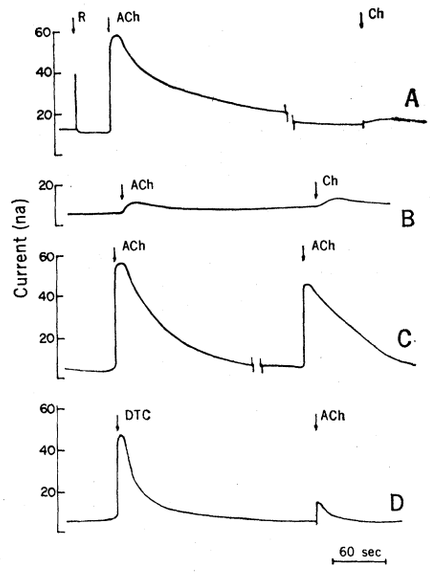

Fig. 1. Records of d-c currents across artificial lipidic membranes containing proteolipids from *Electrophorus*. A, C, and D correspond to peak 3, and B to peak 1. *ACh*, acetylcholine; *Ch*, choline; *DTC*, *d*-tubocurarine; *R*, control; the artifact is due to the injection of the bath solution.

tration failed to elicit such a response (Fig. 1A). When acetylcholine was applied to control membranes or to those containing the "nonreceptor" proteolipid (peak 1) a minimal conductance increase was detected (Fig. 1B). The transient conductance increase induced by acetylcholine may be repeated by successive injections on the same membrane (Fig. 1C). The addition of *d*-tubocurarine ($10^{-3}M$ in the pipette) also elicits a conductance response; however, after this application the action of acetylcholine is much reduced (Fig. 1D). To discard the influence of changes in tonic concentration control experiments were made by injecting different solutions. Thus, with distilled water, 300 m*M* NaCl, or 300 m*M* KCl there were no significant changes in conductance.

Some material of bacterial origin may induce electrical excitability in artificial membranes (*9*). Since the extraction of the proteolipid is done with organic solvents from lyophilized, freshly dissected electric organ this type of contamination can be discarded.

The experimental conditions so far used do not permit us to establish with certainty the initial concentration of the drugs reached at the membrane interface since the 50-μl portions of the drugs applied diffuse and are diluted into the 10-ml content of the chamber and they cannot be washed out. However, those shortcomings do not alter the main facts here reported, and new chambers of better design should decrease these difficulties and permit more elaborate pharmacological experiments.

Del Castillo *et al.* (*10*) reported electrical changes, induced by cholinergic agents, in lipidic membranes with addition of acetylcholinesterase from bovine erythrocytes. In our experiments the proteolipid is devoid of acetylcholinesterase activity and was identified as an independent macromolecular entity (*11*). Unpublished observations with electron microscopy (from this laboratory) have shown that the isolated proteolipid appears as elongated macromolecules which may undergo morphological changes under the action of cholinergic agents. Similar findings were obtained with a "receptor" proteolipid isolated from cerebral cortex (*12*).

Mueller *et al.* (*13*) have shown that some ionophoric antibiotics and the excitability inducing material (*9*), in artificial membranes, were able to induce electrical phenomena resembling those observed in excitable membranes. Our results show that artificial membranes containing the proteolipid of peak 3 may be excited by certain cholinergic agents.

The characteristics of such response provide additional support to the idea that this special proteolipid, which is present in the electroplax membranes (*11*) and which binds cholinergic agents (*4*), may represent a cholinergic receptor.

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Alcohol Breath Tests: Gross Errors in Current Methods of Measuring Alveolar Gas Concentrations

Abstract. *Transitory contact of ethanol with the mucous membranes of the mouth or nasal passages, or both, is sufficient to drastically alter measurements of concentrations of ethanol in so-called "alveolar" gas for more than 20 minutes after such contact. Various concentrations of ethanol were taken into the mouth by human subjects and were expectorated. Readings of so-called "blood alcohol" were then taken at short intervals by means of the Breathalyzer® and were continued up to 1 hour after exposure. These readings were compared with blood-alcohol concentrations measured by quantitative chemical analysis of venous blood. When true concentrations of blood alcohol were at or close to zero (plus possible error of 0.0001 gram per 100 milliliters), readings of greater than 0.40 gram per 100 milliliters were obtained on the Breathalyzer. Repeated mouth washing and gargling with water, changes in the nature of the solvent, and stomach loading each had only a slight effect in diminishing these errors.*

