Persistence of the Natriuretic Response to Isotonic Saline Load During Hemorrhagic Hypotension in the Dog* (33855)

JAIME B. COELHO AND STANLEY E. BRADLEY

Department of Medicine, Columbia University College of Physicians and Surgeons, and the Medical Service, The Presbyterian Hospital, New York, New York 10032

The demonstration (1–3) that intravenous infusion of an isotonic saline solution is promptly followed by an increase in sodium excretion without demonstrable change in plasma composition, glomerular filtration rate, or increased activity of any known humoral agent has recently evoked a spate of experimental studies. The use of micropuncture and recollection techniques in dog (4) and cat (5) showed that the major discrete renal functional change is a fall in fractional sodium reabsorption by the proximal tubule. Special attention was paid to the possibility of the release (or inhibition) of a natriuretic (or antinatriuretic) hormone (third factor) (1–7). DeWardener and others (1, 3, 6) stressed the role of an expansion in extracellular fluid as the major determinant of the response, while Rector *et al.* (7) and Martino and Earley (8) emphasized variously the influence of secondary changes in intrarenal hemodynamics, hydrodynamics, and geometries that may directly affect tubular reabsorption of sodium or that may effectuate the action of "third factor."

It was reported recently (9) that isotonic saline loading maintains urinary output and minimizes ultrastructural damage to the tubules during persistent and severe hemorrhagic shock in dogs. This observation suggests that the natriuretic stimulus may even be capable of overriding the powerful antinatriuretic effect of profound systemic arterial hypotension. Partial occlusion of the aorta with resultant renal arterial hypotension also fails to suppress such a natriuresis in spite of hypofiltration as marked as that observed after blood loss (4). With severe hemorrhage, however, the concomitant reductions in blood volume, cardiac output, and arterial pressure serve as powerful extrarenal stimuli that elicit a widespread neural and humoral response which may profoundly affect sodium and water excretion independently of filtration. Indeed, Cirksena *et al.* (10) observed that the depression in proximal sodium reabsorption induced by sodium loading was completely reversed in dogs after acute intrathoracic vena caval obstruction in association with a "severe hemodynamic disturbance" that presumably resembled shock. A more precise evaluation of the effect of controlled hemorrhagic hypotension upon the natriuretic response therefore appears to be in order.

Methods. Eight female mongrel dogs, weighing between 10 and 18 kg, were employed in this study. All were fasted for 12 hr, allowed free access to water, and given 10 mg of DOCA intramuscularly about 2 hr before control measurements began. Anesthesia was induced with sodium pentobarbital (30 mg/kg iv) and maintained throughout by additional amounts as required. Catheters were placed in the bladder, a femoral artery, and a peripheral vein. The arterial catheter was connected to a mercury manometer and used for the collection of blood samples and for bleeding. After arterial blood and urine blanks were obtained, a priming dose of inulin-^{3}H (1.4–0.7 SD μCi/kg of body wt) was given rapidly by intravenous injection. Thereafter, a constant infusion of inulin-^{3}H (0.021 – 0.007 μCi/kg/min) was maintained. This sustaining infusion was made up in a small volume of isotonic saline, so as to minimize the extent of volume expansion during the preliminary equilibrium (12–25 min) and control phase.

The amount of saline infused throughout the initial control phase, including a slow drip into the femoral artery to keep the catheter patent, never exceeded 150 ml. Since

* Supported by Grants from the National Heart Institute of the United States Public Health Service (HE-05741) and the Heart and Lung Foundation.

the urine flow was very low at this time, 10 ml of distilled water and 10 ml of air were used to wash out the bladder at the end of each of three 10–15-minute clearance periods. An arterial blood sample was obtained near the midpoint of each period. Following completion of the initial phase, 150 ml of NaCl was infused iv at 23 ml/min for 35 min and then at 15 ml/min throughout the remainder of the experiment. After urine flow had attained high and relatively stable levels, three more clearance periods were obtained with air alone being used to insure bladder emptying. Immediately thereafter, the animal was bled until the blood pressure became stable in the vicinity of 50 mm Hg. In order to maintain the arterial pressure relatively constant in the face of continuing saline infusion, blood was withdrawn repeatedly during this phase of the study. (In one animal the blood pressure remained in the vicinity of 80 mm Hg). In general, about 30 min were required to achieve a stable hypotensive phase. Three more clearance periods were then collected, with inulin infusion reduced to one half the control rate. Distilled water was again used for bladder washout. In two dogs blood was retransfused at the end of the study, and the animals were used a second time 20 and 26 days later.

