plications of this hypothesis are mentioned.

1. Padawer, J. and Gordon, A. S., Proc. Soc. Exptl. Biol. Med., **88**, 522 (1955).
2. Padawer, J., Trans. N. Y. Acad. Sci., **19**, 690 (1957).
3. Padawer, J., Ann. N. Y. Acad. Sci., **103**, 87 (1963).
4. Padawer, J., J. Nat. Cancer Inst., **25**, 731 (1960).
5. Padawer, J., Am. J. Physiol., **200**, 1340 (1961).
6. Padawer, J., Life Sci., **1**, 411 (1962).
7. Ashley, J. N. and Harris, J. O., J. Chem. Soc., 677 (1944).
8. Randolph, T. J., J. Lab. Clin. Med., **34**, 1696 (1949).
9. Padawer, J., J. Cell Biol., **29**, 176 (1966).
10. Allen, A. M., personal communication.
11. Fawcett, D. W., Anat Record, **121**, 29 (1955).
12. Kelényi, G., Acta Morphol. Acad. Sci. Hung., **4**, 345 (1954).
13. Brues, A. M., J. Clin. Invest., **21**, 646 (1950).
14. Walaszek, J. and Kocsis, J. J., Federation Proc., **15**, 495 (1956).
15. Malawista, S. E., Arthritis Rheumat., **8**, 752 (1965).
16. Midgley, A. R., Pierce, B., and Dixon, F. J., Science, **130**, 40 (1959).
17. Eigsti, O. J. and Dustin, P., Jr., "Colchicine in Agriculture, Medicine, Biology and Chemistry," Iowa State Coll. Press, Ames (1954).
18. Padawer, J., J. Gerontol., **20**, 515 (1965).
19. Padawer, J., Mitt. Naturforsch. Ges., Bern, **22**, 225 (1965).

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Effect of WY-5256 on Renal Hemodynamics* (32655)

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WY-5256 (4-amino-7-(2-methoxyethylamino)-*N*-(2-methoxyethyl)-2-phenyl-6-pteridine carboxamide) is a new orally active pteridine diuretic reported to have approximately the same activity and potency as hydrochlorothiazide in saline loaded rats and dogs (1).

Inasmuch as other pteridine agents appear to affect renal hemodynamics (2), the purpose of the present study was to determine the effect of this agent on renal hemodynamics and to determine if the increase in sodium excretion produced by WY-5256 could be related to such changes. Changes in renal hemodynamics were determined with an electromagnetic flow meter in conjuction with *p*-aminohippurate (PAH) and inulin clearances.

Methods. The studies were carried out on dog kidneys *in situ*. Mongrel dogs weighing 15–29 kg were anesthetized with sodium pentobarbital, 30 mg/kg. A tracheal cannula was inserted to insure free passage of air. The kidney to be utilized was exposed by a flank incision and the renal artery was cleared of the surrounding tissue.

A flow-meter probe (model EMP-411, Electromagnetic Probe Company, lumen size, 11-mm circumference) was placed around the exposed renal artery and renal blood flow was monitored with a square-wave electromagnetic flow meter (model *321*, Carolina Medical Electronics). The flow meter was set to record mean blood flow and was adjusted to zero flow by briefly occluding the renal artery distal to the flow-meter probe. Blood pressure was monitored from the carotid artery. Both blood flow and blood pressure were recorded on an Offner recorder. A solution containing 0.9% NaCl, 0.4% inulin, and 0.08% *p*-aminohippurate (PAH) was infused into the right femoral vein at a rate of 0.25 ml/kg/min for at least ½ hour before and throughout the entire experiment.

WY-5256 was administered intravenously as a suspension in saline. A priming dose of 0.25 mg/kg plus an infusion of 0.25 mg/kg/hour or a priming dose of 2.5 mg/kg plus an infusion of 2.5 mg/kg/hour were given.

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TABLE I. Effect of WY-5256 on Renal Function (0.25 mg/kg Prime + 0.25 mg/kg/hour Infusion).

	Control periods	WY-5256 periods	Differences ± SE
Sodium excretion (μEq/min)	86	253	167 ± 40[a]
Inulin clearance (ml/min)	50	45	−5 ± 3
Blood pressure (mm Hg)	116	117	1 ± 2
Resistance (mm Hg/ml/min)	0.36	0.31	−0.05 ± 0.012[a]
Total renal blood flow (ml/min)	325	377	52 ± 11[a]
PAH calculated blood flow (ml/min)	234	237	3 ± 19
Total flow—PAH calculated flow (ml/min)	91	140	49 ± 15[a]

[a] Indicates a significant difference. Average dog wt. = 24 kg, $N = 6$.

