CHANGES IN PLASMA AND URINE PHOSPHATE CONCENTRATIONS INDUCED BY DOPAMINE

by

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A Thesis

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CHAPTER I

Introduction

Dopamine is a drug used clinically to raise blood pressure and increase cardiac output. It is used in patients with cardiogenic shock, low cardiac output syndrome, and bacteremic shock (Goldberg, 1972). One of the effects of dopamine is to cause an increase in urinary phosphate excretion (Cuche, Marchand, Greger, Lang, and Knox, 1976). The phosphate excretion persists after dopamine infusion has been stopped. The duration of this phosphaturia, and the effect of the phosphaturia on serum concentrations of phosphate and calcium are not known. Hypophosphatemia may develop if the phosphaturia continues for a sufficient period with or after dopamine infusion. Because the serum concentration of phosphate has so many effects on the cardiovascular as well as other systems,I studied the effects of dopamine on renal handling of phosphate and on plasma phosphate concentrations.

Although calcium is of critical importance and closely related to changes in phosphate balance, this study deals only with phosphate balance. Changes in calcium concentrations and excretion rate made during this study will be reported separately.

Review of the Literature

Dopamine

Dopamine is a vasoactive drug that was released for use in 1974 (Goldberg, 1974). It has been used in low doses (1.3 to 3.6 μg./kg./min.) intravenously to reduce blood pressure and improve sodium excretion in patients with congestive heart failure. (McDonald, Goldberg, McNay, and Tuttle, 1964). It has been used in higher doses (5 to 30 μg./kg./min.) to raise blood pressure and increase cardiac output (Wilson, Sibbald, Jaanimagi, 1976). Recently Cuche, Marchand, Greger, Lang, and Knox (1976) reported that dopamine caused an increased rate of phosphate excretion. Furthermore this increased rate of excretion persisted for a period of time after dopamine infusion was discontinued.

The duration of this phosphaturia, and the effect of the phosphaturia on serum concentrations of phosphate were not reported. Nevertheless it seems reasonable to postulate that hypophosphatemia may develop if the phosphaturia continues.

Chemistry and Duration of Action of Dopamine

Dopamine is the immediate precursor in the synthesis of norepinephrine in the body. Dopamine is formed by hydroxylation and decarboxylation of phenylalanine and tyrosine. When dopamine is infused into the body, one-fourth to one-third is converted to norepinephrine, while the rest is metabolized to homovanillic acid (HVA) by monoamine oxidase and catechol-O-methyltransferase. The HVA is excreted in the urine (Ganong, 1971).

Dopamine has a half-life of 105 seconds, and the pressor effects are evident within two to four minutes of intravenous administration.

Actions of Dopamine

Dopamine has unique cardiovascular and renal effects which are probably due to action on specific receptors. This evidence is based on the use of specific agents which block the action of dopamine but not that of epinephrine or norepinephrine (Goldberg, 1972). It appears that dopamine acts on receptors that behave like beta-adrenergic receptors. It is not likely that these are the same receptors stimulated by epinephrine.

Dopamine has both alpha-adrenergic and beta-adrenergic effects. It increases myocardial contractility and heart rate by action on beta-adrenergic receptors. Vasoconstriction in all vascular beds occurs when large doses are given intravenously (alpha-adrenergic effects). It is reported to be effective in the treatment of cardiogenic shock (Holzer, Karliner, O'Rourke, Pitt, and Ross, 1973; Karliner, 1975), low cardiac output syndrome (Holloway, Stinson, Derby, and Harrison, 1975), and bacteremic shock (Winslow, Loeb, Rahimtoola, Kamath, and Gunnar, 1973).

Response to intravenous dopamine varies with the dosage used (Goldberg, 1972, 1974). At doses of 2-5 μg./kg./min. or less, renal blood flow and urine output may increase significantly but there may be little or no increase in blood pressure, cardiac output, or stroke volume (Beregovich, Bianchi, Rubler, Lomnitz, Cagin, and Levitt, 1974). Dopamine infusions of 5-30 μg./kg./min. cause increases in blood pres-

sure, cardiac output, stroke volume, and myocardial contractility. There may be even greater increases in urine output and renal blood flow (Wilson, et al., 1976).

The mechanism of the selective vasodilation at various dosages is unclear but appears to be due to specific receptors for dopamine in renal and mesenteric vascular beds (Goldberg, 1974).

