interstitial disease. These antibodies appear to fix C3. Mononuclear cells (predominantly macrophages) surround the tubules almost simultaneously with signs of tubular cell damage. Activated macrophages are present (*11*). Antibodies may be necessary for macrophages to effectively phagocytose the TBM and tubules (*12*).

We thus have developed two contrasting models of autoimmune renal damage. In sheep, autoantibodies to GBM damage glomeruli (*13*). Mononuclear cells were not involved. In guinea pigs autoantibodies to TBM selectively damage tubules and interstitium but not glomeruli. Mononuclear cells participate in the progressive tissue injury initiated by autoantibodies to TBM. Thus, the pathogenesis of these two diseases is different. The specificity of these autoantibodies and tissue injury indicate that unique target antigens are in the GBM and TBM.

The serum transfer induces fatal disease in the recipients and reproduces in the recipients all of the cellular manifestations present in the disease of the donor. The transfer of cell-free serum seems to exclude delayed-type cellular hypersensitivity as a mechanism for mononuclear cell participation. Hence, the serum transfer should provide a useful, easy means for studying a new and fatal mechanism of tissue injury initiated by autoantibodies to TBM and involving mononuclear cells, and for studying humoral-cellular interactions in vivo.

This new model of renal tubulointerstitial disease may be applicable to the study in man and domestic animals of tubulointerstitial disease which may be superimposed on glomerular lesions (*14*), renal homograft reactions (*15*), and certain kinds of tubulointerstitial diseases whose pathogenesis is presently obscure.

RAYMOND W. STEBLAY
ULRICH RUDOFSKY
New York State Kidney Disease Institute and *Department of Pathology, Albany Medical College, Albany, New York 12208*

References and Notes

1. R. W. Steblay and U. Rudofsky, *J. Immunol.* **107**, 589 (1971); R. W. Steblay, *Fed. Proc.* **29**, 237 (1970). The cortical tubular disease is characterized by a linear continuous deposit of autoantibodies along the cortical TBM, by degeneration, necrosis, and regeneration of tubular epithelium, by destruction of tubules and TBM, and by peritubular mononuclear cells, giant cells, interstitial hemorrhage, occasional nuclear debris, and polymorphonuclear cells. Animals develop glucosuria, proteinuria, hemoglobinuria, and uremia and die in acute or chronic renal disease.
2. We have found (*1*) animals with severe tubulointerstitial disease and no evidence of antibodies to GBM. Therefore, the incidence of renal tubular disease was correlated only with the deposition of autoantibodies to TBM and was independent of whether or not antibodies to GBM were found.
3. All guinea pigs (Albany strain) weighed 350 to 450 g and were obtained from the New York State Department of Health.
4. A. H. Coons, *Gen. Cytochem. Methods* **1**, 399 (1958). The titers of serum antibodies were the greatest dilution of serum that still gave positive staining along the TBM or GBM by indirect immunofluorescence.
5. Glucose, protein, and hemoglobin were tested in urine with Labstix (Miles Laboratory, Elkhart, Indiana).
6. Fluorescein-conjugated rabbit antiserum to guinea pig IgG was prepared as in (*1*).
7. Rabbit antiserum to complement (C3) was donated by Dr. M. M. Mayer, Johns Hopkins University. Fluorescein-conjugated goat antiserum to rabbit IgG was purchased from Hyland Laboratories, Los Angeles, and absorbed with guinea pig IgG before use.
8. In preliminary experiments, if insufficient amounts of donor autoantibodies to TBM were injected, little or no deposition of autoantibodies to TBM occurred, and no significant tubular disease developed (see also controls in Table 1).
9. In these experiments, neither donors nor recipients had detectable glomerular damage. Moreover, the amount or kinds (or both) of autoantibodies to GBM present did not influence the course of the tubulointerstitial disease. Under different experimental conditions, we could produce a diffuse, progressive, and fatal glomerulonephritis in guinea pigs.
10. The disease in both donors and recipients was characterized by a linear deposit of autoantibodies along the cortical TBM, by tubular damage, and by interstitial changes [as described in the text and (*1*)]. By day 21, 25 percent of donors were moribund; approximately 50 to 60 percent had ecchymoses, glucosuria, proteinuria, and hemoglobinuria; and in 40 to 50 percent the BUN was greater than 75 mg per 100 ml. By the 14th day, 4 of 9 recipients were moribund, 6 of 9 had ecchymoses, 8 of 9 had glucosuria, 6 of 9 had proteinuria, and 4 of 9 had hemoglobinuria, with a BUN greater than 75 mg per 100 ml.
11. Cytochemical stains for β-glucuronidase and acid phosphatase were done by Dr. R. Wylie, Johns Hopkins University.
12. D. S. Nelson, *Macrophages and Immunity* (Elsevier, New York, 1969); in *Mononuclear Phagocytes*, R. Van Furth, Ed. (Davis, New York, 1969). Additional possibilities for humoral-cellular interaction are numerous; they include anti-TBM antibodies acting as opsonins or cytophylic antibodies for macrophages. Macrophages may have receptor sites for the immune complex consisting of TBM, antibody to TBM, and complement. Other humoral factors in donor serum or host may facilitate the emigration, proliferation, and phagocytosis of macrophages, and giant cell formation.
13. R. W. Steblay, *J. Exp. Med.* **116**, 253 (1962); R. A. Lerner and F. J. Dixon, *ibid.* **124**, 431 (1966); R. W. Steblay and U. Rudofsky, *Nature* **218**, 269 (1968).
14. We found linear staining for IgG and C3 along the cortical TBM in glomerulonephritis in sheep [R. W. Steblay and U. Rudofsky, *Science* **160**, 204 (1968)] and in rats, and identified autoantibodies to TBM in rats [R. W. Steblay and U. Rudofsky, *Fed. Proc.* **28**, 426 (1969)].
15. G. M. Williams, H. M. Lee, R. F. Weymouth, W. R. Harlan, K. R. Holden, C. M. Stanley, G. A. Millington, D. M. Hume, *Surgery* **62**, 204 (1967).
16. Supported by PHS grant HL-13389. We thank Drs. C. Pirani and W. Kaufman for assistance.