Urine samples from the exposed kidney were obtained from a renal pelvic catheter introduced into the ureter by way of the retroperitoneal incision. Although urine samples were collected only from the kidney to which blood flow was measured, the other ureter was cannulated via a midline incision to insure free urine flow from the contralateral kidney. All collection periods were 5 min in length. Average control values were determined from three collection periods just prior to administration of WY-5256. Average treated values were determined from six collection periods following administration of WY--5256. Blood samples were obtained from the right femoral artery.

At the end of each experiment the renal artery was cannulated in order to calibrate the flow meter. The flow-meter probe was maintained in its original position while blood was collected into a graduated cylinder for 15 sec. The volume of blood collected was then used to calculate ml/min of blood flow represented by each millimeter of recorder-pen deflection.

Four animals were treated with a priming dose of 0.05 mg/kg plus an infusion of 0.05 mg/kg/hour and a variable-resistance clamp (Blalock Clamp, Tomac) was placed around the aorta just proximal to the left renal artery. The aortic clamp was used to control renal blood flow during drug administration. An additional blood pressure was monitored via the left femoral artery in order to measure perfusion pressure below the aortic clamp. Urine was collected for four clearance periods during clamping and for three clearance periods after release of the clamp. All other procedures were identical to that previously described.

All data were analyzed with Student's t-test paired comparison (3). A p value less than 0.05 was used as the level of significance.

Analytical methods. Inulin was determined by the method of Shreiner (4) and PAH was determined by the method of Smith *et al.* (5).

Sodium concentration was determined with a Coleman flame photometer.

PAH calculated blood flow was determined from PAH clearance and hemotracrit. PAH

$$\text{calculated blood flow} = \frac{\text{PAH clearance}}{(1-\text{Hct})}.$$

Total flow minus PAH calculated flow is the difference between blood flow as determined with the flow meter and PAH calculated blood flow. It represents blood flow to the kidney from which PAH is not cleared.

Systemic blood pressure/total renal blood flow (mm Hg/ml/min) was used to estimate renal vascular resistance.

Results. A 0.25 mg/kg prime plus a 0.25 mg/kg/hour infusion of WY-5256 given intravenously produced a significant increase in sodium excretion (Table I). Inulin clearance and blood pressure were not altered. Total renal blood flow was significantly increased by 16% as renal vascular resistance decreased by 0.05 units. This dose did not significantly alter PAH calculated blood flow, but total flow minus PAH calculated flow was significantly increased by 49 ml/min. Total flow—PAH calculated flow increased to a value 1.5 times the control value.

The effect of a 2.5 mg/kg prime plus a 2.5 mg/kg/hour infusion of WY-5256 (iv) is shown in Table II. With this dose, sodium

TABLE II. Effect of WY-5256 on Renal Function (2.5 mg/kg Prime + 2.5 mg/kg/hour Infusion).

	Control periods	WY-5256 periods	Differences ± SE
Sodium excretion (μEq/min)	31	307	276 ± 105[a]
Inulin clearance (ml/min)	43	44	1 ± 2
Blood pressure (mm Hg)	121	121	0 ± 2
Resistance (mm Hg/ml/min)	0.38	0.30	−0.08 ± 0.012[a]
Total renal blood flow (ml/min)	336	420	84 ± 11[a]
PAH calculated blood flow (ml/min)	263	308	45 ± 14[a]
Total flow—PAH calculated flow (ml/min)	73	113	40 ± 12[a]

[a] Indicates a significant difference. Average dog wt. = 25 kg, $N = 6$.

excretion increased by 276 μEq/min. Inulin clearance and blood pressure remained unaltered. Renal vascular resistance was significantly decreased by 0.08 units producing a 25% increase in total renal blood flow. PAH calculated blood flow as well as total flow minus PAH calculated flow were significantly increased with this higher dose. PAH calculated blood flow increased to a value only 1.2 times the control value while total flow minus PAH calculated flow increased to a value almost 1.6 times that of the control.

Fig. 1 shows a tracing obtained when WY-5256, 0.05 mg/kg prime plus a 0.05 mg/kg/min infusion, was administered and renal blood flow was held constant. The middle tracing represents the blood pressure distal to the aortic clamp and shows the reduction in perfusion pressure required to hold renal blood flow constant. Upon release of the aortic clamp and continued drug infusion, renal blood flow was increased.