McDonald, et al. (1964) studied the effects of dopamine on normal human volunteers. They tested each subject to determine the highest dose of dopamine that could be given without increasing mean arterial pressure. This predetermined dose was then used for each subject in the experiment. The dose for all subjects ranged from 2.6 to 7.1 μg./kg./min. They found that dopamine in these volunteers increased the glomerular filtration rate (inulin clearance), effective renal plasma flow (PAH clearance), sodium excretion, and osmolar clearance. They found that potassium excretion and urine flow varied among the subjects tested.

Welsh, Heistad, and Abboud (1978) reported that dopamine infusion in normal human subjects consistently depressed ventilation during normoxia and hypoxia. This depression is thought to be mediated by inhibition of arterial chemoreceptors.

In studies on patients with congestive heart failure the urinary sodium excretion increased significantly with dopamine (1-10 μg./kg./min.) infusion (Beregovich, et al., 1974; Goldberg, McDonald, and Zimmerman, 1963; McDonald, et al., 1964).

A previous study evaluating the effects of catecholamines on urinary phosphorus and calcium concentrations in rats did not include

dopamine (Morey and Kenny, 1964). However, in this study epinephrine produced a variable phosphaturic response in the rat. Norepinephrine consistently produced hyperphosphaturia.

Cuche, et al. (1976) studied phosphate excretion in dogs given dopamine (1.05 μg./kg./min.) infused directly into one kidney via the renal artery. The investigators divided dogs into two groups. In group I the dogs had intact parathyroid glands. In group II parathyroid glands had been removed. Dogs in group II received parathormone by intravenous infusion at 10 μU/kg./min. throughout the entire protocol. This latter procedure was done to study the effects of dopamine independent of changes in parathormone concentrations in blood. The authors found that dopamine produced a phosphaturia independent of changes in parathormone (PTH), calcitonin, renal blood flow, or sodium excretion. There were no significant changes in plasma phosphate concentrations and glomerular filtration rate (GFR, inulin clearance). The dopamine-produced increment in renal blood flow and sodium excretion returned to control levels soon after the infusion was stopped. The phosphate excretion was still significantly elevated above control periods 30 minutes after the infusion was stopped. Cuche, et al., (1976) state that the persistence of a phosphaturia after the dopamine infusion is discontinued may indicate "activation of a phosphaturic mechanism that does not depend upon the continued presence of increased dopamine levels".

Action of Dopamine in Dogs

The effects of dopamine in dogs are similar to those observed in man. Renal, mesenteric, and coronary vasodilation occur when dopamine

is infused in dogs in low doses. Large doses of dopamine cause vasoconstriction (Goldberg, 1972). The hemodynamic effects of dopamine have been studied extensively in the dog (Black and Rolett, 1968; Brooks, Stein, Matson, and Hyland, 1969; Cuche, et al., 1976; McNay, MacCannell, Meyer, and Goldberg, 1966; McNay and Goldberg, 1966).

The effects of dopamine on sodium excretion in the dog have also been studied. Meyer, McNay, and Goldberg (1967) found that dopamine infused at 6 μg./kg./min. in dogs increased sodium and potassium excretion and osmolal excretion. Serum sodium, potassium, and osmolality concentrations did not change significantly. Average urine flow did not change significantly from control values. In six of the nine dogs studied GFR was significantly higher during dopamine infusion as compared to control and post-infusion values.

McNay, McDonald, and Goldberg (1963) reported that dopamine infused at 6 μg./kg./min. caused increased sodium excretion and increased GFR over pre- and post-infusion controls. Urine volume was not affected.

Davis, Walter, and Murdaugh (1968) studied the effects of dopamine infusion (10 μg./kg./min.) on proximal tubular reabsorption of sodium in dogs. In a series of micropuncture experiments (recollection technique) on six dogs they found no change in the $(TF/P)_{In}$ ratio. Hence, they concluded that dopamine did not alter sodium reabsorption in the proximal tubule in dogs. In the same paper the authors reported no measurable change in whole kidney GFR. Mean sodium excretion was more than doubled during dopamine infusion (39 μEq/min. during control period to 82 μEq/min. during dopamine infusion).

