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10. Adult female *B. marinus*, native to the Dominican Republic, were obtained from Connecticut Valley Biological Supply, Southhampton, Mass., or National Reagents, Bridgeport, Conn., and were maintained, unfed, on moist bedding at room temperature until decapitation.
11. We produced antibody (655) to somatostatin by injecting rabbits with somatostatin coupled to methylated bovine serum albumin. Goat antibody to rabbit gamma globulin (G-ARGG), rabbit PAP, and fluorescein isothiocyanate-linked goat antibody to rabbit gamma globulin (FG-ARGG) were obtained from Cappel Laboratories; normal goat serum was obtained from Miles Laboratories; and normal rabbit serum was obtained from nonimmunized (control) rabbits. Cyclic synthetic somatostatin was obtained from Peninsula Laboratories, synthetic arginine vasopressin and 3,3′-diaminobenzidine (DAB) were obtained from Sigma Chemical, and synthetic arginine vasotocin was obtained from Calbiochem.
12. Freshly frozen sections (8 to 10 μm) were cut in a cryostat, mounted on gelatinized slides, dried in air overnight, postfixed in 10 percent neutral buffered formaldehyde for 10 minutes, and processed by immunofluorescence techniques. [A. H. Coons, in *General Cytochemical Methods*, Y. F. Danielli, Ed. (Academic Press, New York, 1958)]. The sections were washed twice in 10 m*M* phosphate-buffered saline (PBS) (*p*H 7.4) for 20 minutes, incubated in normal goat serum (1:20) for 30 minutes, in antibody to somatostatin for 24 hours at 4°C at titers of 1:50, 1:100, 1:200, and in FG-ARGG (1:10) for 30 minutes. Then they were thoroughly washed in PBS, mounted on glass slides under PBS and glycerin (1:1), and examined for immunofluorescence with a Zeiss microscope. Adjacent sections were incubated overnight with antibody to somatostatin [preabsorbed with somatostatin (200 μg/ml)].
13. Formaldehyde-fixed tissues were embedded in paraffin, cut in 4-μm sections, mounted on albumin-coated slides, and treated according to the PAP methods of L. A. Sternberger, P. H. Hardy, J. J. Cuculis, and H. G. Meyer [*J. Histochem. Cytochem.* **18**, 315 (1970)]. The sections were then treated with methanol and H_2O_2 (9:1) for 30 minutes to remove endogenous peroxidase activity, washed in PBS for 20 minutes and in normal goat serum (1:20) for 30 minutes, and incubated with antibody to somatostatin for 24 hours at 4°C at titers of 1:750, 1:1000, and 1:5000. Next, the sections were incubated with G-ARGG (1:50) for 60 minutes, PAP (1:50) for 30 minutes, and DAB-H_2O_2 [7.5 mg of DAB per 10 ml of 0.2*M* tris-HCL (*p*H 7.4) plus three drops of 3 percent H_2O_2] for 5 to 10 minutes. Then the sections were counterstained with methyl green, dehydrated through xylene, mounted with Permount, and examined for brown reaction product. Adjacent sections were incubated with antibody to somatostatin [preabsorbed with somatostatin (200 μg/ml)] overnight at 4°C. Sections were also exposed to somatostatin similarly preabsorbed with arginine vasopressin (200 μg/ml) and arginine vasotocin (200 μg/ml).
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17. We thank J. Bollinger for expert technical assistance and A. Duflot-Vignery for her excellent photographs. This work was performed during J.L.B.'s tenure as a National Research Service Fellow (IF 32 AM 06018) and J.N.F.'s tenure as an established investigator of the American Heart Association (77-228); D.B.P.G. is an established investigator of the American Heart Association (77-173). Supported by NIH grants AM 16684, AM 17433, AM 19813; American Heart Association grant 77-103; and National Research Service award IF 32 AM 06018.

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Liquid-Phase Dehydration of Aqueous Ethanol-Gasoline Mixtures

Abstract. *Two-phase mixtures of gasoline, water, and ethanol were dehydrated with both starch and saponified starch-g-polyacrylonitrile (HSPAN). Whereas starch absorbed ethanol as well as water, HSPAN selectively absorbed the water component, allowing ethanol to dissolve in the gasoline phase.*

Ladisch and Dyck (*1*) have shown that the ratio of the combustion energy of ethanol to the distillation energy as a function of the percentage of ethanol in the distillate drops rapidly above about 92 percent ethanol. As one approaches 95 percent ethanol, the input of energy begins to approach the amount of energy gained through combustion. Azeotropic distillation to produce the nearly anhydrous ethanol that is needed for blending with gasoline (to avoid phase separation) is a further detriment to the energy balance. The water tolerance of a blend of 10 percent ethanol in gasoline is about 0.3 percent (*2*). Ladisch and Dyck (*1*) successfully dehydrated aqueous ethanol by passing the vapor through a column packed with a number of inexpensive dehydrating agents, such as starch, cracked corn, cellulose, and carboxymethyl cellulose. The use of saponified starch-*g*-polyacrylonitrile (*3*) (HSPAN, sometimes referred to as super slurper) was also suggested.

