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.

STANLEY I. RAPOPORT
Laboratory of Neurophysiology, National Institute of Mental Health, Bethesda, Maryland 20014

MASAHARU HORI*, IGOR KLATZO
Laboratory of Neuropathology and Neuroanatomical Sciences, National Institute of Neurological Disease and Stroke, Bethesda 20014

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.
* Visiting scientist from the Institute of Higher Nervous Activity, Osaka University Medical School, Osaka, Japan.

17 June 1971

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.

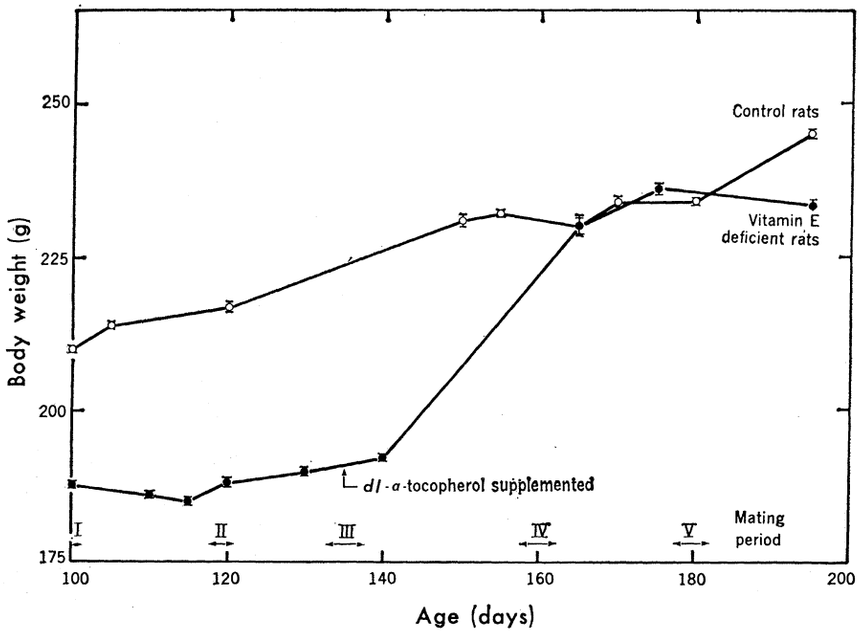

Fig. 1. Change in body weight of female rats on vitamin E–deficient and –supplemented diets. Each point represents mean ± standard deviation. *dl*-α-Tocopherol was added in the supplemented diet in the form of acetate.

Four female rats of the Wistar strain, at an early stage of pregnancy, were placed on a diet deficient in vitamin E (*15*). A corresponding number of pregnant rats were fed a control diet supplemented with 2.5 g of *dl*-α-tocopheryl acetate (2500 international units) per kilogram of diet. After delivery, the mothers and the newborn littermates in the experimental group were continued on the vitamin E–deficient diet for 21 to 23 days. The young females from control and experimental groups were placed in individual cages and their respective diets were continued for a total period of 135 days. The males from both groups were maintained separately on normal laboratory chow diet for breeding purposes. Food and water were supplied freely to all animals.

Between 95 and 100 days, when females from the control group reached their mature body weight of about 210 g and the experimental animals about 180 g, each female was allowed with one male during five different mating periods as described in Table 1. The results clearly show that in the control group the total percentage of successful conception leading to pregnancy was 68, of which 12 percent conceived during the first 12-hour mating period, 16 percent during the second 36-hour mating period, and 40 percent during the subsequent 96-hour mating period. In the experimental group none became pregnant after any one of the above-mentioned mating treatments. After further matings for two subsequent 96-hour periods after 30 and 50 days following supplementation with 2.5 and 5.0 g of *dl*-α-tocopheryl acetate, respectively, these animals still failed to conceive.

The change in body weight of these animals during mating periods is represented in Fig. 1. The control animals maintained a constant body weight throughout the period. On the other hand, females from the vitamin E–deficient group maintained lower body weights throughout the deficiency period. When vitamin E was supplemented to the diet, the body weight increased sharply and reached the level of control animals within 30 days of supplementation.

All animals were killed at the age of 195 days and their uteri were dissected. On examination, no indications of definite sites of implantation or resorption were obtained in the vitamin E–deficient females. A large amount of fat deposition was observed in the abdomen of these females. The vitamin E–deficient females showed distinct pigmentation, uniformly spread all through the uterus and the fallopian tubes, which persisted even after the supplementation of vitamin E for up to 60 days. A similar type of brown pigment formation was first noticed by Martin and Moore (*16, 17*) and was characterized as "ceroid pigment." A possible relation between these pigments and vitamin E deficiency was contributed by Dam and Granados (*18*). According to these investigators, if rats are fed a diet containing a large amount of cod liver oil, they can develop peroxides in their adipose tissues. Neither peroxides nor visible pigments developed when vitamin E was present in the diet.