Blood samples were drawn with clean, disposable syringes and transferred to heparinized tubes. Hematocrits were obtained for almost every sample. The activity of ^{3}H in plasma and urine was measured in a Packard Tri-Carb model 3003 liquid-scintillation spectrometer. Equal volumes (0.5 ml) of plasma and hydroxide of hyamine were mixed and allowed to stand overnight after which 10 ml of Bray's solution were added. Urine specimens were appropriately diluted with distilled water, and 0.5 ml of the final dilution was mixed with 10 ml of Bray's solution by means of a mechanical vibrator. All samples were processed in duplicate. Frequent internal standards, also in duplicate, were prepared by adding 50 μl of the inulin-^{3}H infusion to samples of both urine and plasma prepared as described above. Owing to differences in quenching between urines and plasmas, correction factors were introduced on the basis of such recoveries. Sodium and potassium were determined by flame photometry, osmolality by the Fiske osmometer, and plasma protein concentration by a hand refractometer.

Results. Table I summarizes the data from each experiment. The values are averages of at least two (generally three) collection periods for each of three successive phases; viz., control (C), isotonic saline loading before (S), and after (H) bleeding. There was good intraphase agreement from period to period during control and initial saline-loading and somewhat more variability during the hypotensive phase, probably owing to the greater difficuly in maintaining a stable blood pressure. The degree of hemodilution is shown by the extent of the fall in hematocrits and plasma protein concentrations.

During the initial saline-loaded phase, increments in urine flow, inulin-^{3}H clearance and sodium and potassium excretion rates were observed. Arterial pressure tended to fall somewhat or to remain unchanged in spite of a net gain of 570–2100 ml of saline together with a fall in hematocrit in every experiment.

During the hypotensive phase, an initial negative fluid balance attributable to the rapid blood loss was followed by a continued gain, so that the final net fluid balance did not differ greatly from that obtaining at the end of the initial saline-loaded phase. In general, therefore, clearance measurements were made in both phases under similar conditions of overall fluid balance. It may be presumed, however, that a material reduction in blood volume and cardiac output accounted for continued hypotension and that the extracellular, extravascular spaces were further expanded. With the reduction in arterial pressure, inulin clearances tended to fall in all but two (Clara and Blinky, Table I) to levels below the initial control values. The sodium excretion decreased sharply in every instance but never to values as low as those observed during the control normotensive periods. The sodium–inulin clearance ratios behaved similarly; the percentage of filtered

TABLE I. Renal Functional Changes before and after Hemorrhage in Normal Hydrated Dogs during Isotonic Saline Load.[a]

