ward through the skin to the atmosphere (sink 2). These exposures led to formation of gas bubbles in the skin and in cutaneous blood vessels.

In the physiological studies (*8*), more than 50 such animals were exposed to counterdiffusion conditions for periods of at least 6 hours. In 43 cases where the pig was surrounded by helium and the normoxic (0.21 atm) breathing mixture contained nitrous oxide at atmospheric pressure, or argon or nitrogen at pressures ranging from 1 to 10 atmospheres absolute, skin lesions occurred after an average exposure time of 80 minutes. In the animals where conditions were reversed (argon or nitrous oxide surrounded the pig while the inspired gas was a helium-oxygen mixture), no lesions were observed in any animal throughout the 6-hour duration of the experiment. In the "reversal" experiments we selected those conditions of gas and pressure which had produced the most severe lesions, but reversed the direction of the gas gradients. The most pronounced effects were seen with a nitrous oxide–oxygen mixture for the breathing gas at atmospheric pressure and with an argon-oxygen mixture at 10 atm pressure. Details of the in vivo studies, including implications for gaseous anesthesia, will be described elsewhere (*8*).

The tissue layers involved in the development of lesions were found to include the adipose subcutaneous tissue, the capillary plexus, and the keratinized outermost layers of the skin, each of which is traversed by the permeating gases (*8*). Constituents of such structures include lipids, lipoprotein membranes, and aqueous solutions, and we have demonstrated bubble formation in a lipid-aqueous system. Finally, the in vivo experiments with pigs were found to obey the condition demanded by Eq. 3, namely, lesions form when the gases counterdiffuse in one direction but do not form when the gases are reversed.

The consequences for physiology and anesthesiology of bubble formation in tissues and vessels are apparent. Less obvious, perhaps, is how membrane transport phenomena might be affected by factors such as the increased reaction rates suggested above. These brief examples should alert specialists in various disciplines to a few of the unusual properties of countertransport in composites. We suggest three potential applications. The study of nucleation phenomena should profit from the opportunity now presented to achieve known steady-state supersaturation levels in specific regions of a system. The conversion of free energy of mixing to work has already been achieved in our crude two-membrane device and remains to be further exploited. By operating the two-membrane device "backward" (that is, supplying a gas mixture to the intramembrane space and removing two relatively purified product streams from either side of the membranes), a separation device with interesting properties can be constructed.

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Amphotericin B Methyl Ester Hydrochloride and Amphotericin B: Comparative Acute Toxicity

Abstract. *In single-dose parenteral studies with mice and dogs, the methyl ester hydrochloride of amphotericin B proved to be significantly less toxic than the parent compound, especially to the kidney.*

Intravenously administered amphotericin B (IAB) is the most effective agent currently available for the treatment of many systemic fungal infections in man. This drug is used in the treatment of a number of mycoses that, prior to its availability, were invariably fatal (*1, 2*). The use of IAB, however, is restricted to a significant degree by a variety of toxic side effects (*2, 3*), the most serious of these being nephrotoxicity. Over 80 percent of the patients treated with IAB develop decreased renal function, and it is often the degree of kidney malfunction, and not the patient's therapeutic response, that determines the duration of treatment (*2*).

Amphotericin B methyl ester hydrochloride (AME), a water-soluble derivative of amphotericin B, was recently developed by scientists at the Rutgers Institute of Microbiology. This compound not only retains the antifungal activity of the parent compound, but also, based on limited single-dose studies in mice, appears to be less toxic than IAB (*4*). The objective of this report is to summarize results of more extensive acute toxicologic comparisons of AME and IAB in mice and dogs. The studies were carried out with AME supplied by Rutgers (*5*) and with IAB supplied as marketed Fungizone Intravenous (*6*). Doses were given as milligrams of AME or IAB per kilogram of body weight.

Table 1. Acute intravenous (IV) and intraperitoneal (IP) lethalities of amphotericin B methyl ester hydrochloride (AME) and amphotericin B (IAB) in mice.

Drug	Route	LD_{50} (mg/kg)	LD_2 (mg/kg)	Slope function
AME	IV	106	75	1.20
IAB	IV	4	2	1.45
AME	IP	1320	620	1.45
IAB	IP	88	40	1.42

The results of acute lethality tests in male Charles River CD-1 mice are presented in Table 1, which shows that AME is 1/25 and 1/15 as toxic as IAB by the intravenous and intraperitoneal routes of administration, respectively.