It has been assumed that accurate measurements of the concentration of ethanol in blood can be determined by measurements of ethanol fractions in "alveolar" gas (*1*, p. 29). "Alveolar" gas is usually defined as the last sample

collected at the end of a forced expiration. Various commercial devices for breath analysis are used in most states in the United States and in many other countries to determine, for legal purposes, the state of intoxication of a driver detained under suspicion of driving while "drunk." These tests have been documented as highly reliable by respected physiologists and physicians and have been accepted as legally valid, after many court tests, in most states in the United States (*1–3*).

In this report it is demonstrated that such tests are totally unreliable for at least the first 20 minutes after exposure to alcohol, unless very large correction factors are applied to readouts on these (breath analysis) instruments. The so-called alveolar gas sample is, in fact, gas that has passed through the bronchi and the oral-pharyngeal cavities and has been contaminated by gas in solution in the lining of these spaces.

Three experiments were conducted on two human subjects, as follows.

Experiment 1. Sips of varying concentrations (6 to 25 percent, by volume) of ethanol, U.S.P., diluted with water or concentrated frozen orange juice, or both, were taken into the mouth with or without gargling of the solutions and were then expectorated without swallowing. This procedure was continued for 1, 2, or 4 minutes. The time periods were based on observations of times required by other subjects to comfortably consume amounts of mixed drinks with equivalent concentrations of alcohol. Time zero (t_0) is defined as the time when the last sip of ethanol solution was expectorated. Readings of ethanol concentration in alveolar gas (as defined above) were taken at measured intervals of 4 to 6 minutes on the Breathalyzer® (*4*) until the readout was zero, or until the reading was $\leqslant 0.01$ g/100 ml. Mouthpieces were discarded after each test. Test solutions were discarded before the total cumulative exposure reached 0.30 g/100 ml. Control readings were also taken prior to the exposure to ethanol.

Experiment 2. The same procedure that was used in experiment 1 was followed with the exception that, starting at t_0, the subject rinsed his mouth with tap water, as follows: a mouthful of water, swished about and then expectorated, and two more mouth rinses, in the same fashion, followed by a gargle with tap water. This entire procedure of three rinses and one gargle was repeated two more times, thus adding up to nine mouth rinses and three gargles. This washing procedure lasted from 1 to 2 minutes. Readings were taken as above.

Experiment 3. Experiments 1 and 2 were repeated; this time, samples of blood were drawn from the veins in the antecubital area into vacuum tubes, at intervals before and after the exposures to ethanol. The skin in each case was washed with nonalcoholic fluid. The blood samples were analyzed by the autoclave diffusion (dichromate reduction) technique, a chemical procedure for quantitative determination of alcohol (*1*).

Figure 1 shows typical families of curves of "blood-alcohol" readings on the Breathalyzer. In most cases, at $t_0 + < 4$ minutes, the readings were above scale (> 0.40). If these were true readings, the subject should have been totally inebriated, possibly at severely toxic or even lethal levels. However, neither subject displayed any evidence of performance or behavioral deficit, nor did either subject feel any subjective evidence of ethanol influence. Note that the solution in which the ethanol was dissolved, as well as the concentration of ethanol in the solution, each played a role in determining the rate of extinction of this effect, but all the curves are almost parallel and displaced only slightly by such changes in solvent or concentrations.

Results after mouth washings with water were strikingly similar to those without washings, with the curves