Subject and body wt (kg)	Phase	V (ml/min)	C_{IN} (ml/min)	$U_{Na}V$ (μeq/min)	$\frac{U_{Na}V_{100}}{C_{IN}P_{Na}}$ (%)	U_KV (μeq/min)	$\frac{U_KV_{100}}{C_{IN}P_K}$ (%)	HCT (%)	BP (mm Hg)	Fluid balance (ml)	$T^c_{H_2O}$ (ml/min)	$\frac{T^c_{H_2O}}{C_{IN}}$ (%)	Protein (g/100 ml)
Lucia (15)	C	0.16	70	59.0	0.58	16.4	6.3	36	105	150	—	—	—
	S	6.50	73	1515.0	14.70	78.3	33.4	25	110	2250	4.42	6.05	—
	H	0.33	48	93.0	1.34	5.3	3.5	11	48	1600	0.57	1.19	—
Carol (12)	C	0.77	40	4.2	0.07	11.8	8.5	36	125	—20	0.93	2.32	—
	S	7.50	41	667.0	10.40	42.1	31.8	25	125	550	—0.89	—2.17	—
	H	3.40	34	438.0	8.10	26.8	24.5	16	82	300	0.28	0.82	—
Blackie (14)	C	0.25	61	38.0	0.43	35.4	18.8	45	105	0	0.93	1.52	6.1
	S	7.70	72	1246.0	11.70	51.1	23.0	30	112	1400	1.46	2.03	3.7
	H	1.70	55	207.0	2.60	40.2	20.8	20	65	1200	1.50	2.72	1.6
Clara (13)	C	0.10	39	5.2	0.09	14.0	9.4	49	140	0	0.29	0.74	6.0
	S	2.50	35	517.0	9.80	38.7	38.2	25	120	1150	1.33	3.80	2.4
	H	0.30	39	78.0	1.10	14.0	11.9	27	60	1350	0.46	1.18	0.8
Alma (10)	C	0.05	33	2.7	0.55	16.2	10.4	46	170	—30	0.28	0.85	—
	S	2.40	37	436.0	7.68	18.9	15.2	30	156	1400	0.73	1.97	—
	H	0.42	23	104.0	3.02	10.3	14.8	13	52	1300	0.39	1.70	—
Blinky (10)	C	0.12	31	14.0	0.31	20.6	20.3	35	140	—30	0.48	1.55	—
	S	1.90	56	356.0	4.20	21.3	12.0	24	115	1400	0.80	1.43	—
	H	0.25	44	62.0	1.00	12.3	8.1	13	56	1300	0.50	1.14	—
Myrna[I] (13)	C	0.14	50	13.2	0.17	28.2	15.8	44	142	0	—	—	6.0
	S	5.80	67	1286.0	12.60	90.3	43.1	34	130	1400	—	—	3.8
	H	0.18	76	15.5	1.60	7.6	31.0	13	55	1100	—	—	0.6
Janet[I] (14)	C	0.10	43	5.5	0.09	11.2	6.7	37	135	0	0.27	0.63	4.6
	S	4.60	47	701.0	10.20	70.3	49.7	27	130	1880	0.84	1.79	2.5
	H	0.08	26	10.9	0.27	7.6	6.3	12	52	1450	0.13	0.50	0.5
Myrna[II] (14)	C	0.15	46	3.8	0.06	18.3	9.6	34	130	0	0.29	0.63	5.7
	S	5.00	49	749.0	10.40	81.5	55.1	28	130	1300	0.46	0.94	3.8
	H	1.20	35	224.0	4.30	66.9	48.5	6	60	1400	0.72	2.06	0.2

TABLE I (continued).

Subject and body wt (kg)	Phase	V (ml/min)	C_{IN} (ml/min)	$U_{Na}V$ (μeq/min)	$\frac{U_{Na}V_{100}}{C_{IN}P_{Na}}$ (%)	U_KV (μeq/min)	$\frac{U_KV_{100}}{C_{IN}P_K}$ (%)	HCT (%)	BP (mm Hg)	Fluid balance (ml)	$T^c_{H_2O}$ (ml/min)	$\frac{T^c_{H_2O}}{C_{IN}}$ (%)	Protein (g/100 ml)
Janet[11] (13)	C	0.13	32	8.0	0.17	18.0	15.2	38	122	100	0.50	1.56	4.5
	S	4.10	52	539.0	6.90	67.1	42.1	28	120	1800	—0.22	—0.42	2.6
	H	0.11	17	29.7	1.16	15.3	21.1	10	54	2300	0.24	1.41	0.5

[a] All values are averages of determinations during two or more 10-min urinary collection periods. After the initial 30-min control phase (C), 150 m*M* NaCl was infused iv at 23 ml/min for 35 min and thereafter at 15 ml/min. With stabilization of urine flow (V), three more urine periods (S) were obtained. Blood was then removed from an artery until the arterial pressure (BP) stabilized close to 50 mm Hg and three additional urine periods (H) were obtained. Glomerular filtration was measured as the inulin clearance (C_{IN}). Sodium ($U_{Na}V$) and potassium (U_KV) outputs, the percentage of the filtered load of sodium ($U_{Na}V/C_{IN}P_{Na}$) and potassium ($U_KV/C_{IN}P_{Na}$) excreted in the urine, and the arterial hematocrit (HCT) was measured in every period. The tubular reabsorption of solute-free water—absolute ($T^c_{H_2O}$) and relative to filtration ($T^c_{H_2O}/C_{IN}$)—and plasma protein concentration were determined whenever possible.

sodium excreted in the urine remained much higher than during control despite blood loss and hypotension. A persistent natriuretic response was thus evident during marked hemorrhagic hypotension.

