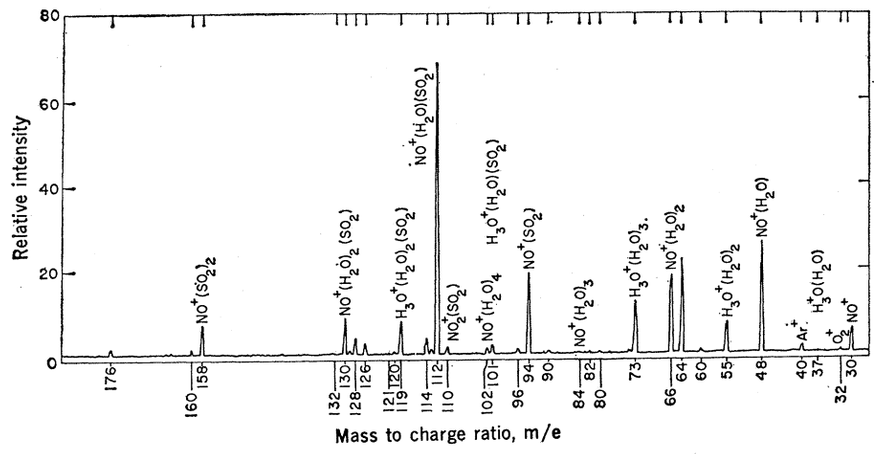

Fig. 2. Clustering of SO_2 and H_2O on oxonium and nitric oxide ions.

mation of the following hitherto unobserved species: $NO^+(SO_2)$, $NO^+(SO_2)_2$, $NO^+(SO_2)_3$, $NO^+(H_2O)(SO_2)$, $NO^+(H_2O)(SO_2)_2$, and $NO_2^+(SO_2)$. A typical recorder tracing for the mass spectrometer output is shown in Fig. 1. The sulfur-containing species were conclusively identified as a result of the doublet in the appropriate spectrum owing to the presence of both the ^{32}S and ^{34}S isotopes. Other species that were observed included $O_2^+(SO_2)$, also reported by Adams *et al.* (*9*), and $O_2^+(SO_2)_2$, as well as the known species NO^+, O_2^+, and $NO^+(H_2O)$. In experiments made in the absence of SO_2, $NO_2^+(H_2O)$ was found, which suggests that the peak observed at a mass-to-charge ratio (*m/e*) of 128 is partially due to $NO_2^+(H_2O)(SO_2)$. The relative contributions of SO_2^+ or O_4^+ to the peak at *m/e* 64 and corresponding clusters with SO_2 molecules contributing to the peak at *m/e* 128 have not been established. Likewise, the peak at *m/e* 192 may be due to both $SO_2^+(SO_2)_2$ and $NO_2^+(H_2O)(SO_2)_2$.

Additional experiments were carried out to establish the identity of the major species formed in air containing both H_2O vapor and SO_2 at concentrations up to 1 percent. During the course of this work, we discovered species involving mixed clusters of both hydrated protons and SO_2. As shown in Fig. 2, the species $H_3O^+(H_2O)_n(SO_2)$ where *n* is equal to 1 and 2, are clearly visible at *m/e* 101 and 119. The mixed cluster $NO^+(H_2O)(SO_2)$, as well as other species also seen in Fig. 1, are present in the spectrum shown in Fig. 2. Other species that have been reported by earlier investigators (*9*) are included. Preliminary results obtained from the work presented here indicate that the stabilities of the species $NO^+(H_2O)_2$, $NO^+(H_2O)(SO_2)$, and $NO^+(SO_2)_2$ are nearly equal; the respective standard free energies of formation are ~14 kcal/mole.

De Paz *et al.* (*10*) have made theoretical calculations on the basis of molecular-orbital theory and have shown that chainlike structures for the hydrated protons are equally as feasible as symmetrical clusters resulting from electrostatic-dipole interactions of water molecules with a central ion. By analogy, the mixed SO_2 and H_2O vapor clusters may also have chainlike structures. This reasoning, together with the fact that the mass of the mixed clusters is relatively large owing to the presence of SO_2, leads one to expect that the mobilities of the mixed clusters would be rather small.