A 0.05 mg/kg prime plus a 0.05 mg/kg hour infusion of WY-5256 did not significantly alter PAH clearance and inulin clearance while renal blood flow was held constant or upon release of the aortic clamp (Table III). A decrease in perfusion pressure of approximately 14 mm Hg was required to hold renal blood flow constant. Upon release of the clamp, perfusion pressure returned to slightly above the control value while renal blood flow was significantly increased. Sodium excretion significantly increased from 46 to 99 μEq/min while renal blood flow was held constant. An additional increase to 159 μEq/min was observed when the clamp was released and renal blood increased.

Discussion and Conclusions. The results showed that WY-5256 increases renal blood flow in the dog. Furthermore, it was found that total renal blood flow, as measured with the flow meter, was increased to a greater extent than indicated by calculating blood flow from the clearance of PAH. The increase in non-PAH extracting flow produced by WY-5256 was observed to be proportionally greater than the increase in PAH calculated flow. Exactly what this change in non-PAH

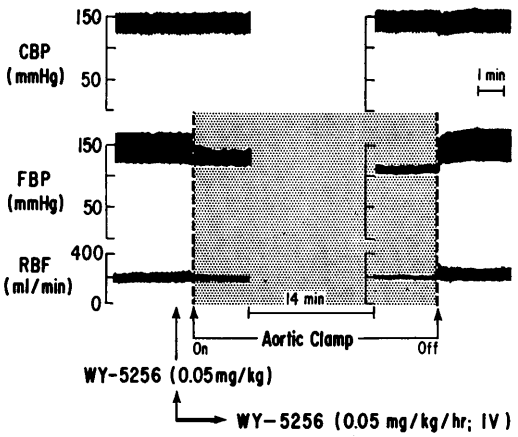

FIG. 1. Blockage of WY-5256-induced increase in renal blood flow by use of an aortic clamp. Carotid blood pressure (CBP) is shown by the top tracing. The bottom tracing represents renal blood flow (RBF). The middle tracing (FBP) represents blood pressure distal to the aortic clamp (obtained from the femoral artery) and shows the reduction in perfusion pressure required to hold renal blood flow constant after drug administration. An intravenous injection of 0.05 mg/kg of WY-5256 (administered at the arrow) was followed by an infusion of WY-5256 at 0.05 mg/kg/hour. The stippled area represents tightening of the aortic clamp as necessary to hold renal blood flow at the control level.

TABLE III. The Effect of WY-5256 on Renal Function When Renal Blood Flow Was Held Constant (0.05 mg/kg Prime + 0.05 mg/kg/hour Infusion).

	Control periods	WY-5256 periods (aorta clamped)	WY-5256 periods (clamp released)
PAH clearance (ml/min)	111	121	124
		10 ± 5[a]	13 ± 10[a]
			4 ± 12[b]
Inulin clearance (ml/min)	34	33	34
		−1 ± 2	0 ± 3
			1 ± 2
Renal blood flow (ml/min)	251	255	291
		4 ± 6	40 ± 11[c]
			37 ± 8
Sodium excretion (μEq/min)	46	99	159
		53 ± 9[c]	113 ± 28[c]
			61 ± 19[c]
Femoral blood pressure (mm Hg)	132	118	137
		−14 ± 5	5 ± 3
			19 ± 3[c]

[a] Difference from control periods ± SE.
[b] Difference from clamped aorta values ± SE.
[c] Indicates a significant difference. $N = 4$.

extracting flow represents remains unanswered. It has been suggested that non-PAH extracting blood flow represents "noncortical plasma flow" (6). Since noncortical plasma flow is in part medullary blood flow an increase in non-PAH extracting flow could indicate an increase in medullary blood flow. It has been postulated that superficial areas of the cortex are extremely inefficient in the extraction of sodium (7). Perhaps this is also true for PAH. Considering this suggestion it is equally possible that an increase in non-PAH extracting blood flow could represent an increase in flow to such superficial cortical areas. Therefore, whether the increase in non-PAH extracting blood flow produced by WY-5256 represents an increase in medullary flow, superficial cortical flow, or a combination of the two cannot be definitely stated at this time.