This enhanced output of sodium in response to isotonic sodium loading under circumstances seemingly conducive to sodium retention was associated with persistent reabsorption of solute-free water and with a relative kaliuresis. In nine studies in which osmotic clearances were followed, $T^c_{H_2O}$ was found to range from 0.27 to 0.93 ml/min during the control phase. With saline loading, this value increased by 0.17–1.04 ml/min or by 57–360% in all but two (Janet[11] and Carol, Table I) in whom a free water clearance of 0.22 and 0.89 ml/min were observed. During hemorrhage plus saline load, $T^c_{H_2O}$ rose on four occasions and fell or remained unchanged relative to control values on four. It is noteworthy that the percentage of filtered water reabsorbed as solute-free water, rose from an average control level of 0.94% (range: 0.63–1.55%) to 1.44% (0.5–2.9%) during hemorrhage in salt-loaded animals. The clearance of potassium also increased relative to the inulin clearance in 9 out of 10 dogs (from an av of 12.1 to 34.4%) during saline load and fell with hemorrhage in all but not to less than control values in eight, *i.e.*, from an average control of 12.1 to 19.1% after blood loss.

Discussion. These observations appear to be consonant with the conclusion that hemorrhagic shock does not eliminate the natriuretic response to an isotonic saline load. The response was somewhat obtunded but sodium output was usually greatly in excess of that observed during the control unloaded period when the glomerular filtration rate was much higher. It may be inferred, therefore, that reduction in fractional proximal tubular reabsorption occured even in the face of severe blood loss, in company with maintenance or an increase in distal reabsorptive rate (11–14) that was evident in relative increments in the reabsorption of solute-free water and in potassium–sodium exchange.

Analysis of the mechanisms which govern

the natriuretic response is always complicated by the simultaneous operation of multiple potential determinants. The present study is certainly no exception. Although total extracellular fluid volume was kept close to the control, the blood volume undoubtedly decreased together with arterial blood pressure and cardiac output. The associated fall in glomerular filtration rate, reactive release of mineralocorticoids and adrenergic substances, and increased autonomic activity (15–17) might all have been expected to diminish sodium and water excretion to the vanishing point. Replacement of the blood loss with isotonic saline resulted in a marked reduction in the hematocrit and the plasma protein concentration. Both of these changes could conceivably have impaired the tubular reabsorption of sodium and contributed to natriuresis.

There is increasing agreement, however, that isotonic saline infusion produces its effect in normal animals through an alteration in some aspect of body water volume (1, 3, 6, 18–20). The weight of the evidence points increasingly to an expansion of blood volume *per se* as the prime mediator but the possibility of an integral change in an interstitial fluid volume cannot be set aside. In the experiments set forth herewith, it is highly probable that blood volume was greatly reduced, although the difficulty of making precise measurements of blood volume under the circumstances of the study precluded absolute certainty on this point. Nevertheless, the persistent reduction in arterial blood pressure following blood loss is hard to explain in any other way and it may be inferred that the added saline was distributed preponderantly to the extravascular compartment. The striking dilution of the plasma proteins may be presumed to have been largely responsible for this shift, serving to permit the movement of water from the capillaries despite the fall in intravascular hydrostatic pressures. Such an expansion of the interstitial spaces, independent of the blood volume, both in control and in hypotensive animals, following infusion of isotonic saline might be important in eliciting release of a natriuretic hormone from some undetermined tissue site.

The kidney itself cannot be eliminated either as the source of the hypothetical hormone or as responsive directly to hemodilution and increased interstitial fluid volume. The fall in hematocrit unquestionably entailed a corresponding reduction in oxygen delivery that was further diminished by the hypotensive renal ischemia. Although moderate anemia *per se* does not seem to depress tubular reabsorption (20), hematocrits ranging from 6 to 27% might well have been associated with sufficient oxygen deficit to impair cellular function. It may be noted, however, that sodium reabsorption is well maintained in dogs receiving hypertonic glucose solutions during equally severe hemorrhagic shock and anemia (21). Moreover, measurements were made promptly after equilibration of the arterial pressure in the present study, so that tubular cell damage must have been minimal or absent. Even a more prolonged exposure under these circumstances has proved to produce relatively little cellular injury that can be detected by electron microscopy (9).