The results presented here have established the existence and identity of mixed clusters of H_2O and SO_2 about common ions such as NO^+ and H_3O^+ which are present in electrical discharges. These results provide an explanation for the heretofore unexplained observations reported in the literature (*6*, *7*). However, the observed clusters are part of a complex ion-molecule reaction sequence and would not necessarily be terminal ions under all conditions.

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References and Notes

1. R. S. Narcisi and A. D. Bailey, *J. Geophys. Res.* **70**, 3787 (1965).
2. V. Mohnen, in *Planetary Electrodynamics*, S. C. Coroniti and J. Hughes, Eds. (Gordon & Breach, New York, 1970), pp. 197–206; R. Siksna, in *ibid.*, pp. 207–230; K. G. Vohra and P. V. N. Nair, *J. Aerosol Sci.* **1**, 127 (1970); V. A. Mohnen and J. P. Lodge, Jr., in *Proceedings of the 7th International Conference on Condensation and Ice Nuclei* (Academia, Prague, 1970), pp. 69–91; V. J. Schaefer, *Science* **170**, 851 (1970).
3. E. E. Ferguson and W. F. Libby, *Nature* **229**, 37 (1971).
4. W. J. Megaw and R. D. Wiffen, *Geofis. Pura Appl.* **50**, 118 (1961).
5. J. Bricard, F. Billard, G. Madelaine, *J. Geophys. Res.* **73**, 4487 (1968).
6. J. Bricard, M. Babane, G. Madelaine, in *Planetary Electrodynamics*, S. C. Coroniti and J. Hughes, Eds. (Gordon & Breach, New York, 1970), pp. 243–247.
7. L. B. Loeb, *Advan. Geophys.* **13**, 223 (1969).
8. A. Good, D. A. Durden, P. Kebarle, *J. Chem. Phys.* **52**, 222 (1970).
9. N. G. Adams, D. K. Bohme, D. B. Dunkin, F. C. Fehsenfeld, E. E. Ferguson, *ibid.*, p. 3133.
10. M. De Paz, S. Ehrenson, L. Friedman, *ibid.*, p. 3362.
11. We thank Prof. D. R. Olander and Dr. W. J. Siekhaus of the University of California, Berkeley, for helpful suggestions regarding the design of ion optics. This work was performed under the auspices of the U.S. Atomic Energy Commission.

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Reversible Osmotic Opening of the Blood-Brain Barrier

Abstract. *Reversible breakdown of the blood-brain barrier is produced by a class of electrolytes and nonelectrolytes which have little or no lipid solubility but which differ in chemical and ionic properties. These agents may osmotically shrink barrier cells, possibly the vascular endothelium, and reversibly open spaces between them. Lipid-soluble nonelectrolytes damage the barrier irreversibly.*

It has been hypothesized (*1*) that the blood-brain barrier can be opened by osmotically shrinking barrier cells and opening spaces between them to the passage of ions and large molecules. In this report we show, in the rabbit, that a group of electrolytes and relatively lipid-insoluble nonelectrolytes will open the barrier reversibly to the intravascular Evans blue–albumin complex. The action of these substances on the barrier conforms to four experimental conditions (*1*, *2*) which are required if they should act osmotically: (i) the agent should open the barrier independently of a specific chemical or drug action, (ii) its effect on the barrier should increase with increasing osmolality or concentration, (iii) its effect should be related inversely to its ability to penetrate the cell membrane, and (iv) its effect should be reversible.

Rabbits weighing 1 to 1.5 kg were anesthetized with urethan (1.6 g/kg, intraperitoneally), the pia-arachnoid of the parietal cortex was exposed, and 4 ml of Evans blue in Ringer solution (2 g/100 ml) per kilogram was injected into the femoral vein. This quantity of dye is completely bound to plasma albumin (*3*). A circular filter paper pledget, 6 mm in diameter, was placed on a cortical region and kept saturated with a test solution for 10 minutes (*1*), then removed, and the region was observed and photographed. Different osmotic concentrations of a test substance dissolved in distilled water were applied to different cortical regions to find the threshold for barrier opening, which was defined as the lowest osmolality which produced a blue staining outside of the pial vessels (*4*) (usually small veins). Electrolyte solutions were tested in steps of about 0.25 molal and nonelectrolyte solutions in steps of 1 molal or 2 molal (for concentrations above 5 molal). At concentrations above threshold, staining was more diffuse and around the larger veins and arterioles, as well as around small veins. This increased damage conforms to condition ii of the osmotic hypothesis.