Absorption and Excretion of Phosphate

Phosphorus plays an important role in maintaining normal homeostasis. In biologic fluids phosphorus exists either as organic or inorganic phosphate (Massry, Friedler, Coburn, 1973). It is present in plasma and extracellular fluid, collagen, and bone tissue. Phosphate is primarily in an inorganic form in the extracellular fluid (Peach, 1975). Approximately 90% of the ingested phosphate is absorbed from the gastrointestinal tract and excreted in the urine, the remaining 10% is excreted in feces (Knochel, 1977; Peach, 1975).

In the kidney 90% or more of the plasma phosphate is filtered (Karliner, 1975). Phosphate reabsorption takes place only in the proximal tubule and is probably not secreted in any part of the nephron (Massry, et al., 1973; Schneider, Strandhoy, Willis, and Knox, 1973; Mudge, Berndt, and Valtin, 1973). Furthermore, the tubule has a transport maximum for phosphate reabsorption. The amount of phosphate appearing in the urine, therefore, represents the difference between the amount filtered and the amount reabsorbed in the proximal tubule (Pitts and Alexander, 1944; Massry, et al., 1973).

Other Factors Affecting Phosphate Excretion

Steele (1970) found that rapid extracellular fluid volume expansion by isotonic saline caused phosphaturia. In 16 normal human subjects given a rapid saline infusion to expand extracellular space by approximately 10%, GFR, plasma phosphate concentration, and filtered load of phosphate remained stable. However, the mean rate of phosphate excretion more than doubled over control values.

Renal tubular handling of phosphate also depends to a great ex-

tent on parathormone serum levels (Massry, et al., 1973). Parathormone inhibits the proximal tubular reabsorption of sodium, calcium, and phosphate. The reduced proximal reabsorption causes increased delivery of these ions to the distal tubule where calcium and sodium are reabsorbed. Since there is no distal tubular reabsorption of phosphate, phosphaturia results.

Fasting will cause phosphate to disappear from the urine of man and dogs. Humans have a diurnal variation in phosphate excretion with low excretion between 9 AM and 12 noon. The peak excretion rate occurs about 12 midnight. In dogs there is a wide variation in phosphate excretion and no diurnal rhythm. Furthermore, dogs have wide variations of phosphate excretion during prolonged anesthesia (Massry, et al., 1973).

Hypophosphatemia

Knochel (1977) defines the normal range of serum concentrations of phosphate in adults as varying from 2.7 to 4.5 mg./100 ml.

Moderate hypophosphatemia is defined as serum concentrations between 1.0 and 2.5 mg./100 ml. (Knochel, 1977). Conditions associated with causing moderate phosphate depletion include: gram-negative bacteremia, hyperparathyroidism, starvation, and sodium bicarbonate administration.

Severe hypophosphatemia is defined as serum concentrations less than 1.0 mg./100 ml. Knochel (1977) describes a distinct clinical syndrome found in patients with serum phosphate concentrations below 1.0 mg./100 ml. The symptoms include anorexia, weakness, and bone pain. There are seven conditions associated with severe phosphate

depletion:

1. Alcohol withdrawal
2. Diabetic ketoacidosis
3. Pharmacologic binding of phosphate
4. Recovery/diuretic phase after severe burns
5. Hyperalimentation
6. Nutritional recovery syndrome
7. Severe respiratory alkalosis

Extracellular phosphate is essential in regulation of rate of glycolysis and the concentration of ATP and 2,3-DPG in vivo (Lichtman, Miller, Cohen, and Waterhouse, 1971). A decrease in inorganic phosphate reduces overall glycolytic rate, limits high energy organic phosphate synthesis, reduces concentrations of ATP and 2,3-DPG, and thereby affects tissue oxygenation. Several investigators (Lichtman, et al., 1971; Newman, Neff, and Ziporin, 1977; Sheldon, 1973; and Sheldon and Gryzb, 1975) have noted a left shift in the oxy-hemoglobin dissociation curve and decreased amounts of ATP and 2,3-DPG in patients with low serum phosphate concentration.

Jacob and Amsden (1971) noted that red cells deprived of ATP became rigid and had decreased survival time. Thus, hemolytic anemia can result from the low ATP concentration.

Respiration as a whole is vulnerable to three specific complications of hypophosphatemia (Newman, et al., 1977). Reduced oxygen delivery to the tissues results from hemolytic anemia and left shift in the oxy-hemoglobin curve. The lung tissue itself is vulnerable to infection since leukocyte dysfunction results from ATP depression

(Newman, et al., 1977).