The most characteristic ultrastructural change observed (9) in dogs similarly receiving isotonic saline loads during hemorrhagic shock has been an expansion of the extracellular phase of the basal infoldings of the proximal convoluted tubules. This accumulation of interstitial fluid was not observed in hypotensive dogs in whom urine flow was maintained by osmotic diuretics. Other workers (22) have described a similar distention of the basal cisternae during excretion of a sodium load in normal animals. Curran and MacIntosh (23) recently suggested that such an intercellular compartment, bounded by the cellular plasma membrane and by a more permeable basal lamina propria, could operate to couple osmotic and hydraulic pressure gradients in promoting a net iso-osmotic flow across the intestinal mucosa. Anatomical evidence conforming with the Curran "serial membrane" model has now been reported for several tissues active in sodium transport (24, 25). Although the correlation of tubular

sodium rejection (rather than enhanced reabsorption) with expansion of the basal labyrinth is difficult to understand in terms of the Curran hypothesis, the appearance of the clefts in the saline-loaded hypotensive dogs is consistent with the natriuresis observed and suggests that localized hydrodynamic responses were not deranged by intrarenal hemodynamic rearrangements. A number of workers (26–28) have presented evidence that peritubular pressure may be a major normal determinant of sodium reabsorption, perhaps by governing the movement of fluid from the cisternae. Measurements of deep intrarenal pressures would therefore be of considerable interest in view of the possibility that the exaggeration of the imbalance in transcapillary forces by saline loading could actually result in an increase in "effective" interstitial pressure and thus interfere with transport of electrolyte and water out of the clefts, even in the face of arterial hypotension.

The redistribution of the blood flow within the kidney during hemorrhagic hypotension appears to be associated with complete or partial cessation of function of the more peripheral cortical nephrons (21, 29). This heterogeneity of perfusion results both from the hypotension itself and from a nonuniform vasoconstrictive response. Assuming heterogeneity of nephron function, it is possible that the antinatriuresis of shock arises, in part at least, from the functional removal of that portion of the nephron population in the cortex which is most active in sodium excretion. If so, then the infusion of isotonic saline could have its effect by preventing or moderating afferent arteriolar constriction so that filtration is maintained in a moiety of sodium-excreting nephrons, with resultant production of a natriuresis. Such an explanation would require a much more marked functional heterogeneity with respect to sodium excretion, however, than has been evident in micropuncture studies (14). An infusion of saline does seem to evoke a moderate intrarenal vasodilation (27) but there is as yet no evidence that it is nonuniform or that it can occur during hemorrhagic hypotension. Further work is necessary to explore these possibilities.

Summary. Water, sodium, and potassium outputs increased well above control values in normal hydrated fasting dogs during the intravenous administration of isotonic saline solution. With the reduction in arterial pressure to a constant level close to 50 mm Hg by controlled hemorrhage in these animals, all three outputs decreased in association with diminished glomerular filtration rate (inulin clearance), hematocrit, and plasma protein concentration but never to levels as low as those observed during the control phase. Since the blood loss was more than replaced by the isotonic saline infused, it may be presumed that extracellular extravascular fluid volume increased while blood volume fell. The persistence of natriuresis indicates continued tubular rejection of sodium, possibly as a result of a change in interstitial volume or vascular perfusion of the kidney.

The authors wish to acknowledge with thanks the technical assistance of Misses Eleanor Bachthaler, Linda Green, and Barbara Jones.