Studies in dogs were conducted with a total of 12 unanesthetized and six anesthetized (pentobarbital sodium) purebred beagles. They received single intravenous doses of either AME or IAB injected during a period of 1 minute. In unanesthetized animals, AME was administered to two dogs each, at dosages of 6, 12, or 24 mg/kg, and to

Table 2. Acute intravenous toxicologic comparison of AME and IAB in dogs.

Effect	Minimal dose (mg/kg) producing indicated effect		Ratio of minimal doses AME/IAB
	AME	IAB	
Unanesthetized dogs			
Death	48	12	4
Serum urea nitrogen; increase	6	0.75	8
Renal tubular degeneration	24	1.5	16
Intravascular hemolysis	12	6	2
Hemoglobinuria	24	12	2
EKG; T-wave aberration	12	1.5	8
Serum potassium changes; increase followed by decrease	>24	6	>4
Serum glutamic pyruvic transaminase; increase	12	3	4
Bloody diarrhea	24	6	4
Anesthetized dogs			
Renal blood flow; depression	12	1.5	8
Glomerular filtration rate; depression	12	1.5	8
Intravascular hemolysis	12	6	2
Arterial pressure; elevation	6	1.5	4
Arterial pressure; depression	24	6	4
Bradycardia	24	1.5	16
EKG; T-wave aberration	24	3	8
Serum potassium; increase	6	1.5	4
Urinary potassium excretion; increase	6	1.5	4
Hematocrit; increase	12	6	2

one dog at 48 mg/kg; IAB was given to one dog each at dosages of 0.75, 1.5, 3, 6, or 12 mg/kg. Serum samples for biochemical analyses were taken 0, 4, 24, and 48 hours after dosage. Electrocardiograms and clinical signs were recorded periodically until 72 hours after dosage. At this time, nine animals were necropsied for gross and micropathologic examinations of the kidneys. In anesthetized animals, the dosages given were: AME 6, 12, or 24 mg/kg, and IAB 1.5, 3, or 6 mg/kg. The electrocardiogram (lead II), renal blood flow (electromagnetic flow probe), and carotid arterial pressure were recorded continuously for 90 minutes after injection. Blood and urine samples for biochemical analyses were taken at 15-minute intervals throughout this period. Clearance of exogenous creatinine was used as a measure of the glomerular filtration rate.

To facilitate comparisons of the two compounds, the minimal doses of each that caused changes in monitored parameters were determined. These doses and their ratios (AME/IAB) are shown in Table 2. The ratios range between 2 and 16, but usually are 4 or 8. Most importantly, the ratios for elevated serum urea nitrogen, depressed renal blood flow and glomerular filtration rate, and renal tubular degeneration are at least 8, indicating that AME is, at most, only one-eighth as nephrotoxic as IAB. Moreover, the increase in serum urea nitrogen was proportional to dose with IAB (maximal value 162 mg/100 ml), but not with AME (maximal value 46 mg/100 ml), and the probable cause of death after the high dose of AME was not nephrotoxicity, as was expected with IAB, but massive intravascular hemolysis. Hemolysis produced by disruption of the membrane structure exemplifies the mechanism of action of amphotericin B and polyene macrolide antifungal agents in general. Since the dose ratio of renal tubular degeneration to intravascular hemolysis is 2 (24/12) for AME and 0.25 (1.5/6) for IAB, it is conceivable that AME will not be nephrotoxic at therapeutic doses.

These preliminary studies indicate that AME has a significant safety advantage over IAB; however, the potential clinical worth of this new agent will be determined only after more extensive tests of efficacy, stability, and repeat-dose toxicity have been completed.

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Noradrenergic Stimulation of Cyclic Adenosine Monophosphate in Rat Purkinje Neurons: An Immunocytochemical Study

Abstract. *A specific immunofluorescent histochemical method for cyclic adenosine monophosphate was used to study rat cerebellum. After topical treatment with norepinephrine or stimulation of norepinephrine-containing afferents from locus coeruleus, there was a striking increase in the number of Purkinje cells with strong cyclic adenosine monophosphate reactivity. Other putative inhibitory transmitters had no significant effect on staining of Purkinje cells. The results provide the first histochemical support for the hypothesis that cyclic adenosine monophosphate can be generated postsynaptically in central neurons in response to noradrenergic stimuli.*

The discovery that catecholamines stimulate production of adenosine 3′,5′-monophosphate (cyclic AMP) in brain homogenates (*1*) or slices (*2*) has suggested that cyclic AMP may take part in central adrenergic neurotransmission