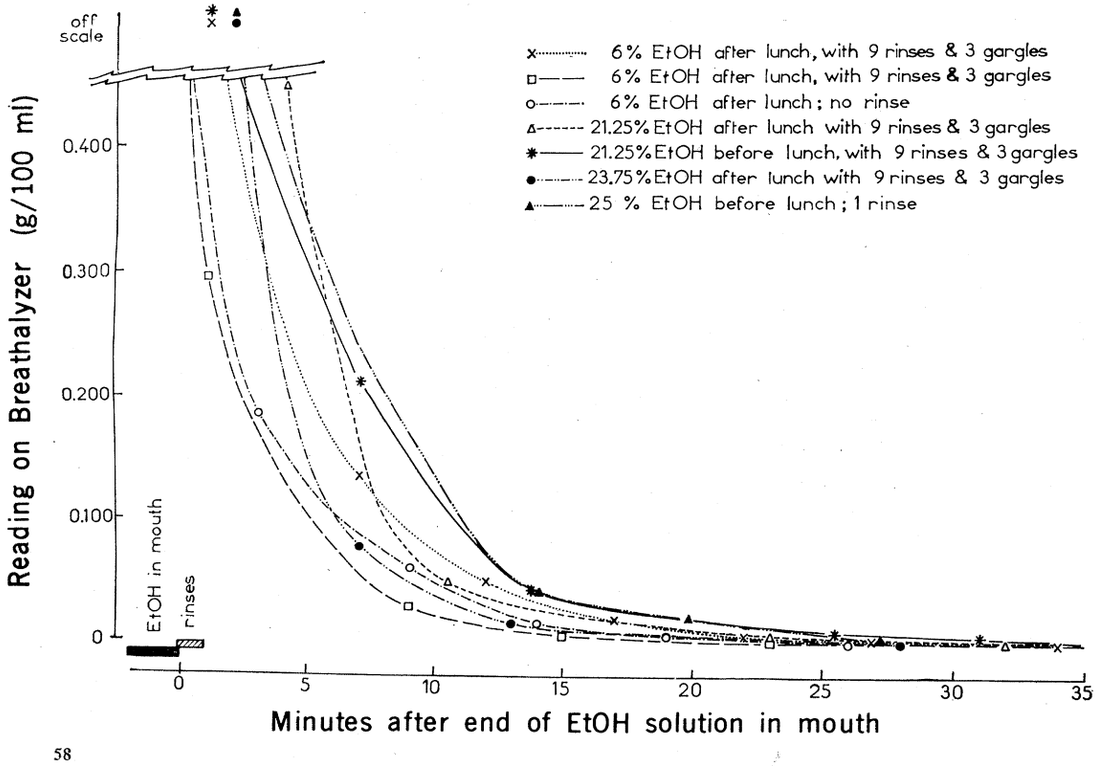

Fig. 1. Readings on the Breathalyzer of concentrations of "blood - alcohol" based on concentration of ethanol in the last portion of gas collected during forced expiration. Sips of various solutions were taken into the mouth and then expectorated, during a period of 2 minutes ending at t_0. At t_0, the mouth was rinsed with tap water, and the subject gargled (where indicated) intermittently for 1 minute.

shifted only slightly to the left. Also, there were little differences between the results recorded just before lunch and those obtained just after lunch. Dilution of ethanol with orange juice, as compared with dilution in tap water, produced no appreciable change in the results.

From data in Fig. 2 it can be seen that the actual concentration of ethanol in the blood was either at zero or extremely close to zero and in no way followed the Breathalyzer readings. The control reading for subject G.M., prior to ethanol exposure, was at 0.000099 g/100 ml, the highest of the four blood sample readings. Thus it appears that the quantity of ethanol absorbed into the blood via mucous membranes of the mouth was negligible.

Several authorities have made claims for accuracy and consistency of the Breathalyzer, and other such tests, as remarkable as $\pm$ 0.024 g/100 ml (2 standard deviations; weight/volume) (*2*, p. 101). There have been occasional warnings about the inaccuracy of Breathalyzer or other alveolar gas tests (*2*, p. 166), but these are rare, generally nonquantitative, and inaccurate. In only one case, of many cited, was the legality of such tests challenged on the basis of a time factor (*5*) and, in this case, the factor was given as 6 minutes, rather than 30. Borkenstein (*4*), one of the inventors of the Breathalyzer, makes no mention of these time factors in his book of instructions and states that when the instructions for proper use are followed "errors are all but impossible."

My data demonstrate clearly that determinations of the concentration of ethanol in so-called alveolar gas are highly inaccurate for at least the first 20 minutes after exposure to ethanol. Indeed, in other experiments, it was found that merely sniffing three times, with the nose at 2 cm from the edge of a flask containing 50 percent ethanol, resulted in off-scale (> 0.40) readings on the Breathalyzer at $t_0 + 2$ minutes. It has also been demonstrated that rinsing the mouth with water does not eliminate these false readings.