Craddock, Yawata, Van Santen, Gilberstadt, Silvis, and Jacob (1974) found depression of leukocyte chemotactic, phagocytic, and bactericidal activity in patients with severe hypophosphatemia.

Hypophosphatemia is also associated with seven abnormalities of platelet function and structure (Yawata, Hebbel, and Silvis, 1974):

1. Thrombocytopenia
2. Increase in platelet diameter suggesting shortened platelet survival
3. Megakaryocytosis of the marrow
4. Five- to ten-fold acceleration of the rate of labelled platelet disappearance from the blood
5. Impairment of clot retraction
6. A 44% to 57% reduction in platelet ATP content
7. Hemorrhage into the gut and skin

Encephalopathy may also be a complication of decreased serum phosphate concentrations. Symptoms of irritability, apprehension, muscular weakness, numbness, paresthesias, dysarthria, confusion, obtundation, convulsive seizures, and coma have been reported in severely hypophosphatemic patients (Knochel, 1977).

Statement of the Problem

Much is known about the effect of dopamine on the cardiovascular system. However, less is known of the effect of dopamine on phosphate balance in the body. There is evidence that dopamine may cause hypophosphatemia (Cuche, et al., 1976).

Several of the conditions associated with decreased phosphate concentrations may occur concurrently in patients requiring dopamine infusions. If dopamine does further decrease phosphate levels, patients could manifest any of the serious consequences of hypophosphatemia.

In this study three questions were addressed:

1. What is the time to onset of phosphaturia after beginning dopamine infusion?
2. What is the duration of the phosphaturia after the dopamine infusion is discontinued?
3. What is the magnitude and time course of any changes in the serum phosphate concentrations that occur during or as a result of phosphaturia?

CHAPTER II

Methods

Protocol

Eight mongrel dogs weighing between 18 and 33 kg were used for this study. They were allowed free access to water but food was withheld approximately 12 to 14 hours before the experiment. The dogs were anesthetized with sodium pentobarbital (30 mg./kg. body weight) and endotracheal tubes were placed in the trachea. Catheters were placed in a femoral artery and vein. Blood pressure was continuously monitored and samples of blood were obtained via the arterial catheter. The venous catheter was used for infusion of maintenance fluid and dopamine.

A midline abdominal incision was made, both ureters were ligated and cannulated. The abdominal incision was then closed by suture and covered with saline saturated gauze to keep insensible loss of fluid to a minimum.

Initially, 250 ml.of saline was infused over 25 to 30 minutes. Then a maintainance infusion (5% dextrose in a 0.2% sodium chloride solution with 1.75 gm. of inulin per liter) was begun at 2 ml./min and continued throughout the experiment by Harvard infusion pump. The pump was calibrated prior to the experiment.

Clearance periods were begun 30 to 45 minutes after the start of the maintainance fluid infusion. The clearance periods lasted 20 to 60 minutes depending on urine flow. Twenty milliliters of blood was withdrawn from the arterial catheter at the midpoint of each clearance

period. The blood was immediately centrifuged and the plasma separated and refrigerated.

Dopamine dissolved in isotonic saline was begun after three control clearance periods were completed. Three dogs received dopamine in a dose of 1.05 µg./kg./min. Three dogs received dopamine in a dose of 10 µg./kg./min. Two dogs served as control animals and did not receive dopamine. The dopamine was infused at 0.3 to 0.5 ml./min. by Harvard infusion pump. Rate of infusion was dependent on the body weight of the dog. The control dogs received an equivalent volume of saline without dopamine. Four clearance periods were completed with dopamine infusion, the dopamine was then discontinued and three or four post-infusion clearance periods were completed.

At the completion of the experiment the dog was sacrificed and the kidneys were removed, weighed, and examined for gross abnormalities.

Chemical Methods

Standard spectrophotometric methods for analysis of blood and urine were used (Fiske and Subba Row, 1925; Sunderman and Sunderman, 1969). Samples were read using a Beckman Model 25 spectrophotometer. See Appendix for a detailed discussion of methods.

CHAPTER III

Results

Results are discussed in eight categories. These are: urine flow, glomerular filtration rate, plasma phosphate concentration, filtered load of phosphate, clearance of phosphate, excretion rate of phosphate, transport of phosphate, and fractional excretion of phosphate.