In this report, we describe a method for the dehydration of ethanol in the liquid phase, in which an absorbent polymer, particularly HSPAN, is contacted at room temperature with a two-phase mixture of aqueous ethanol and unleaded gasoline (Table 1). The HSPAN used was a commercial sample (SGP 502S, Henkel Corporation) (*4*) that was screened to isolate the fraction passing 20-mesh but held by 40-mesh. It contained 10 percent water. The aqueous ethanol solutions contained 95, 90, and 80 percent ethanol (by volume) [93.7, 86.7, and 75.9 percent (by weight)], and the ratios of aqueous ethanol to gasoline were chosen to give typical gasohol blends of 10:90 by volume [11.1 to 11.3 percent ethanol (by weight)], after removal of water. The weight of polymer added was slightly in excess of that needed to absorb all of the lower, water-containing layer.

Gas chromatographic analysis for ethanol was made difficult by the numerous components present in gasoline; however, acceptable results were obtained on a Bendix 2500 gas chromatograph with a flame ionization detector (2 m by 0.32 cm glass column packed with 15 percent Carbowax 20*M* on Gas-Chrom Q). The column temperature of 65° to 190°C was programmed at 15°C per minute. We constructed a standard curve of detector response versus the percentage of eth-

Table 1. Dehydration of aqueous ethanol-gasoline mixtures: analysis of the gasoline phase. Gasoline (90 ml) was mixed with sufficient aqueous ethanol to give 90:10 (by volume) gasohol, after removal of water. Polymer was added and the mixture shaken overnight at room temperature. An ethanol content in gasoline of 10 percent (by volume) corresponds to 11.1 percent ethanol (by weight) because of the higher density of ethanol. For this calculation, the density of gasoline was equated with the density of octane.

Aqueous ethanol used, % by volume (% by weight)	Controls, no polymer added		Cornstarch* added			HSPAN† added		
	Water (% by weight)	Ethanol (% by weight)	Amount (g)	Water (% by weight)	Ethanol (% by weight)	Amount (g)	Water (% by weight)	Ethanol (% by weight)
95 (93.7)	0.51	10.5	2	0.60	10.6	0.5	0.50	11.1
90 (86.7)	0.39	7.4	8	0.43	8.6	5	0.41	10.8
80 (75.9)	0.20	5.0	10	0.18	5.2	10	0.41	10.6

*Water content, 12 percent. †Water content, 10 percent.

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anol (by weight) by analyzing a series of known solutions of ethanol in gasoline. Integration was done by digital computer. Water was determined by Fischer titration (*5*).

Addition of HSPAN to the two-phase mixtures of gasoline, ethanol, and water resulted in selective absorption of water with nearly complete solution of the ethanol component into the gasoline phase (Table 1). Corn starch did not exhibit this selectivity. Because the HSPAN was not dry but contained 10 percent water, one should be able to reuse the polymer after air-drying it at room temperature and low relative humidity, and the energy required to regenerate HSPAN would thus be negligible [HSPAN air-dries readily (*3*), and the resulting polymer remains highly absorbent]. As expected, residual water increased with the final ethanol content of the gasohol. Water was removed more efficiently when gasoline-ethanol-water mixtures were passed through a column of HSPAN. For example, water contents of 0.24 and 0.28 percent (by weight) were found with ethanol contents of 10.4 and 10.9 percent (by weight), respectively, and these water contents are within the tolerance limit for a 90:10 ethanol-gasoline blend.

It apparently is not sufficient for HSPAN to merely absorb all of the lower aqueous ethanol phase; rather, enough HSPAN must be used so that the water present in the system is actually bound to the polymer by strong hydrogen bonds. The amount of polymer needed to dehydrate a particular system thus depends on the total water present and not on the degree of swelling or absorbency (*6*) of HSPAN in the ethanol-water mixture. Low-proof ethanol will require a large amount of HSPAN, even though much smaller amounts will absorb and solidify the aqueous ethanolic solution. Since the high-proof ethanol systems of Table 1 are essentially nonswelling media for HSPAN, addition of sufficient polymer to absorb all of the aqueous ethanol layer provides about the correct ratio of polymer to water to ensure its strong hydrogen bonding.

