18. D. L. Matthies, *Anat. Rec.* **159**, 55 (1967).
19. K. Kohmoto and H. A. Bern, *J. Endocrinol.* **48**, 99 (1970); H. L. Buttle, I. A. Forsyth, G. S. Knaggs, *ibid.* **53**, 483 (1971).
20. D. F. H. Wallach and V. B. Kamat, *Methods Enzymol.* **8**, 164 (1966).
21. E. W. Emmart, R. W. Bates, P. G. Condliffe, W. A. Turner, *Proc. Soc. Exp. Biol. Med.* **114**, 754 (1963).
22. We thank Dr. A. E. Wilhelmi (Emory University) and Dr. R. W. Bates (NIH) for providing the bovine and ovine prolactin preparations which have been bioassayed by the pigeon crop sac assay; Dr. B. Wentworth (University of Wisconsin) for turkey pituitaries; Dr. M. S. Raben (New England Medical Center Hospitals, Boston) for human ACTH; Dr. A. G. Frantz (Columbia University) for performing the in vitro mouse mammary gland assay; and National Institute of Arthritis and Metabolic Diseases for some of the hormone preparations used in this study. The secretarial help of Miss Francine Dupuis and the preparation of the figures by Mrs. Jean Parodo are much appreciated. This research was supported by the Medical Research Council of Canada MA-2525, the PHS child health and human development grant HD-01727-07, and the National Cancer Institute N.C.I. E-C-72-26.

5 January 1973; revised 14 February 1973

Osmotic Opening of the Blood-Brain Barrier in the Monkey without Associated Neurological Deficits

Abstract. *Hypertonic urea or lactamide solutions osmotically open the blood-brain barrier in the monkey without producing gross neurological deficits if the blood supply to the brain is not compromised. The brain is perfused via the left lingual artery when the external and common carotid arteries are clamped temporarily. Hypertonic perfusion, which opens the barrier by opening tight junctions between cerebrovascular endothelial cells, can thus be used to study barrier function and brain pharmacology.*

In a previous demonstration that the blood-brain barrier (BBB) could be opened in the monkey by internal carotid perfusion of hypertonic urea solution, it was noted that neuronal damage often occurred (*1*). Hypertonic perfusion reversibly opens tight junctions between cerebrovascular endothelial cells, probably by shrinking the cells osmotically (*2–4*), and would be useful for studying barrier function and brain pharmacology if unaccompanied by neuronal damage.

The perfusion method in the monkey, which included permanent ligation of the common carotid artery and was associated with transient hypotension (*1, 5*), may have predisposed the homolateral brain to ischemic damage (*6*). To test this, we altered the method so as not to ligate the common carotid artery nor to compromise permanently cerebral blood flow (*4, 7*).

Twenty-one monkeys (*Macaca mulatta*) weighing 2.5 to 3.5 kg were anesthetized and given intravenous Evans blue tracer (*1*). Filtered 2*M* buffered urea (*1*), *p*H 7.4 (Ureaphil, Abbott), 2*M* DL-lactamide (*2*), *p*H 5.0 (Sigma), or 0.9 percent NaCl was perfused into the left lingual artery for 20 seconds at a fixed rate between 0.74 and 0.86 ml/sec. The left external and common carotid arteries were clamped temporarily during perfusion, so that the perfusate went into the internal carotid and then into the left hemisphere of the brain (*4*). The animal was ventilated artificially if transient apnea appeared.

The monkey was permitted to recover and was examined for changes in gross motor function or behavior. It was killed 5 to 10 days later to determine the extent of Evans blue staining of the perfused brain (evidence of BBB opening) and for brain histology.

Table 1 summarizes the observations and compares them with experiments in which 2*M* urea was perfused for 30 seconds into the internal carotid when the common carotid was permanently ligated (*1*). With permanent ligation, urea opened the BBB in 9 of 12 animals, usually in the distribution of the left middle cerebral artery, but only 2 of 9 were neurologically normal.

Table 1. Effect of perfusion with hypertonic solutions on the blood-brain barrier with and without permanent ligation of the common carotid artery.

Flow rate (ml/sec)	Solution	Staining (No. of animals)		
		None	Left hemisphere	
			Total	Normal*
Common carotid ligated†				
0.4 –1.7	2*M* urea	3	9	2
	0.9% NaCl	5	1	0
Common carotid temporarily clamped				
0.76–0.86	2*M* urea	2	6	6
	2*M* lactamide	1	6	5
	0.9% NaCl	5	1	1

* Normal neurologically. † Observations from Rapoport, Bachman, and Thompson (*1*).

With lingual perfusion and temporary carotid clamping, however, the barrier was opened by both urea and lactamide without associated right-sided weakness or gross behavioral changes in 11 of 12 animals. Both urea and lactamide produced diffuse and even brain staining, but isotonic saline did not. Urea-perfused brains often had superimposed darkly stained regions, perhaps due to opening of large intracerebral vessels as well as of capillaries (*3*). Frozen histological sections stained with cresyl violet showed no evidence of brain necrosis in the normal monkeys.

Electroencephalograms (EEG) taken 1 day after urea perfusion showed decreased amplitude on the left side in 4 of 6 animals with BBB opening. At the time the animals were killed, EEG records did not differ from controls prior to perfusion for normal animals perfused with urea, lactamide, or isotonic saline. As was noted previously, the anterior and posterior chambers of the left eye were stained by Evans blue after hypertonic perfusion (*1, 8*).

Osmotic opening of the BBB therefore can be produced without gross neurological injury if the vascular supply to the perfused brain is not compromised permanently and if adequate ventilation is maintained. This method can be considered now for a more detailed study of brain and barrier function (*9*).

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

References and Notes

1. S. I. Rapoport, D. S. Bachman, H. K. Thompson, *Science* **176**, 1243 (1972).
2. S. I. Rapoport, *Amer. J. Physiol.* **219**, 270 (1970); ———, M. Hori, I. Klatzo, *ibid.* **223**, 323 (1972); Z. M. Rap, M. Spatz, S. I. Rapoport, in preparation.
3. S. I. Rapoport, M. W. Brightman, T. S. Reese, *Biophys. Soc. Abstr. 17th Annu. Mtg.* **13**, 230a (1973); M. W. Brightman, T. S. Reese, S. I. Rapoport, in preparation.
4. S. I. Rapoport, in *Pharmacology and Pharmacokinetics: Problems and Perspectives, Fogarty International Center*, Proceedings No. 20, T. Teorell, R. L. Dedrick, P. Condliffe, Eds. (Plenum, New York, in press).
5. O. Steinwall, *Acta Radiol.* **49**, 281 (1958).
6. S. Levine and W. Stypulkowski, *Neurology* **9**, 407 (1959); S. Levine, *Amer. J. Pathol.* **36**, 1 (1960).
7. C. G. Hartman and W. L. Straus, *The Anatomy of the Rhesus Monkey* (Hafner, New York, 1933); F. Coceani, I. Libman, P. Gloor, *Electroencephalogr. Clin. Neurophysiol.* **20**, 542 (1966).
8. In one eye examined histologically ½ hour after perfusion by 2*M* lactamide, the ciliary epithelium appeared vacuolated (S. Okisaka, T. Kuwabara, S. I. Rapoport, unpublished).
9. We thank Dr. E. Streicher for helpful comments, Dr. C. Ajmone-Marsan for reading the EEG's, and S. Sudler for doing brain histology.

11 December 1972; revised 15 March 1973