Reversibility of barrier opening was studied as follows. In the absence of intravascular Evans blue, concentrated solutions at threshold and above were applied with continuously saturated pledgets to regions on one cortical hemisphere for 10 minutes, after which the cortex was washed for 30 minutes with Ringer solution at 37°C. Evans blue then was injected and the treated cortical regions observed for staining after 10 to 15 minutes. After this, the other hemisphere was exposed and the same damaging concentrations of test substance were applied to it for 10 minutes. The second hemisphere acted as a control, since solutions were applied to it *after* giving Evans blue.

Barrier opening was defined as reversible if the first side, to which the concentrated solutions were applied 30 minutes before Evans blue, was not stained, in contrast to the control side. Barrier opening was irreversible if both hemispheres were stained.

Thirteen solutes were studied in 65 rabbits (Table 1). The molecular weights of the nonelectrolytes were less than 100, so as to minimize diffusion differences between them (*1*). The substances are rank ordered in Table 1 with respect to their olive oil/water distribution coefficients, which represent lipid solubilities (*5*), and the thresholds and reversibility observations also are listed. Molality was converted to osmolality with use of published osmotic and freezing point coefficients (*1, 6*).

Table 1. Threshold and reversibility of blood-brain barrier opening as related to lipid solubility.

Substance	Olive oil/water distribution	Threshold		Reversibility of opening
		Molal	Osmolal	
LiCl	*	0.76	1.5	Yes
Na_2SO_4	*	.76	1.5	Yes
NaCl	*	.87	1.6	Yes
Glycerol	0.00007	5.0	5.7	Yes
Urea	.00016	3.4	3.0	Yes
Methyl urea	.00044	5.0	4.3	Yes
Ethylene glycol	.0005	17.5	19.6	No
Formamide	.00083	5.0	4.6	Yes
Acetamide	.0009	7.0	6.4	Yes
Cyanamide	.0045	3.0	2.5	No
Propylene glycol	.0056	20.5	27.5	No
Ethanol	.023	10.9	12.3	No
Urethan	.074	2.0	1.6	No

* Close to zero (*2, 5*).

The irreversible agents, according to condition iv of the osmotic hypothesis, would not be expected to act by shrinking barrier cells. With the exception of ethylene glycol, they have distribution coefficients greater than 0.001 (Table 1), and may therefore damage cell membranes because of lipid solubility or solvent properties (*1, 5*). The position of ethylene glycol in Table 1 would be between acetamide and cyanamide if the substances were ranked by ether/water distributions, another indicator of lipid solubility (*7, 8*).

The irreversible agents also may kill cells by denaturing protein or by metabolic inhibition (*1*), although lower concentrations of some inhibitors—NaCN, Na iodoacetate, NaF, NaN_3—do not damage the barrier to HCO_3^- under similar experimental conditions, and the barrier to trypan blue and to HCO_3^- is resistant to anoxia (*9*). The barrier to trypan blue is undamaged in a *p*H range of 1 to 9 (*9*). The *p*H of the solutions in Table 1 was between 4 and 8.

The reversible agents appear to act osmotically. For the following, thresholds and lipid solubilities increase in the same order, salts < urea < methyl urea ≦ formamide < acetamide. Lipid solubility and cell membrane permeability are correlated, but not exactly, because the small nonelectrolytes may traverse aqueous membrane channels and have permeabilities greater than expected from lipid solubility alone (*5, 8*). Nevertheless, a rough relation of reversible threshold to solubility is consistent with the condition that the osmotic effect of these reversible agents is related inversely to membrane permeability. The high threshold of glycerol may be due to its small diffusion coefficient, about one-half that of urea at the respective thresholds of the two solutions (*6, 10*).