4 December 1972; revised 12 March 1973

Radioreceptor Assay for Prolactin and Other Lactogenic Hormones

Abstract. *A radioreceptor assay with a sensitivity of 5 nanograms per milliliter has been developed for mammalian and avian pituitary prolactin, placental lactogenic hormones, and human growth hormone, using a membrane receptor preparation isolated from rabbit mammary glands. Prolactin preparations inhibited the binding of [^{125}I]prolactin to receptors in direct proportion to the biological potency of these preparations. Thus, the radioreceptor assay provides a convenient and simple assay for hormones which have lactogenic activity.*

The currently available prolactin bioassays, namely, the pigeon crop sac assay (*1*) and the in vitro rabbit and mouse mammary gland assays (*2*), while adequate, are difficult to perform, time-consuming, and costly. With the demonstration that prolactin linked to Sepharose is biologially active in mouse mammary tissue in vitro (*3*) and that ovine [^{125}I]prolactin is taken up by rabbit mammary tissue in vitro and in vivo (*4*), it appeared feasible to develop a membrane receptor assay for prolactin, as has been done for other polypeptide hormones (*5*). Indeed, the successful development of such an assay by using membranes obtained from midpregnant mouse mammary tissue has been presented (*6*). The same group of investigators subsequently reported that their initial findings with regard to sensitivity of their assay were not reproducible (*7*). In view of the limited amount and the heterogeneity of mammary tissue which can be obtained from a pregnant mouse, we turned our attention to the midpregnant rabbit mammary gland as a source of membrane receptors. Previously we had demonstrated that midpregnant rabbits injected intramuscularly with 10 mg of human placental lactogen and 5 mg of hydrocortisone daily for 4 days exhibited a remarkable development of their mammary glands (*8*). An average of 100 g of fresh mammary tissue can readily be dissected from one rabbit treated in this manner. Moreover, histo-

logically the mammary tissue obtained is homogeneous, being composed almost exclusively of alveolar and ductal elements which are distended with milk (*8*).

