumor formation in humans should be done with great caution because of the differences in incidence among species. The national cancer survey now in progress should record details (type, brand, and so forth) about the alcoholic beverages consumed so that inferences can be made about the effects of congeners and nitrosamines.

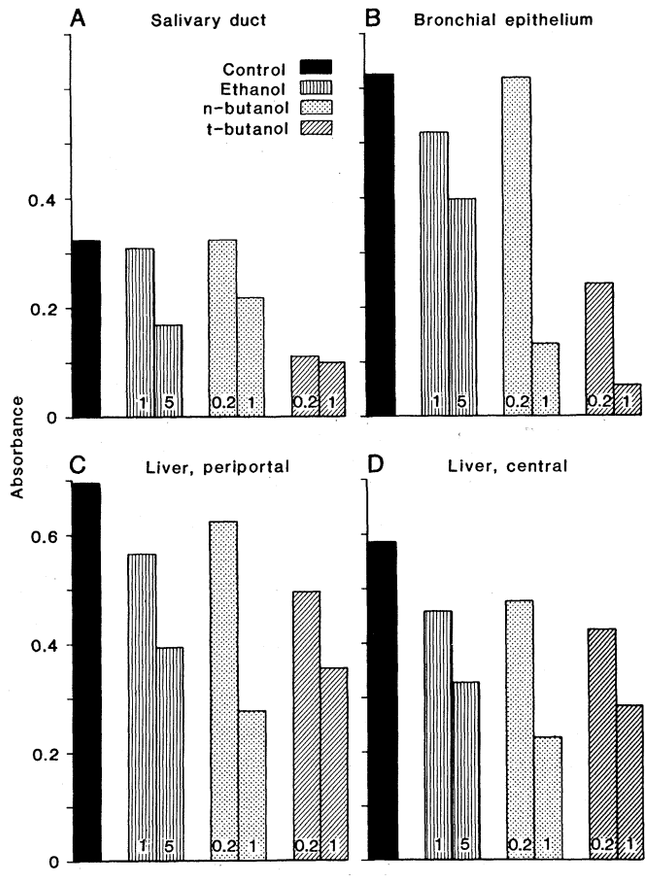

Fig. 2. Mean absorbances for the four areas in which the localization of radioactivity was inhibited. The number in each bar represents the dose (in grams per kilogram) of the alcohol, that was administered orally 20 minutes before the intravenous injection of [^{14}C]nitrosonornicotine. The means for each mouse were obtained from 15 measurements of random areas of a given site (five absorbances for each of three autoradiographs) after setting the blood in each equal to zero. The control value is the mean for six mice, the *n*-butanol value at 1 g/kg is for two mice, and the other means are for one mouse. The coefficient of variation of each mean is less than 10 percent. All measurements were made at one occasion by the same observer, who had no knowledge of the treatment of each randomly selected autoradiograph.

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References and Notes

1. W. J. Waddell and C. Marlowe, *Cancer Res.* **40**, 3518 (1980).
2. D. Hoffmann, R. Raineri, S. S. Hecht, R. Maronpot, E. L. Wynder, *J. Natl. Cancer Inst.* **55**, 977 (1975); G. M. Singer and H. W. Taylor, *ibid.* **57**, 1275 (1976); S. S. Hecht, C. B. Chen, N. Hirota, R. M. Ornaf, T. C. Tso, D. Hoffmann, *ibid.* **60**, 819 (1978).
3. W. J. Waddell and C. Marlowe, in *Drug Fate and Metabolism: Methods and Techniques*, E. R. Garrett and J. L. Hirtz, Eds. (Dekker, New York, 1977), pp. 1–25.
4. M. Windholz, Ed., *The Merck Index* (Merck & Co., Rahway, N.J., ed. 9, 1976), p. 557.
5. M. J. Hardonk, *Histochemie* **5**, 234 (1965).
6. L.-E. Appelgren, *Acta Physiol. Scand. Suppl.* **301**, 77 (1967).
7. E. T. Morgan, D. R. Koop, M. J. Coon, *J. Biol. Chem.* **257**, 13951 (1982).

8. K. K. Hajek and R. F. Novak, *Biochem. Biophys. Res. Commun.* **108**, 664 (1982).
9. C. B. Chen, S. S. Hecht, D. Hoffmann, *Cancer Res.* **38**, 3639 (1978).
10. R. C. Baker, S. M. Sorensen, R. A. Deitrich, *Alcohol. Clin. Exp. Res.* **6**, 247 (1982).
11. G. D. McCoy, S. S. Hecht, S. Katayama, E. L. Wynder, *Cancer Res.* **41**, 2849 (1981).
12. D. Schottenfeld, R. C. Gantt, E. L. Wynder, *Prev. Med.* **3**, 277 (1974).
13. W. Schmidt and R. E. Popham, *Cancer (Philadelphia)* **47**, 1031 (1981); W. Schmidt and J. de Lint, *Q. J. Stud. Alcohol* **33**, 171 (1972); R. R. Williams and M. S. Horn, *J. Natl. Cancer Inst.* **58**, 525 (1977).
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Is Titan Wet or Dry?