The similar thresholds among the three salts on the one hand, and among the reversible nonelectrolytes on the other, show that the reversible agents act independently of chemical composition or individual drug effects (condition i of the osmotic hypothesis). The nonelectrolytes have different sizes, diffusion coefficients, reflection coefficients, extracellular-intracellular distributions, and hydrogen bonding properties (*8, 11*); their thresholds and reversibility characteristics would be expected to depend on these factors as well as on lipid solubility. The wide range of thresholds of the irreversible agents may arise also from different ways in which they may damage the barrier.

Although topical threshold concentrations are unphysiological, the topical method helps to define classes of agents which produce irreversible and reversible barrier damage, and which can be tested with intracarotid perfusion. Intracarotid perfusion, for 30 seconds, of 1 to 2 ml of NaCl, urea, ethanol, or propylene glycol, at concentrations about one-half topical thresholds, will also damage the barrier (*9, 12*). Because of the short diffusion time, damage is probably at the level of the endothelial cell of the brain vascular endothelium (*4, 9*) (also see below). After passing through the brain and damaging the barrier, the intracarotid

solutions will be diluted by a factor of 50 or more in the plasma volume during circulation (*13*).

We have demonstrated reversible breakdown of the blood-brain barrier by substances which have little or no lipid solubility but which differ in chemical and ionic properties. Although reversible damage with hypertonic NaCl has been shown after 24 hours (*14*), the demonstration of reversibility within at least 30 minutes for damage by a whole class of substances may make it possible to use reversible osmotic opening as an experimental tool in the study and modification of barrier permeability, perhaps in relation to central nervous system chemotherapy.

The observations support the hypothesis that the reversible agents act osmotically, perhaps by shrinking barrier cells and reversibly opening spaces between them. These spaces may be at the tight junctions between endothelial cells of the cerebral blood vessels (*15*). The more lipid-soluble agents appear to act irreversibly, perhaps by destroying cell membranes or killing cells. The observations also support the suggestion (*1*) that the blood-brain barrier to HCO_3^-, trypan blue (*4*), and Evans blue–albumin complex, as well as to horseradish peroxidase (*15*), arises because of close contiguity of barrier cells.

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References and Notes

1. S. I. Rapoport, *Am. J. Physiol.* **219**, 270 (1980); *Int. J. Neurosci.* **1**, 273 (1970).
2. E. Overton, *Vierteljahresschr. Naturforsch. Ges. Zurich* **40**, 159 (1895).
3. F. B. Freedman and J. A. Johnson, *Am. J. Physiol.* **216**, 675 (1969).
4. T. Broman and A. M. Lindberg-Broman, *Acta Physiol. Scand.* **10**, 102 (1945).
5. R. Collander and H. Barlünd, *Acta Bot. Fenn.* **11**, 1 (1933); R. Höber, *Physical Chemistry of Cells and Tissues* (Blakiston, Philadelphia, 1945); R. Collander, *Physiol. Plant.* **2**, 300 (1949).
6. *International Critical Tables* (McGraw-Hill New York, 1926); G. Scatchard and S. S. Prentiss, *J. Am. Chem. Soc.* **56**, 1486 (1934); H. M. Chadwell and F. W. Politi, *ibid.* **60**, 1291 (1938); H. K. Ross, *Ind. Eng. Chem.* **46**, 601 (1954); R. A. Robinson and R. H. Stokes, *Electrolyte Solutions* (Butterworths, London, ed. 2, 1959); V. Crescenzi, F. Quadrifoglio, V. Vitagliano, *Ric. Sci.* **37**, 529 (1967); R. H. Stokes and R. A. Robinson, *J. Phys. Chem.* **70**, 2126 (1967).
7. R. Collander, *Acta Chem. Scand.* **3**, 717 (1949).
8. E. M. Wright and J. M. Diamond, *Proc. Roy. Soc. Ser. B* **172**, 227 (1969); J. D. Fenstermacher and J. A. Johnson, *Am. J. Physiol.* **211**, 341 (1966).
9. T. Broman, *The Permeability of the Cerebrospinal Vessels in Normal and Pathological Conditions* (Munksgaard, Copenhagen, 1949); S. I. Rapoport, *J. Physiol. London* **170**, 238 (1964); *J. Pharmacol. Exp. Ther.* **144**, 310 (1964).
10. F. H. Garner and P. J. M. Marchant, *Trans. Inst. Chem. Eng.* **39**, 397 (1961); L. J. Gosting and D. F. Akeley, *J. Am. Chem. Soc.* **74**, 2058 (1952).
11. I. R. Fenichel and S. B. Horowitz, *Acta Physiol. Scand.* **60**, Suppl. 221 (1963).
12. M. Hori, S. I. Rapoport, I. Klatzo, unpublished.
13. P. L. Altman and D. S. Dittmer, *Blood and Other Body Fluids* (Federation of American Societies for Experimental Biology, Washington, D.C., 1961).
14. E. Streicher, D. P. Rall, J. R. Gaskins, *Am. J. Physiol.* **206**, 251 (1964).
15. T. S. Reese and M. J. Karnovsky, *J. Cell Biol.* **34**, 207 (1967); M. W. Brightman, I. Klatzo, Y. Olssen, T. S. Reese, *J. Neurol. Sci.* **10**, 215 (1970).
16. We thank Drs. Eugene Streicher and Samuel Horowitz for critically reading this manuscript.
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Ceroid Pigment Formation and Irreversible Sterility in Vitamin E Deficiency