Mammary tissue obtained from primed rabbits was rinsed in ice-cold 0.3*M* sucrose solution and cut into smaller fragments before being homogenized in 5 volumes of sucrose solution. Homogenization was carried out at 4°C for 5 minutes with a Virtis homogenizer with the dial set at "medium." The homogenate was filtered twice, first through four layers and then through eight layers of cheesecloth. The filtrate was centrifuged at 1500*g* for 20 minutes at 4°C. The supernatant was centrifuged at 15,000*g* for 20 minutes, and the pellet was discarded. The supernatant was again centrifuged at 100,000*g* for 90 minutes to obtain the total microsomal pellet which contains most of the broken cell membranes. Of the total prolactin-binding activity, 76 percent was recovered in this fraction. The microsomal pellet was resuspended in 0.025*M* tris-HCl buffer, *p*H 7.6, containing 10 m*M* $CaCl_2$ or $MgCl_2$, because these salts were found to promote binding of prolactin. The volume of buffer added was such that 0.1 ml of the suspension contained 100 to 300 μg of protein as determined by the Lowry procedure (*9*). At this point the prolactin-binding activity was stable at −20°C for as long as 6 months. When required for assay, the suspensions were thawed and the particles were dispersed with a glass homogenizer immediately before use. Repetitive thawing and freezing of the receptor suspensions resulted in a decrease of prolactin binding. Membrane suspensions from one rabbit provide sufficient material for as many as 1000 determinations.

Human [^{125}I]prolactin (hPRL) was prepared according to the method of Thorell and Johansson (*10*) with lactoperoxidase and hydrogen peroxide. The specific activity of the labeled prolactin was 60 to 80 μc/μg.

The assay procedure which was employed to demonstrate binding of [^{125}I]-hPRL to mammary gland membrane receptors is outlined in the legend to Fig. 1, left. Human prolactin as well as prolactin of several other species, human growth hormone, and human placental lactogen inhibited the binding of [^{125}I]-hPRL to receptors. This finding is consistent with the reports by Forsyth (*11*) and Frantz *et al.* (*12*) that human growth hormone and human placental lactogen produce lactogenic responses in the in vitro rabbit and mouse mammary gland assays. Several other polypeptide hormones did not inhibit the binding of [^{125}I]hPRL. The slight displacement caused by the very high concentrations of human thyroid-stimulating hormone and human adrenocor-