Comparisons are made between control animals (no dopamine) and those receiving 1.05 and 10 μg./kg./min. A t-test was used to test if differences between means were significant.

In general, plasma inulin concentrations did not change during the course of any given experiment (see Figure 1). The results from all experiments are summarized in Table I.

Urine Flow (Refer to Figure 2)

Urine flow did not vary in a predictable fashion during the experiments. Control dogs (#4 and #9) had urine flows ranging from 0.130 ml./min. to 0.3 ml./min. per one hundred grams of kidney weight.

Dogs receiving dopamine at 1.05 μg./kg./min. had urine flows ranging from 0.10 ml./min. to 1.50 ml./min. per one hundred grams of kidney weight. Dog #8 had a sharp rise in urine flow before and after dopamine infusion with the lowest urine flow during dopamine infusion.

Dogs receiving dopamine infusion at 10 μg./kg./min. had urine flows ranging from approximately 0.10 ml./min. to 0.60 ml./min. per one hundred grams of kidney weight.

LEGEND FOR TABLE 1

* Significantly different from control values

** Significantly different from infusion values

1. $P < 0.05$
2. $P < 0.001$
3. $P < 0.01$

Where P is the probability the values are identical

N = 3 unless noted

C is control period

D is infusion period

P is post-infusion period

GFR - glomerular filtration rate

C_{PO_4} - clearance of phosphate

FE_{PO_4} - fractional excretion of phosphate

L_{PO_4} - filtered load of phosphate

T_{PO_4} - reabsorptive rate of phosphate

P_{PO_4} - plasma phosphate concentration

P_{IN} - plasma inulin concentration

TABLE 1. SUMMARY OF RESULTS FOR ALL EXPERIMENTS

MEAN ± STANDARD DEVIATION

		GFR ml/min	C_{PO_4} ml/min	FE_{PO_4}	L_{PO_4} mg/min	T_{PO_4} mg/min	P_{PO_4} mg%	P_{IN} mg%
Dog #4 Control	C $\bar{X}$	26.0	3.4	0.14	1.8	1.6	7.0	7.1
	SD	± 9.4	± .6	± .07 (*1)	± .7	± .7	± .3	±1.5
	D $\bar{X}$	35.8	10.7	0.31	2.8	2.1	8.0	6.4
	SD	± 8.8	± 1.4	± .06	± .6	± .6	± .3	± .1
	P $\bar{X}$	45.3	13.9	(*1) 0.3	3.7	2.7	(*3) 8.1	6.4
	SD	±12.6	± 3.2	± .02	±1.0	± .8	± .1	± .5
Dog #9 Control	C $\bar{X}$	58.3	13.8	0.25	3.4	2.6	5.8	3.7
	SD	+23.5	± 3.7	±0.06	±1.4	±1.2	± .2	± .6
	D $\bar{X}$	(*1) 101.8	21.0	0.21	6.4	(*1) 5.1	6.3	3.2
	SD	± 9.1	± 1.6	± .03	± .7	± .7	± .2	± .3
	P $\bar{X}$ N=2	112.8	19.0	0.17	7.2	(*1) 6.0	(*1) 6.4	3.0
	SD	± 7.4	± 1.2	± .001	± .5	± .4	± 0	± .2
Dog #2 1.05 µg/kg/min dopamine	C $\bar{X}$	40.6	6.5	0.16	2.6	2.2	6.4	7.1
	SD	±13.0	± 3.6	± .05	± .9	± .7	± .2	± .1
	D $\bar{X}$	52.3	14.1	0.32	3.2	2.4	6.0	5.8
	SD	±13.4	± 3.7	± .1	±1.2	±1.1	± .8	± .8
	P $\bar{X}$ N=2	60.8	24.7	(*3) 0.41	2.9	1.8	(*3) 4.8	5.9
	SD	±15.2	± 4.3	± .03	± .6	± .5	± .1	± .5

TABLE 1. (Continued)