Abstract. *Female rats maintained on a diet deficient in vitamin E for a prolonged period of 100 to 135 days, starting from birth, failed to conceive in spite of repeated matings. Dietary vitamin E supplementation for a period of 60 days following prolonged deficiency was ineffective in reversing the sterility, although a definite growth response was observed. These observations suggest that the tissue damage caused by lipid peroxidation, as evidenced by distinct brown ceroid pigment in the uterus and fallopian tubes, may be responsible for the irreversible loss of fertility observed in the vitamin E–deficient female rats.*

The relation of vitamin E to reproduction in female rats was first recognized by Evans and Bishop (*1*) who discovered an unknown antisterility "factor" termed vitamin E (*2, 3*). The reproductive failure in female rats was characterized by the malformation or resorption of the fetus in vitamin E deficiency (*1–14*). However, it still remains a question whether permanent sterility is possible in female rats by depriving animals of vitamin E for an extended period of time starting from birth. The present study was undertaken to find the long-term effect of vitamin E deficiency on the incidence of sterility in the female rats and the nature of such sterility with respect to its reversibility by vitamin E supplementation in the diet.

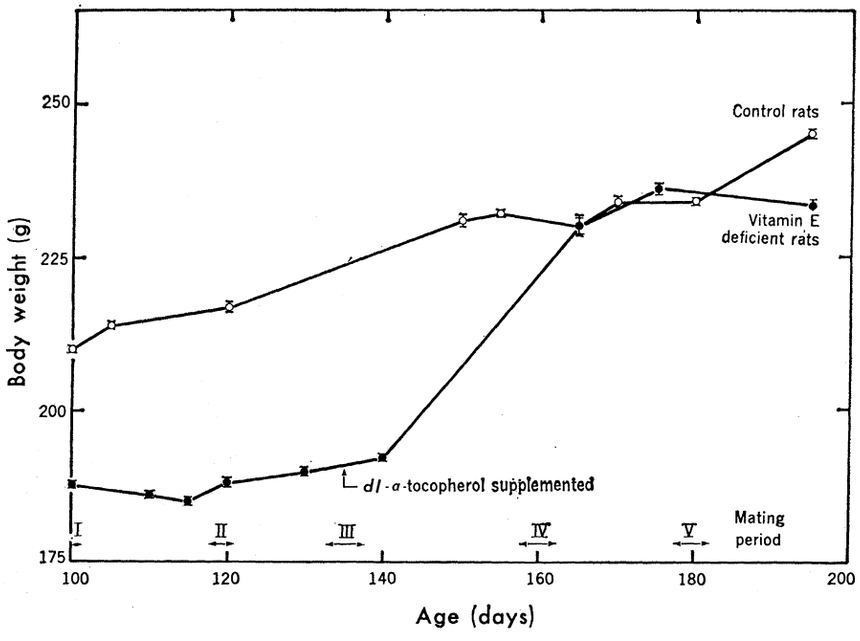

Fig. 1. Change in body weight of female rats on vitamin E–deficient and –supplemented diets. Each point represents mean ± standard deviation. *dl*-α-Tocopherol was added in the supplemented diet in the form of acetate.