It is likewise clear that it is virtually impossible by such tests to obtain uncontaminated samples of gas from the alveoli. The sample taken at the end of an expiration must necessarily pass through the bronchi and oral-pharyngeal cavities and in so doing may gain or lose gases to the gas-fluid interfaces of the mucous membranes.

A subject might be pronounced "legally drunk" (that is, > 0.1 or 0.15 g of alcohol per 100 ml of blood) by such tests even if his true alcohol level is zero. Values obtained by the Breathalyzer or similar devices may still be very useful, if correction factors are applied, as shown in Fig. 3 (*6*). These correction factors may have a different value for each subject and for each set of conditions.

A word should be added about the legal and sociological implications of these findings. Alcohol intoxication is involved in a very large number of automobile, aircraft, and many other types of accidents. It is hoped that, although the results of these experiments might be used at first for evasion of penalties by intoxicated drivers, in the long run, better laws will be enacted, drivers and pilots will be at first coerced and eventually educated to safety, and that more research will be done on the basic problems of the etiology and physiology of alcoholism.

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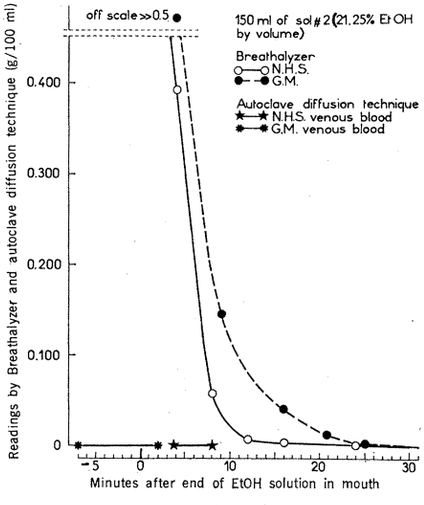

Fig. 2. Mouth exposure to alcohol for 2 minutes as in Fig. 1. The concentration of ethanol was the same for both subjects (21.25 percent). Readings on the Breathalyzer (open and closed circles) are compared with readings obtained by quantitative chemical analysis (autoclave diffusion technique) of venous blood (asterisks and stars). Both experiments were conducted in midafternoon, after lunch. N.H.S. drank 250 ml of coffee with milk just prior to ethanol exposure; G.M. had no liquid to drink after lunch. Neither subject rinsed his mouth after exposure.

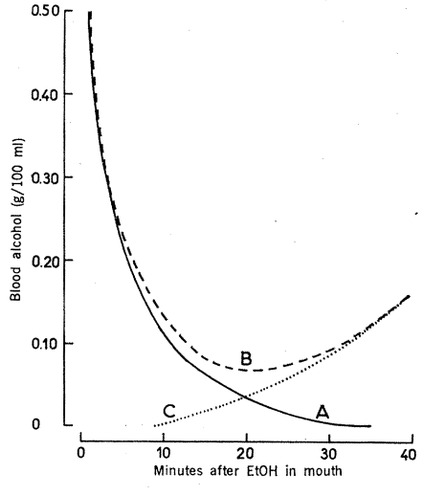

Fig. 3. Values obtained by the Breathalyzer (curves A and B) and values with correction factors (curve C). (Curve A) Breathalyzer reading after EtOH in mouth only; (curve B) Breathalyzer reading after swallowing EtOH solution; and (curve C) corrected curve (B minus A) showing true blood alcohol concentrations after swallowing EtOH solution.

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6. No attempt has been made in these experiments to verify the claims of the manufacturers that the photometric reactions used in the Breathalyzer and other such instruments are highly specific for ethanol and insensitive to other volatile substances. Obviously, a positive reaction to acetaldehyde, a metabolic product of ethanol, or paraldehyde, used in the treatment of alcoholism, would also alter the accuracy of the readout.
7. Data for this report were collected while the author was at the Medical College of Virginia, Richmond. I thank Dr. Grady V. Maraman for his collaboration and participation, and Dr. Jim Scow and his staff at NASA, Langley Research Center, Hampton, Va., for their cooperation. A partial report was presented at the 16th International Institute on the Prevention and Treatment of Alcoholism, Lausanne, Switzerland, 1 June 1970. Supported in part by NSF and NIH grants.
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