WY-5256 was studied with an aortic clamp in an attempt to assess drug action in the absence of blood-flow alterations. It was demonstrated that sodium excretion after drug administration and with renal blood flow held constant was significantly greater than predrug values. These results indicate that WY-5256 can increase sodium excretion in the absence of blood-flow alterations. Upon release of the aortic clamp the drug produces an additional increase in sodium excretion. Since inulin clearance was not altered throughout the procedure while sodium excretion was increased during aortic clamping and again upon release of the clamp, it follows that per cent of filtered sodium excreted was increased in both cases. These results indicate that WY-5256 acts in part by directly inhibiting sodium reabsorption and in part via renal blood flow alterations.

Summary. WY-5256 was administered to dogs to determine the effect of this agent on renal hemodynamics. Changes in renal hemodynamics were estimated with an electromagnetic flow meter in conjunction with clearances of *p*-aminohippurate (PAH) and inulin. It was observed that WY-5256 increased renal blood flow, that the increase was greater than that indicated by PAH calculated blood flow, and that increases in non-PAH extracting flow were greater in proportion than increases in PAH calculated blood flow. The effect of WY-5256 on renal function was also studied when renal blood flow was held con-

stant with an aortic clamp. It was demonstrated that the drug increased sodium excretion in the absence of blood flow alterations. The increase was less than that seen without the clamp. These data are interpreted as indicating that this agent acts in part by directly inhibiting sodium reabsorption and in part by altering renal hemodynamics to increase sodium excretion.

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1. Rosenthale, M. E., and Osdene, T. S., Arch. Intern. Pharmacodyn., 164, 11 (1966).

2. Rosenthale, M. E., J. Pharmacol., 147, 399 (1965).

3. Snedecor, G. W., "Statistical Methods," 5th ed., Iowa State College Press, Ames (1956).

4. Schreiner, G. E., Proc. Soc. Exptl. Biol. Med., 74, 117 (1950).

5. Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., and Graber, M., J. Clin. Invest., 24, 388 (1945).

6. Earley, L. E. and Friedler, R. M., J. Clin. Invest., 44, 929 (1965).

7. Barger, A. C., Ann. New York Acad. Sci., 139, 276 (1966).

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Differences in Electrophoretic Migration of Mouse $7S\gamma_{2a}$ and $7S\gamma_{2b}$ Globulins (32656)

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The mouse immunoglobulin system consists of four major classes of globulins—$7S\gamma_1$, $7S\gamma_2$, γ_{1A}, and γ_{1M} (1) and the $7S\gamma_2$ class has two antigenically distinct subclasses, the $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins (2). The $7S\gamma_2$ subclasses are of considerable interest because of differences in their isoantigens (3–6) and biologic functions (7). However, studies with these two subclasses have been limited to the use of $7S\gamma_{2a}$ and $7S\gamma_{2b}$ myeloma proteins because a technique for separating normal $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins has not been described. This report describes differences in the electrophoretic migration of $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins of various inbred strains of mice and shows that partial separation of these two proteins may be obtained by electrophoretic methods.

Materials and Methods. Inbred mice and mouse serums were obtained from Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine. Noninbred mouse serums were secured from Colorado Serum Company (CSC), Denver, Colorado and from RML mice maintained at the Rocky Mountain Laboratory.

Hen albumin (HEA) was obtained from K and K Laboratories, Inc., Jamaica, New York and iodinated with I^{131} (E. R. Squibb and Sons, Inc., New Brunswick, New Jersey), as previously described (8). Myeloma proteins LPC-1, MPC-31, and MPC-47 representing the $7S\gamma_{2a}$, $7S\gamma_{2b}$, and $7S\gamma_1$ groups, respectively, were obtained from Dr. J. L. Fahey and Dr. R. Asofsky (NIH, Bethesda, Maryland).

New Zealand white rabbits and Targhee sheep from local sources were used for the production of antiserums. Rabbit antiserums to Balb/c $7S\gamma_1$ globulins and mouse antiserums to HEA were prepared as previously described (9). Antiserums to $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins were prepared in rabbits by injection of the respective myeloma protein in complete Freund's adjuvant. Specificity was achieved by absorption with the heterologous $7S\gamma_2$ myeloma protein and verified by gel-diffusion analysis with $7S\gamma_{2a}$, $7S\gamma_{2b}$, and $7S\gamma_1$ myeloma proteins. Rabbit and sheep antiserums to mouse serum were prepared by injecting whole mouse serum emulsified in complete Freund's adjuvant and then 3–4 weeks later starting weekly injections of the antigen in incomplete