12. Carlson, L. A., Orö, L., *Metabolism,* 1963, v12, 132.

13. Felts, J. M., In: *Fat as a Tissue,* McGraw-Hill, in press.

14. Wade, O. L., Bishop, J. M., *Cardiac Output and Regional Blood Flow,* Blackwell Scientific Publications, Oxford, 1962.

15. Holst, E. J., *Acta physiol. Scand.,* 1944, v7, 69.

16. Clark, B., Hübscher, G., *Nature,* 1962, v195, 599.

17. Bragdon, J. H., Gordon, R. S., Jr., *J. Clin. Invest.,* 1958, v37, 574.

18. Havel, R. J., Felts, J. M., Van Duyne, C. M., *J. Lipid. Res.,* 1962, v3, 297.

19. Wadström, L., *Nature,* 1957, v179, 259.

Received May 16, 1963. P.S.E.B.M., 1963, v113.

Unilateral Renal Hemodynamic Changes with Hypertonic Mannitol.* (28540)

LEONARD B. BERMAN, KEMAL ÖNEN, AND GEOFFREY CHISHOLM

Kidney Research Laboratory, Loma Linda University Service, Los Angeles County Hospital, Los Angeles, Calif.

The renal hemodynamic effects of mannitol have recently become of interest in prevention of renal ischemia under various clinical circumstances(1,2). Harvey has reported that, in the excised perfused dog kidney, hypertonic mannitol infusions reduce the resistance to blood flow. Hypertonic glucose has a similar effect, while urea does not(3). Lilienfield & Braun(4,5) found that 20% mannitol infused intravenously into anesthetized dogs increased PAH clearance while decreasing both the inulin clearance and the extraction fraction of PAH. They suggested that mannitol had produced dilatation of the efferent arterioles of juxta-medullary glomeruli. The same authors also found, by clearance techniques, that hypertonic mannitol infused during, or after the induction of hemorrhagic shock, prevented or restored the decrease in PAH and inulin clearances observed during the hypotensive period. Teschan and his colleagues(6) confirmed these effects of both hypertonic mannitol and glucose, using direct and electromagnetic flow meter measurements of renal blood flow. A decreased postglomerular resistance was suggested by these authors. Finally, Goldberg and Lilienfield have reported that, while 20% mannitol reduces renal resistance to blood flow, 6.6% mannitol does not(7). They speculate that the effect of the 20% solution is on some extra-renal volume receptor which causes release of a renal vasodilating substance. The present experiments were undertaken to examine this last point.

Materials and methods. Group I. Eight, anesthetized mongrel dogs weighing 18-22 kg were prepared with bilateral ureteral cannulas and a needle in one renal artery. Priming and sustaining infusions of PAH, and creatinine, were begun in the usual manner for estimating renal plasma flow and filtration rate. After a 20-minute waiting period, PAH and creatinine clearances were measured in each kidney (2 clearance periods of about 10 minutes each). Twenty per cent mannitol was then infused into the renal arterial needle by constant infusion pump at the rate of 300 mg/min for 15 minutes. Clearances were again measured in each kidney.

Group II. Five dogs were prepared as in Group I. Control clearances were measured in each kidney. The dogs were then bled from a femoral artery to a total loss of 30-35 cc/kg in 15 minutes. Separate clearances were again measured (single periods of 15 minutes) although urine flows were extremely small. Twenty per cent mannitol was then infused into the renal arterial needle at a rate of 480 mg/min for 10 minutes, followed by a third set of separate clearances (3 pe-

* This work partially supported by grants from N.I.H. and Don Baxter, Inc. We gratefully acknowledge the technical assistance of Miss Elizabeth Vinciguerra.

TABLE I. Separate Renal Clearances in Anesthetized Dogs Before and After Unilateral Renal Arterial Infusion of 20% Mannitol. (Mean and one standard deviation are shown.)

	Control	Mannitol
	Infused kidney	
C_{creat}	28 ± 7	29 ± 8
C_{pah}	75 ± 18	73 ± 26
F.F.	.37	.40
	Non-infused kidney	
C_{creat}	30 ± 9	33 ± 13
C_{pah}	71 ± 19	78 ± 28
F.F.	.42	.42

riods of 5-10 minutes each). The clearance values presented under "Results" are the last of the periods—at a time when urinary creatinine and PAH values had fallen sharply signifying the collection of newly formed urine. Femoral arterial pressure was monitored directly with a mercury manometer throughout the procedure.

Group III. Four anesthetized dogs were prepared with bilateral ureteral cannulas. In addition, 2 of the dogs (no. 1 & 2, Table III) had a needle in their left renal artery and 2 (no. 3 & 4, Table III) had the needle in the right renal artery. All 4 dogs had a shunt established between the left renal and left femoral veins by means of plastic cannulas with a connecting piece. A side arm provided access for direct collection of left renal flow using a cylinder and a stop watch. The side arm was clamped when not in use, and opened simultaneously with closure of the femoral vein catheter when renal blood flow was being measured.