Table 1. Fertility in female rats fed vitamin E–deficient and –supplemented diets.

Group	Fertility at various periods of mating* (%)					Total fertility (%)	Brown ceroid pigment in uterus
	I	II	III	IV	V		
Control rats†	12	16	40			68	–
Vitamin E–deficient rats‡	0	0	0			0	+
Vitamin E–deficient rats supplemented with vitamin E§				0	0	0	+

* Exposure time for mating periods: 12 hours for I, 36 hours for II, and 96 hours for III, IV, and V. † Two groups each consisting of four animals. ‡ Three groups each consisting of four animals. § Supplementation of vitamin E during mating periods IV and V was 2.5 and 5.0 g of *dl*-α-tocopheryl acetate per kilogram of diet, respectively.

Martin and Moore (*17*) earlier reported that the pigmentation formed as a result of long-term vitamin E deficiency in rats could be minimized by refeeding vitamin E to the animals. However, none of these previous investigators tried to examine the fertility of their animals under the experimental conditions reported by them. In the present experiment, we could reverse neither the pigmentation nor the sterility in our deficient animals even after refeeding them with vitamin E for a period of 60 days, although a distinct growth response was clearly evident.

It appears that during long-term vitamin E deficiency irreversible tissue damage, as evidenced by brown ceroid pigmentation, is caused, which interferes with the conception and implantation in the uterus and thereby induces infertility in the female rats. Such irreversible free radical damage to tissue constituents during lipid peroxidation in vitro and the chemical nature of such damage has been demonstrated earlier by Desai and Tappel (*19*). Roubal and Tappel (*20, 21*) subsequently showed that proteins and enzymes in aqueous solution, when subjected to lipid peroxidation, undergo major reactions of polymerization, polypeptide chain scission, and irreversible changes in the individual amino acids.

It is therefore suggested that during long-term vitamin E deficiency, lipid peroxidation which causes extensive tissue damage, as evidenced by brown ceroid pigment formation in the uterus and fallopian tubes, may be responsible for the irreversible loss of fertility observed in the vitamin E–deficient female rats.

C. RAYCHAUDHURI
I. D. DESAI
Division of Human Nutrition, School of Home Economics, University of British Columbia, Vancouver 8, Canada

References and Notes

1. H. M. Evans and K. S. Bishop, *Science* **56**, 650 (1922).
2. B. Sure, *J. Biol. Chem.* **58**, 693 (1924).
3. ———, *ibid.* **62**, 371 (1924).
4. ———, *ibid.* **63**, 211 (1925).
5. H. M. Evans and G. O. Burr, *Mem. Univ. Calif.* **8**, 1 (1927).
6. D. W. Cheng and B. H. Thomas, *Proc. Iowa Acad. Sci.* **60**, 290 (1953).
7. D. W. Cheng, L. F. Chang, T. A. Bairnson, *Anat. Rec.* **129**, 167 (1957).
8. E. Biavati and R. Matscher, *Quad. Nutr.* **19**, 225 (1959).
9. M. A. Kenney and C. E. Roderuck, *Proc. Soc. Exp. Biol. Med.* **114**, 257 (1963).
10. ———, *Fed. Proc.* **20**, 452 (1961).
11. D. W. King, *Anat. Rec.* **148**, 300 (1964).
12. B. H. Thomas and G. R. Wermus, *J. Anim. Sci.* **26**, 910 (1967).
13. G. R. Wermus, *Diss. Abstr.* **B27**, 3833 (1967).
14. J. Bunyan, J. Green, A. T. Diplock, D. Robinson, *Brit. J. Nutr.* **21**, 137 (1967).
15. H. H. Draper, J. G. Bergan, M. Chiu, A. S. Csallany, A. V. Boaro, *J. Nutr.* **84**, 395 (1964).
16. A. J. P. Martin and T. Moore, *Chem. Ind. (London)* **55**, 236 (1936).
17. ———, *J. Hyg.* **39**, 643 (1939).
18. H. Dam and H. Granados, *Acta Physiol. Scand.* **10**, 162 (1945).
19. I. D. Desai and A. L. Tappel, *J. Lipid Res.* **4**, 204 (1963).
20. W. T. Roubal and A. L. Tappel, *Arch. Biochem. Biophys.* **113**, 5 (1966).
21. ———, *ibid.*, p. 150.
22. Supported by National Research Council of Canada grant A-4686. C.R. is a postdoctoral fellow under this project.

7 July 1971