The presence of the gasoline phase as an extractant for ethanol, as water is being hydrogen-bonded to HSPAN, is essential to the success of our method, since high-proof ethanol in the absence of gasoline was not completely dehydrated by polymer. For example, when ethanol containing 23 percent (by weight) water was passed through 5 g of HSPAN loosely packed into a column 9 by 1.2 cm, the first 5 ml through the column still contained 15 percent water. The water content of the effluent remained fairly constant until about the 15th milliliter and then began to increase, as the column reached its capacity for water.

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Stereospecific Nicotine Receptors on Rat Brain Membranes

Abstract. *A stereospecific binding site for nicotine has been detected on rat brain membranes. Competition studies with cholinergic agonists suggest that this site is a nicotinic cholinergic receptor.*

Nicotine is one of the most widely studied drugs. Its mechanism of action at the neuromuscular junction and at autonomic ganglia is well understood at both the molecular and the cellular levels (*1*). Nicotine also has many effects on the central nervous system: it is a primary reinforcer (*2*); it serves as a discriminative stimulus (*3*); it has anti-nociceptive properties (*4*); and it stimulates vasopressin release (*5*). Although it is usually assumed that these actions of nicotine are mediated by nicotinic cholinergic receptors on central neurons, there is no direct evidence for this assumption. Blockade of these effects by mecamylamine or pempidine is not a sufficient criterion, since these compounds are poor blockers at the neuromuscular junction, and their ganglionic blocking properties are largely noncompetitive and presynaptic (*6*, *7*).

α-Bungarotoxin binds specifically to brain membranes, and its binding site shares ligand specificity and biochemical properties with the acetylcholine recep-

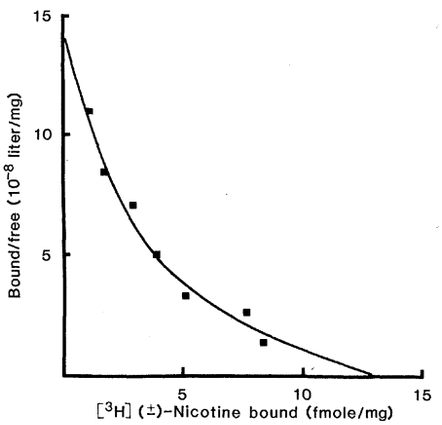

Fig. 1. Scatchard plot of (±)-[^{3}H]nicotine binding to rat brain membranes. Male Simonsen albino rats (Sprague-Dawley derived, Simonsen Laboratories, Gilroy, California), weighing 250 to 350 g were killed by decapitation and the brain was removed. Whole brain homogenate was prepared in ten volumes of ice-cold buffer (Hepes, 50 m*M*; NaCl, 118 m*M*; KCl, 4.8 m*M*; $CaCl_2$, 2.5 m*M*; $MgSO_4$, 1.2 m*M*; and NaOH to *p*H 7.40) and centrifuged at 17,500*g* for 30 minutes. The pellet was suspended in 20 volumes of ice-cold glass-distilled water, allowed to lyse for 60 minutes, and centrifuged as above. The membrane pellet was then suspended in buffer and centrifuged as above. The final pellet was suspended to a final concentration of 40 mg of original tissue per milliliter of buffer; 0.5 ml of the membrane suspension was added to 0.5 ml of buffer containing (±)-[^{3}H]nicotine at the appropriate concentration, in the absence or presence of unlabeled (−)-nicotine at a 1.0 m*M* final concentration (each concentration in triplicate). Tubes were incubated for 40 minutes at 37°C, and then chilled on ice. After 20 minutes the mixture was diluted with 4.0 ml of ice-cold buffer, filtered through polylysine-soaked Whatman GFC filters, and washed with four 4.0-ml portions of cold buffer. To control for residual displaceable binding to filters, parallel tubes were incubated and chilled as above, but (±)-[^{3}H]nicotine was not added until immediately before filtration. Radioactivity was determined by spectrometry in a dioxane-based liquid scintillation mixture at a counting efficiency of 43 percent. Binding not displaceable by 1.0 m*M* (−)-nicotine, and displaceable binding to filters have been subtracted; plotted data represent only displaceable binding to brain membranes. Units for bound ligand are femtomoles per milligram of original tissue and for bound/free are liters per milligram of original tissue. This experiment yielded the following parameter estimates: high-affinity site K_d = 28 n*M*, density = 3.2 fmole per milligram of original tissue; low-affinity site K_d = 0.46 μ*M*, density = 10.4 fmole per milligram of original tissue.

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