			GFR ml/min	C_{PO_4} ml/min	FE_{PO_4}	L_{PO_4} mg/min	T_{PO_4} mg/min	P_{PO_4} mg%	P_{IN} mg%
Dog #3 1.05 µg/kg/min dopamine	C	$\bar{X}$	39.5	2.0	0.05	1.9	1.8	5.0	4.9
		SD	± 5.8	± .9	±.03	± .2	± .2	± .3	± .6
	D	$\bar{X}$	53.0	4.9	0.09	3.4	(*3) 3.1	(*3) 6.4	5.9
		SD	± 6.2	± .9	±.01	± .3	± .2	± .4	± .7
	P	$\bar{X}$	49.0	6.0	(*1) 0.12	3.5	(*1) 3.2	(**1)(*2) 7.2	6.2
		SD	± 7.2	± .8	±.03	± .5	± .5	± .05	± .5
Dog #8 1.05 µg/kg/min dopamine	C	$\bar{X}$	47.0	13.6	0.31	2.0	1.4	4.3	7.0
		SD	± 3.5	± 1.5	±.04	± .1	± .2	± .2	± .9
	D	$\bar{X}$	50.6	18.2	0.35	2.8	1.8	(*3) 5.5	8.5
		SD	± 3.9	± 4.4	±.06	± .4	± .2	± .3	± .3
	P N=2	$\bar{X}$	55.6	26.3	(*1) 0.48	3.2	1.7	(*3) 5.8	7.8
		SD	± 8.9	± 1.4	±.05	± .6	± .5	± 0	±1.5
Dog #5 10 µg/kg/min dopamine	C	$\bar{X}$	48.3	2.7	0.06	3.6	3.3	7.3	7.0
		SD	±16.5	± 1.8	±.02	±1.4	±1.3	± .7	±1.0
	D	$\bar{X}$	61.0	7.8	(*1) 0.14	5.4	4.7	(*1) 8.8	6.3
		SD	±27.5	± 1.3	±.04	±2.3	±2.2	± .2	±1.2
	P N=2	$\bar{X}$	77.4	3.4	0.06	5.3	5.1	(**1) 7.4	5.7
		SD	±34.2	± 2.8	±.06	±1.7	±2.0	± .9	± .7

TABLE 1. (Continued)

			GFR ml/min	C_{PO_4} ml/min	FE_{PO_4}	L_{PO_4} mg/min	T_{PO_4} mg/min	P_{PO_4} mg%	P_{IN} mg%
Dog #6 10 µg/kg/min dopamine	C	$\bar{X}$	40.2	6.0	0.16	2.5	2.1	6.2	4.5
		SD	± 6.9	±3.4	±.1	± .3	± .5	± .4	± .5
	D	$\bar{X}$	(*1) 54.9	21.4	(*1) 0.39	3.7	2.3	6.9	5.4
		SD	± 2.6	±4.5	±.06	± .2	± .3	± .6	± .2
	P	$\bar{X}$	(*3)(**3) 64.8	14.1	(**1) 0.22	4.6	(*3) 3.6	(*1) 7.1	5.1
		SD	± .8	± .5	±.01	± .2	± .2	± .3	± .6
N=1 Dog #7 10 µg/kg/min dopamine	C	$\bar{X}$	47.2	7.1	0.3	1.6	1.2	5.3	2.7
		SD	± 0	±2.5	± 0	± 0	± 0	± .5	±1.1
	D	$\bar{X}$	66.2	22.5	0.3	4.2	2.8	(*1) 6.4	3.4
		SD	± 4.2	±1.5	±.03	± .5	± .5	± .4	± .2
	P N=2	$\bar{X}$	71.1	14.3	(**1) 0.2	4.6	(**2) 3.7	6.3	3.0
		SD	± 5.2	± .6	±,02	± .4	± .4	± .3	± .3

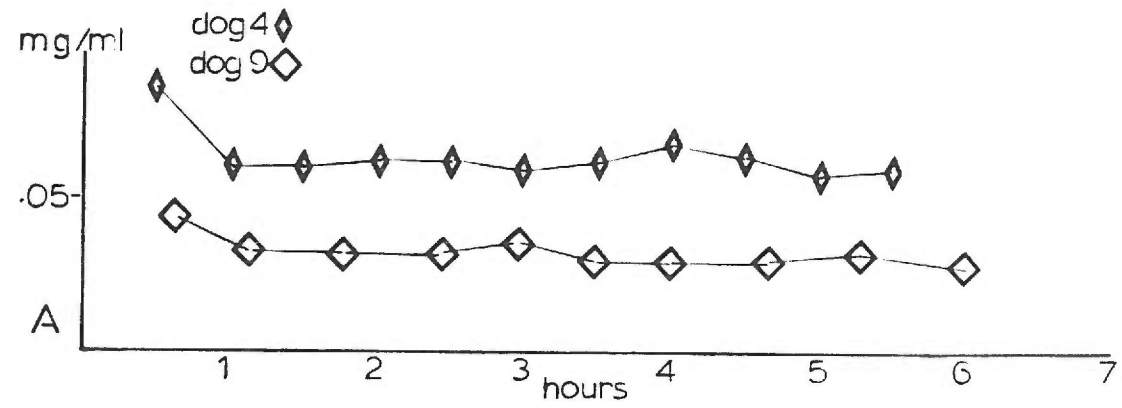
FIGURE 1 Plasma inulin concentration. Values are reported for each group of dogs over the course of the experiment.