After control measurements of left renal blood flow, the dogs were bled to a total of 30-35 cc/kilo in 15 minutes. Left renal blood flows were again measured and 20% mannitol was infused into the left renal artery (2 dogs) and into the right renal artery (2 dogs) at the rate of 480 mg/min for 15-20 minutes. Left renal blood flows were then repeated. Arterial pressure was monitored directly with a mercury manometer throughout the procedure, and renal vein pressure (lateral) was similarly monitored with a saline manometer.

Chemical methods. Creatinines were measured by the method of Bonsnes & Taussky (8). PAH was done by the method of Smith, Smith, and Finkelstein(9).

Results. Group I (Table I). The infused and non-infused kidneys showed similar clearances during the control period. There was no significant change during the mannitol infusion. Urine volumes, however, did reflect the infusion of mannitol, going from a mean of 0.25 ml/min on each side, to a mean of 0.97 ml/min on the infused side, and 0.48 ml/min on the non-infused side.

Group II (Table II). The control measurements were similar on both sides. Following hemorrhage, arterial blood pressure declined to 60 mm of mercury and urine flows were less then 0.2 ml/min in both kidneys. Clearances in these circumstances are known to be grossly unreliable(10). The calculated values which were extremely low (0-16% of control in each kidney) are not presented. With unilateral infusion of mannitol there was a prompt ipsilateral increase in urine flow from mean values of 0.15 ml/min after hemorrhage to 0.7 ml/min after mannitol. Measurements after 15 minutes of infusion indicated a 68% restoration of creatinine clearance and a 97% restoration of PAH clearance on the infused side, without a rise in arterial pressure or a change in large vessel hematocrit. The non-infused kidney showed a very slight increase in urine flow without any significant change in clearances when post-bleeding values were compared

TABLE II. Separate Renal Clearances During Control, Post-Bleeding and Post-Mannitol Infusion into One Renal Artery. (Mean and one standard deviation are shown.)

	Infused kidney			Non-infused kidney		
	C_{creat}	C_{pah}	F.F.	C_{creat}	C_{pah}	F.F.
Control	32 ± 9	89 ± 32	.38	36 ± 7	97 ± 15	.37
Bleeding	Mean urine flow .14 ml/min.*			Mean urine flow .11 ml/min.*		
Mannitol	22 ± 9 (Mean urine flow .7)	87 ± 43	.26	Mean urine flow .20*		

* Calculated clearances below 16% of control values and not reliable at these urine flows.

TABLE III. Direct Measurement of Left Renal Vein Flow, (ml/min) Before and After Hemorrhage, and After Renal Artery Infusion of 20% Mannitol.

	Left renal artery infusion			Right renal artery infusion		
	Dog 1	Dog 2	Mean	Dog 3	Dog 4	Mean
Control	163	231	197	205	172	189
Post-hemorrhage	95	126	111	116	88	102
Mannitol	128	195	167	123	92	108
% increase (hemorrhage)/(mannitol)			50			6

with post-mannitol values.

Group III (Table III). A degree of hemorrhage similar to the preceding group, reduced the blood flow to a little over half of the control value. It should be noted that the Group II dogs, with similar bleeding, had plasma flows erroneously estimated by PAH clearance (without correction for a lowered extraction fraction) of less than one-sixth of control. With unilateral infusion of hypertonic mannitol, the ipsilateral kidneys increased their blood flow by 50% over post-hemorrhage value, while the contralateral kidneys showed only a 6% increase. Arterial pressure and large vessel hematocrit did not change. Renal vein pressures did not vary by more than 2 mm of mercury when post-bleeding measurement is compared with post-mannitol measurement. In 2 additional dogs, not shown on the table, plasma volumes were determined by RISA dilution technique after hemorrhage, and again after infusion of hypertonic mannitol at the rate of 480 mg/min for 15 minutes. There were no significant changes.

Discussion. The results in Group II clearly demonstrate a difference in the behavior of the mannitol-infused kidney as compared with the non-infused. If improvement in post-hemorrhagic renal hemodynamics were initiated by mannitol at some extrarenal site, one might have expected a greater urine flow as well as an increase in clearances on the non-infused side. The increase found for the infused side probably represents an overestimation of degree of improvement of renal blood flow(6,10) because of dead space errors, and the need for longer equilibration times, but the direction of change may be accepted.

The results in Group III provide direct evidence in support of the above statements. Hypertonic mannitol appears to act directly on the kidney to reduce renal vascular resistance under the experimental conditions employed. The decreased resistance to blood flow seen with hypertonic mannitol in the excised dog kidney(3) is in accord with this view. The failure to obtain changes in the Group I dogs may be due to the lower infusion rate of mannitol. A second possibility is that the metabolic alterations in hemorrhagic shock produce a renal vasculature more sensitive to hypertonic mannitol. With regard to the concept that the physiologic state of the recipient influences the response to mannitol, it may be noted that Barry failed to find clearance changes in normal human subject given hypertonic mannitol. Subjects with impaired renal function, on the other hand, showed an increase toward normal(11).