Abstract. *Titan's dense and cold nitrogen atmosphere contains a small amount of methane under conditions at least approaching those at which one or both constituents would condense. The possibility of methane and nitrogen rain clouds and global methane oceans has been discussed widely. From specific features of radio occultation and other Voyager results, however, it is concluded that nitrogen does not condense on Titan and that Titan has neither global methane oceans nor a global cloud of liquid methane droplets. Certain results indirectly support the conjecture that methane does not condense at any location. However, other considerations favor a methane ice haze high in the troposphere, and liquid and solid methane might exist on the surface and as low clouds at polar latitudes.*

Saturn's giant moon Titan is the only planet-sized body in the solar system known to share with Earth the characteristic of a predominantly nitrogen atmosphere (*1–4*). Many scientific and popular accounts propose that Titan shares another fundamental set of terrestrial features, namely global oceans, clouds, rain, snow, rivers, glaciers, and polar ice caps. In this scenario, methane plays the role on Titan that water does on Earth, being present as a liquid and a solid on the surface and in clouds and as a gas in the atmosphere (*1, 5*). In addition, it has been suggested that some of the atmospheric nitrogen itself condenses to the liquid phase (*6*).

Although the initial Voyager reports reinforced speculation about a "wet" Titan, current studies cast doubt on this interpretation and indicate instead that Titan is mostly "dry." The new evidence is based primarily on the Voyager radio occultation measurements as analyzed by Lindal *et al.* (*7*). While there is a global cloud cover, it might not include any condensed methane or nitrogen. Organic molecules, including complex hydrocarbons, are formed photochemically from methane and other constituents in the stratosphere and produce aerosol that obscures the surface to visual imaging (*8*). These particles are believed to settle to the surface, removing methane gas irreversibly from the atmosphere (*8*). Thus whether there are reservoirs of condensed methane depends on the evolutionary history of Titan's hydrocarbon and organic chemistry.

Figure 1 shows the average of the two atmospheric profiles from the Voyager radio occultation experiment. The two profiles, which are based on the assumption that the atmosphere consists entirely of nitrogen (*7*), apply to two positions within 10° latitude of the equator (assumed to be in Titan's orbital plane), one each near the morning and evening terminators at the time of the measurements. For the lowest 30 km of the atmosphere they have a temperature difference of less than 0.2 K (root-mean-square), suggesting a remarkable uniformity for Titan's equatorial troposphere.

The question of the influence of other constituents on the derived profile is discussed elsewhere (*1, 7, 9*). The values in Fig. 1 are near the low end of a possible range of about 4 K in actual equatorial temperatures because of uncertainty about the concentrations of minor constituents, particularly methane and argon. If there is very little argon, as we believe likely (*7*), then the proportion of methane would probably be less than a few percent, in which case the correct values would be very close to those shown in Fig. 1.

Measurements by the Voyager infrared instrument indicate about a 2 K reduction in surface infrared brightness temperature with increasing latitude and only a 2 K hemispheric difference at the wave number that is primarily sensitive to heights near the tropopause (*10*). However, since neither set of measurements includes regions within 20° of the orbital poles, global changes may be somewhat greater. We consider the profile shape in Fig. 1 to be representative, with up to a 3 K decrease for temporal and latitudinal effects and up to a 4 K increase for constituent uncertainties.

Also shown in Fig. 1 is the condensation temperature of nitrogen. It is about 5 K from the atmospheric curve at its closest approach, or a minimum of 2 K under the above uncertainties. We conclude that atmospheric nitrogen does not condense at Titan, either at the surface or in the atmosphere.

At every 4-km height interval up to 20 km in Fig. 1, we have superposed two short line segments representing certain theoretical slopes. In each case the steeper slope is for a nitrogen atmosphere that is saturated with methane and mixed vertically to produce a wet adiabat where the condensate moves with the gas. The accompanying percentages indicate the relative molar abundances of methane needed for saturation. The other segment is for a wet "pseudoadiabat" in which the condensate either does not mix away from where it condenses or else precipitates as rain or snow (*11*). Below about 10 km the slope of the actual curve is less steep than these theoretical segments.

The theoretical dry adiabat (no methane condensation) matches the measured curve for the lowest 4 km of the atmo-

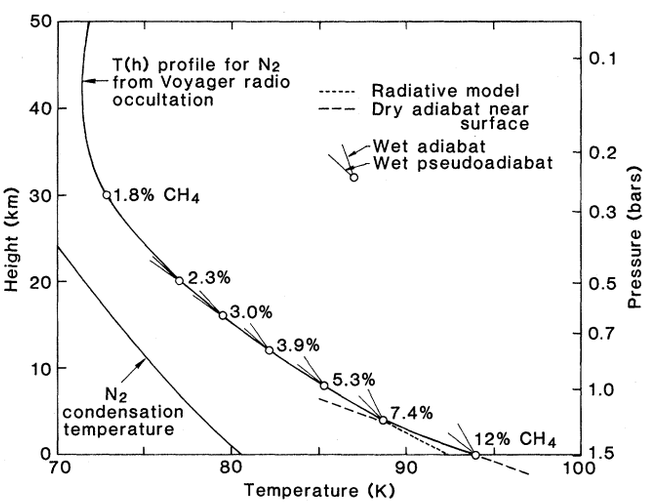

Fig. 1. Temperature as a function of height and pressure in the atmosphere of Titan (*7*), compared with the nitrogen condensation temperature, dry and wet methane adiabats, and a simple radiative model. Approximate relative methane molar abundances for saturation at a number of heights are shown. The point at 30 km is the cold trap.