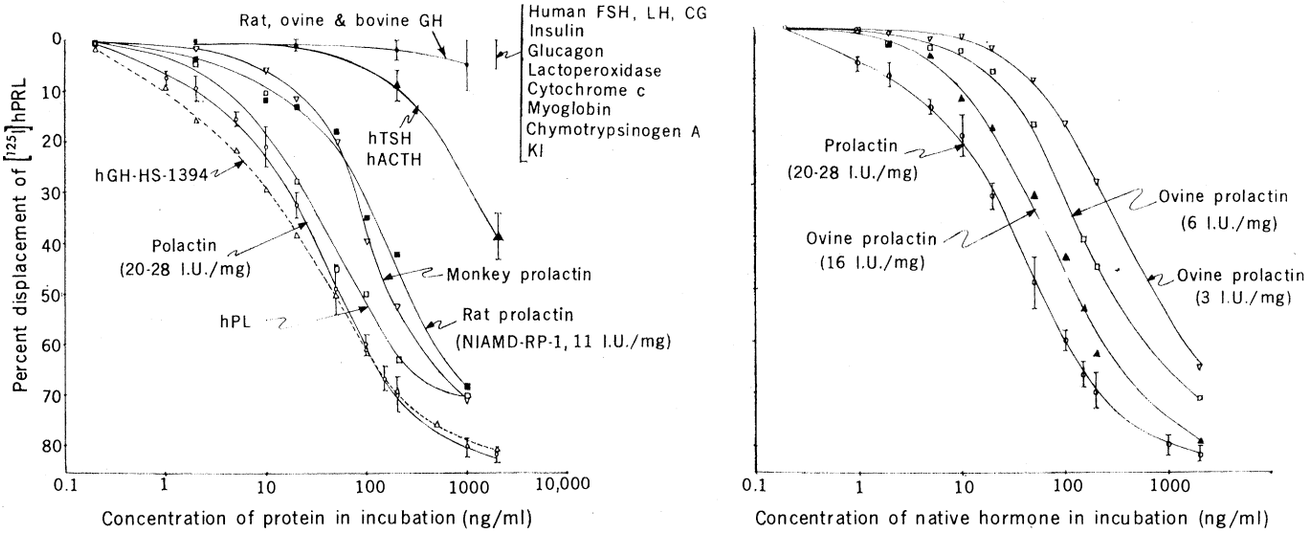

Fig. 1. (Left) Specificity of binding of [^{125}I]hPRL to rabbit mammary receptors. One-tenth milliliter of membrane receptor suspension (containing 100 to 300 μg of protein) was incubated with [^{125}I]hPRL (10^5 count/min) in a final volume of 0.5 ml in a 12 by 75 mm plastic tube. The buffer employed for all dilutions and additions was 0.025*M* tris-HCl, *p*H 7.6, containing 10 m*M* $CaCl_2$ and 0.1 percent (wt./vol.) bovine serum albumin. Native unlabeled hormone, when required, was added in 0.1 ml to give the final concentrations indicated on the abscissa. Incubation was carried out at room temerature (25°C) for 90 minutes with manual shaking every 15 minutes. At the end of the incubation period, 3 ml of ice-cold buffer was added and the contents of each tube were filtered through a Millipore membrane (type EGWP 02500, pore size 0.2 μm) under suction. The membrane was washed twice with 5 ml of the same ice-cold buffer. The filtering procedure normally required less than 30 seconds. The filter membrane was then counted in a plastic tube in a Packard Auto-Gamma spectrometer. An alternative method of separating bound and free hormone was by centrifugation in an IEC PR-2 centrifuge at top speed (750*g*). Plasma membranes stored frozen in the absence of sucrose or glycerol, but in the presence of $CaCl_2$, tend to form irreversible aggregates which sediment at relatively low speed (*20*). At the end of the incubation period, the contents of each tube were diluted with 3 ml of ice-cold buffer and immediately centrifuged for 30 minutes at 4°C. The supernatant was decanted and the mouth of the tube was blotted on absorbent papers. The tube was then counted in the same manner. Under these conditions, 15 to 20 percent of the [^{125}I]hPRL was bound, of which 80 percent could be displaced by a final concentration of 1 μg/ml of native prolactin (25 I.U. mg). (Right) Potency estimates of prolactin by radioreceptor assay, pigeon crop sac assay, and in vitro mouse mammary gland assay. Radioreceptor assay procedures were the same as above. Vertical bars in the prolactin displacement curves indicate the range observed when five different preparations of bovine and ovine prolactin with biological potencies from 20 to 25 I.U./mg by the pigeon crop sac assay (*21*) and a human prolactin preparation having a potency of 28 I.U./mg by the in vitro mouse mammary gland assay (*12*) were asayed in the receptor assay. Monkey prolactin has a potency of 13 I.U./mg. Abbreviations: *hGH*, human growth hormone; *hPL*, human placental lactogen; *hTSH*, human thryoid-stimulating hormone. *hACTH*, human adrenocorticotropic hormone; *FSH*, follicle-stimulating hormone; *LH*, luteinizing hormone; *CG*, chorionic gonadotropin; *GH*, growth hormone.

ticotropic hormone was due to a 2 to 3 percent contamination of these preparations by human growth hormone as shown by radioimmunoassay. Only human growth hormone but not growth hormones of other species competed for the prolactin binding sites. This is also consistent with the previous reports (*12, 13*) that bovine growth hormone has no lactogenic activity. Identical specificity of binding of prolactin was observed when purified plasma membranes prepared from the 1500*g* pellet according to the procedure of Neville (*14*) as modified by Meldolesi *et al.* (*15*) were used in the assay in place of the 100,000*g* material. When ovine and human prolactin ranging from 3 to 28 international units (I.U.) per milligram by conventional bioassays were tested by the receptor assay, an excellent correlation was observed between the estimates of potency obtained by the two assay methods (Fig. 1, right).