1. De Wardener, H. E., Mills, I. H., Clapham, W. F., and Hayter, C. J., Clin. Sci. **21**, 249 (1961).

2. Levinsky, N. G. and Lalone, R. C., J. Clin. Invest. **42**, 1261 (1963).

3. Lichardus, B. and Pearce, J. W., Nature **209**, 407 (1966).

4. Dirks, J. H., Cirksena, W. J., and Berliner, R. W., J. Clin. Invest. **44**, 1160 (1965).

5. Cortney, M. A., Mylle, M., Lassiter, W. E., and Gottschalk, C. W., Am. J. Physiol. **209**, 1199 (1965).

6. Johnston, C. I., Davis, J. O., Howards, S. S., and Wright, F. S., Circulation Res. **20**, 1 (1967).

7. Rector, F. C., Jr., Sellman, J. C., Martinez-Maldonado, M., and Seldin, D. W., J. Clin. Invest. **46**, 47 (1967).

8. Martino, J. A. and Earley, L. E., J. Clin. Invest. **46**, 1963 (1967).

9. Ben-Ishay, Z., Wiener, J., Sweeting, J., Bradley, S. E., and Spiro, D., Lab. Invest. **17**, 190 (1967).

10. Cirksena, W. J., Dirks, J. H., and Berliner, R. W., J. Clin. Invest. **45**, 179 (1966).

11. Eknoyan, G., Suki, W. N., Rector, F. C., Jr., and Seldin, D. W., J. Clin Invest. **46**, 1178 (1967).

12. Stein, R. M., Abramson, R. G., Kahn, T., and Levitt, M. F., J. Clin. Invest. **46**, 1205 (1967).

13. Hayslett, J. P., Kashgarian, M., and Epstein, F. H., J. Clin. Invest. **46**, 1254 (1967).

14. Howards, S. S., Davis, B. B., Knox, F. G., Wright, F. S., and Berliner, R. W., J. Clin. Invest. **47**, 1561 (1968).

15. Goodyer, A. V. N. and Jaeger, C. A., Am. J. Physiol. **180**, 69 (1955).

16. Farrell, G. L., Rosnagle, R. S., and Rauschkalb, E. W., Circulation Res. **4**, 606 (1956).

17. Darby, T. D. and Watts, D. T., Am. J. Physiol. **206**, 1281 (1964).

18. Bahlmann, J., McDonald, S. J., Dunningham, J. G., and De Wardener, H. E., Clin. Sci. **32**, 395 (1967).

19. Bahlmann, J., McDonald, S. J., Ventom, M. G., and De Wardener, H. E., Clin. Sci. **32**, 403 (1967).

20. Knox, F. G., Howards, S. S., Wright, F. S., Davis, B. B., and Berliner, R. W., Am. J. Physiol. **215**, 1041 (1968).

21. Coelho, J. B. and Bradley, S. E., J. Clin. Invest. **43**, 386 (1964).

22. Caulfield, J. B. and Trump, B. F., Am. J. Pathol. **40**, 199 (1962).

23. Curran, P. F. and MacIntosh, J. R., Nature **193**, 347 (1962).

24. Kaye, G. I., Wheeler, H. O., Whitlock, R. T., and Lane, N., J. Cell Biol. **30**, 237 (1966).

25. Schmidt-Nielsen, B. and Davis, L. E., Science **159**, 1105 (1968).

26. Lewy, J. E. and Windhager, E. E., Am. J. Physiol. **214**, 943 (1968).

27. Martino, J. A. and Earley, L. E., Circulation Res. **23**, 371 (1968).

28. Koch, K. M., Aynedjian, H. S., and Bank, N., J. Clin. Invest. **47**, 1696 (1968).

29. Carriere, S., Thorburn, G. D., O'Morchoe, C. C. C., and Barger, A. C., Circulation Res. **19**, 167 (1966).

Received Jan. 15, 1969. P.S.E.B.M., 1969, Vol. 131.

Glycerylphosphorylcholine Diesterase: Inhibition by Nucleotides* (33856)

J. J. BALDWIN[1] AND W. E. CORNATZER

Guy and Bertha Ireland Research Laboratory, Department of Biochemistry, University of North Dakota, School of Medicine, Grand Forks, North Dakota 58201

Glycerylphosphorylcholine (GPC) diesterase (glycerolphosphorylcholine glycerophosphohydrolase, EC 3.1.4.2), which catalyzes the following reaction: GPC + H_2O → L-α-glycerophosphate + choline is found at the end of the phosphatidylcholine degradative scheme. GPC, unlike lysolecithin is not capable of being acylated to form lecithin (1). Consistent with this data is our inability to demonstrate the synthesis of GPC from L-α-glycerolphosphate and choline. It therefore appears in view of available evidence that the sole purpose of GPC diesterase is to hydrolyze GPC.

The rate at which this enzyme operates with respect to the other degradative enzymes in the metabolism of lecithin in mammals could conceivably exert an influence on the rate of metabolism of lecithin due to its unique position in the degradative scheme.

Studies designed to test the effects of various biosynthetic and degradative intermediates in lecithin metabolism on GPC diesterase in our laboratory (2) showed ATP to be a strong inhibitor of the enzyme. The present communication reports the results obtained from studies on the nucleotide inhibition of GPC diesterase from rat kidney.

Materials and Methods. The ATP was purchased from Sigma. The GTP, UTP, and ITP were products of P. L. Biochemicals and all other nucleotides were obtained from Calbiochem. PP_i was the product of the Fisher Chemical Company. Sources of substrates and other reagents were those used in a previous publication (2).

* This work was supported in part by a grant from the U.S. Atomic Energy Commission No AT(11-1)-1513 and is part of a thesis submitted by J. J. B. in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[1] Investigator supported by a Public Health Fellowship Predoctoral Fellow No. 1-FL-6M-32,651-02 from the National Institutes of Health.