The mechanism by which mannitol exerts its action is not clear. Hypertonicity alone is not entirely satisfactory since hypertonic urea fails to lower renal vascular resistance in the excised kidney while isotonic glucose in the intact dog is said to do so(12). On the other hand, in the non-hemorrhaged dog, Goldberg has lowered renal vascular resistance with 20%, but not 6.6% mannitol(7).

A pharmacologic effect of the mannitol upon renal vasculature remains a possibility. In considering this problem, data obtained from other vascular beds cannot safely be translated to the kidney. For example, hypertonic urea reduces vascular resistance in the dog's hind limb(13), but fails to do so in the excised dog kidney(3), or the intact dog kidney (unpublished data, this laboratory).

Finally, as noted by many authors, and

most recently emphasized by Teshan(6) the types of experiment in which renal functions and urine flow are rapidly changing, put clearance methods at their greatest disadvantage. This is particularly true when hemorrhagic shock and mannitol infusions are both important parts of the protocol. Direct or electromagnetic flowmeter measurements of renal blood flow may offer a considerably more accurate assessment of change in the design of future experiments.

Summary. Unilateral renal artery infusions of hypertonic mannitol increase ipsilateral urine flow, but do not change PAH or creatinine clearances in the anesthetized dog, when infused at 300 mg/min for 10 minutes. In the dog with hemorrhagic hypotension, unilateral renal artery infusion of 480 mg/min of hypertonic mannitol produces a 50% increase in renal blood flow ipsilaterally and a 6% increase contralaterally. These effects are seen in the absence of any change in plasma volume or any increase in the hypotensive arterial pressures.

1. Barry, K. G., Cohen, A., LeBlanc, P., *Surgery,* 1961, v50, 335.
2. Barry, K. G., Cohen, A., Knochel, J. P., Whelan, T. J., Jr., Beisel, W. R., Vargas, C. A., LeBlanc, P. C., Jr., *New Engl. J. Med.,* 1961, v264, 967.
3. Harvey, R. B., *Am. J. Physiol.,* 1960, v199, 31.
4. Lilienfield, L., Braun, W., *Clin. Research,* 1962, v10, 67.
5. Braun, W., Lilienfield, L., *ibid.,* 1962, v10, 67.
6. Teschan, P. E., Gagnon, J. A., Murphy, G., *ibid.,* 1963, v11, 241.
7. Goldberg, A., Lilienfield, L., *ibid.,* 1963, v11, 242.
8. Bonsnes, R. W., Taussky, H. H., *J. Biol. Chem.,* 1945, v158, 581.
9. Smith, W. W., Finkelstein, N., Smith, H. W., *ibid.,* 1940, v135, 231.
10. Selkurt, E. E., *Am. J. Physiol.,* 1945, v145, 699.
11. Barry, K., Doberneck, R., McCormick, G., *Clin. Research,* 1962, v10, 245.
12. Bounous, G., Shumacker, H., King, H., *Ann. Surg.,* 1960, v151, 47.
13. Marshall, R., Shepherd, J. T., Jr., *Am. J. Physiol.,* 1959, v197, 951.

Received May 17, 1963. P.S.E.B.M., 1963, v113.

Effect of Crowding on Hypertension and Growth in Rats Bearing Regenerating Adrenals.* (28541)

LEE L. BERNARDIS AND FLOYD R. SKELTON
Department of Pathology, State University of New York, Buffalo, N. Y.

Increased population density has been shown to have both a deleterious effect on growth(1,2,3) and a stimulating action on the anterior pituitary-adrenocortical system (4,5,6,7). Deranged response of the latter system to stress has been implicated rather widely in the genesis of hypertension, further support for which was found in the observation(8) that rats bearing regenerating adrenals develop hypertension and vascular changes similar to human hypertensive disease when renal mass is reduced and excess sodium provided.

The high level of stress incident to modern civilization is blamed by some for the increasing frequency of hypertensive disease and, although evidence is not conclusive, high population density and psychic factors arising therefrom may enter into the genesis of hypertension(9). In fact, Weiss(10) has stated that emotional stress seems at times to precede the onset of hypertension and Ayman(11,12) postulated that the early symptoms of essential hypertension were of psychic origin.

The present study was done to determine whether the stress of population density affected the development of high blood pressure in rats bearing regenerating adrenals.

Methods. Seventy-two female Charles River rats were divided into 6 groups and treated in the following manner: Group 1 (6 rats in single cages) and Group 2 (18 rats, 3 per

* This investigation was supported by a research grant from Nat. Heart Inst., Nat. Inst. Health, P.H.S.