Serial dilutions of crude pituitary extracts obtained from human, rat, mouse, guinea pig, rabbit, and turkey inhibited the binding of [^{125}I]hPRL in a manner parallel to that of the hPRL standard, whereas extracts of cod pituitaries, rat cerebral hemispheres, and rat liver did not exhibit this effect. The prolactin receptor assay can detect concentrations as low as 5 ng/ml in tissue extracts and in crude or purified prolactin preparations.

When serum prolactin concentrations are to be measured by the receptor assay, equivalent amounts of serum from patients who have been surgically hypophysectomized or serum from hypophysectomized rats must be added to the prolactin standards. Under this circumstance, the sensitivity of the assay is 20 ng/ml as compared to 5 ng/ml when samples which contain no serum are assayed. Serum prolactin concentrations have been determined by radioimmunoassay and radioreceptor assay in a number of samples from individuals with different clinical and pathological conditions and after treatment with drugs which increase or decrease prolactin levels. An excellent correlation was obtained between the estimates of prolactin concentrations as determined by radioimmunoassay and radioreceptor assay in 60 samples (correlation coefficient, $r = .98$). In all the samples assayed, human growth hormone concentrations by radioimmunoassay were below 5 ng/ml.

During pregnancy, very high concentrations of a prolactin-like factor were detected in rat serum (Fig. 2). This substance may be identical to the placental luteotropin identified in extracts of rat placenta in 1938 by Astwood and Greep (*16*) or the rat chorionic mammotropin (rCM) reported by Averill *et al.* (*17*). Their finding was later confirmed by Matthies (*18*) with a crude bioassay technique. These investigators suggested that the serum and placental concentrations of this lactogenic factor were maximal around day 12 of gestation (*17, 18*). With the receptor assay for lactogenic hormones, it has been possible to accurately measure serum concentrations of rCM throughout pregnancy. The highest concentration of rCM occurs on day 12, reaching a mean level of 1584 ± 632 ng/ml, and by day 14 has declined to 180 ± 24 ng/ml, followed by a secondary increase on days 17 to 21 to a mean level of 821 ± 276 ng/ml. Serial dilutions of serum samples from either day-12 or day-18 pregnant rats inhibited the binding of [^{125}I]hPRL in a manner parallel to that of the similarly diluted hPRL standard to which hypophysectomized rat serum has been added. Twenty minutes after the ligation of uterine vessels in ether-anesthetized day-18 pregnant rats, serum rCM levels were less than that which could be detected by the assay, suggesting a very rapid disappearance rate for rCM. Serum concentrations of pituitary prolactin determined by radioimmunoassay were lower than 60 ng/ml throughout this period. The radioreceptor assay, therefore, is useful not only for determining the potency of prolactin preparations and serum concentrations of prolactin in many species but even more importantly it provides a convenient method for the identification and accurate measurement of other placental lactogenic hormones which are secreted during pregnancy (*11, 19*).

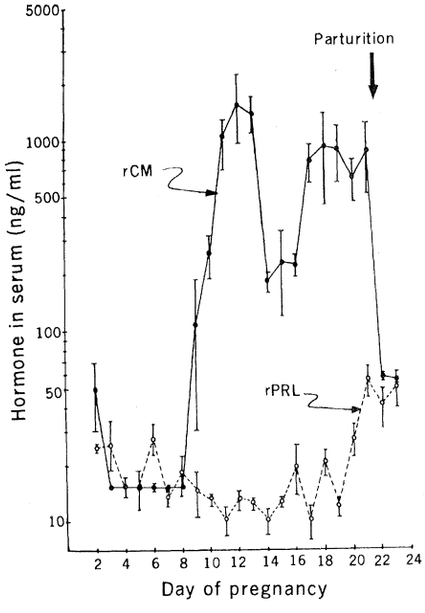

Fig. 2. Serum rCM and prolactin (rPRL) concentrations throughout pregnancy in rats. Blood samples of 1 ml each were obtained by cardiac puncture from animals lightly anesthetized with ether. Blood samples were allowed to clot overnight at 4°C and serum samples were obtained after centrifugation. The rCM was determined by radioreceptor assay and values are expressed as ovine prolactin (25 I.U./mg) equivalents and as means (with vertical bars indicating standard errors) of five animals. The assay procedures were essentially identical to that described in Fig. 1 except that 25 μl of unknown serum sample was used, and to the tubes containing the standards, 25 μl of appropriate amounts of hPRL and 25 μl of hypophysectomized rat serum were added. The volume of diluent was adjusted in each case to give a final volume of 0.5 ml after the additions of receptor suspension and tracer. Serum concentrations of pituitary prolactin were determined by double antibody radioimmunoassay as outlined in the instructions provided in the kit obtained from the National Institute of Arthritis and Metabolic Diseases.

R. P. C. Shiu
P. A. Kelly, H. G. Friesen
Department of Experimental Medicine and McGill University Clinic, Montreal, Quebec, Canada

References and Notes

1. C. S. Nicoll, *Endocrinology* **80**, 641 (1967).
2. I. A. Forsyth and R. P. Myres, *J. Endocrinol.* **51**, 157 (1971); D. Kleinberg and A. G. Frantz, *J. Clin. Invest.* **50**, 1557 (1971); R. W. Turkington, *J. Clin. Endocrinol. Metab.* **33**, 210 (1971); J. E. Loewenstein, I. K. Mariz, G. T. Peake, W. H. Daughaday, *ibid.*, p. 217.
3. R. W. Turkington, *Biochem. Biophys. Res. Commun.* **41**, 1362 (1970).
4. I. R. Falconer, *Biochem. J.* **126**, 8p (1972); M. Birkinshaw and I. R. Falconer, *J. Endocrinol.* **55**, 323 (1972).
5. R. J. Lefkowitz, J. Roth, I. Pastan, *Science* **170**, 633 (1970); K. J. Catt, M. L. Dufau, T. Tsuruhara, *J. Clin. Endocrinol. Metab.* **34**, 123 (1972); A. R. Means and J. Vaitukaitis, *Endocrinology* **90**, 39 (1972); P. Cuatrecasas, *Proc. Nat. Acad. Sci. U.S.A.* **63**, 450 (1969).
6. R. W. Turkington, *J. Clin. Invest.* **50**, 94 (1971).
7. W. L. Frantz and R. W. Turkington, *Abstracts of the Fourth International Congress on Endocrinology* (Washington, D.C., 1972), abstr. 503, p. 200.
8. H. G. Friesen, *Endocrinology* **79**, 212 (1966).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
10. J. I. Thorell and B. G. Johansson, *Biochim. Biophys. Acta* **251**, 363 (1971).
11. I. A. Forsyth, in *Lactogenic Hormones* (Ciba Foundation symposium), G. E. W. Wolstenholme and J. Knight, Eds. (Churchill, London, 1972), p. 151.
12. A. G. Frantz, D. L. Kleinberg, G. L. Noel, in *Lactogenic Hormones* (Ciba Foundation symposium), G. E. W. Wolstenholme and J. Knight, Eds. (Churchill, London, 1972), p. 137; *Recent Progr. Horm. Res.* **28**, 527 (1972).
13. E. B. Barnawell, *J. Exp. Zool.* **160**, 189 (1965).
14. D. M. Neville, *Biochim. Biophys. Acta* **154**, 540 (1968).
15. J. Meldolesi, J. D. Jamieson, G. E. Palade, *J. Cell Biol.* **49**, 109 (1971).
16. E. B. Astwood and R. O. Greep, *Proc. Soc. Exp. Biol. Med.* **38**, 713 (1938).
17. S. C. Averill, E. W. Ray, W. R. Lyons, *ibid.* **75**, 3 (1950).

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