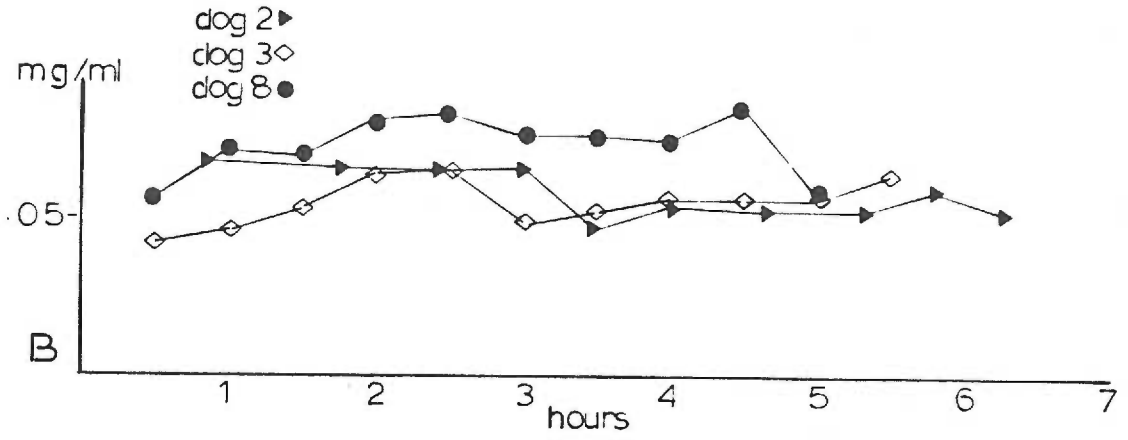
A = control dogs

B = 1.05 µg./kg./min. dopamine

C = 10 µg./kg./min. dopamine

FIGURE 1 P_{inulin}





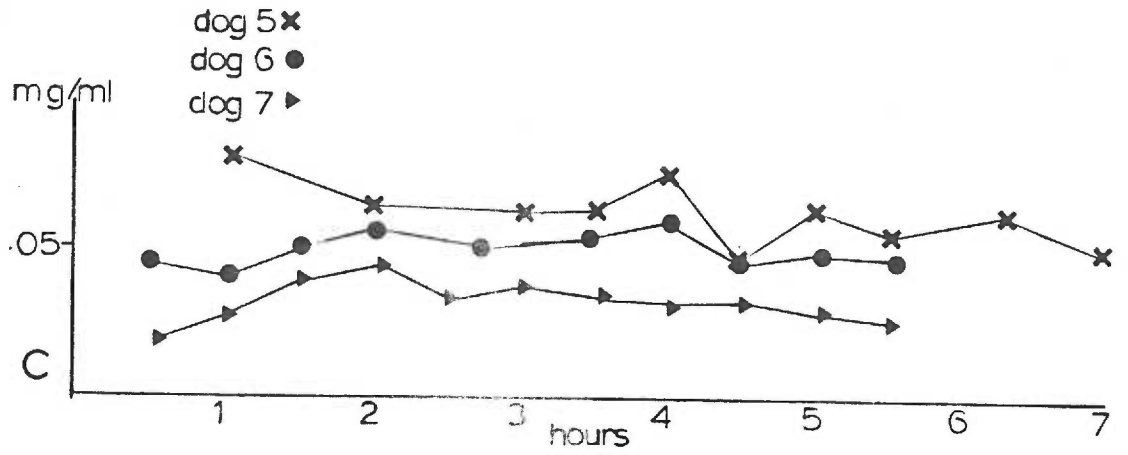


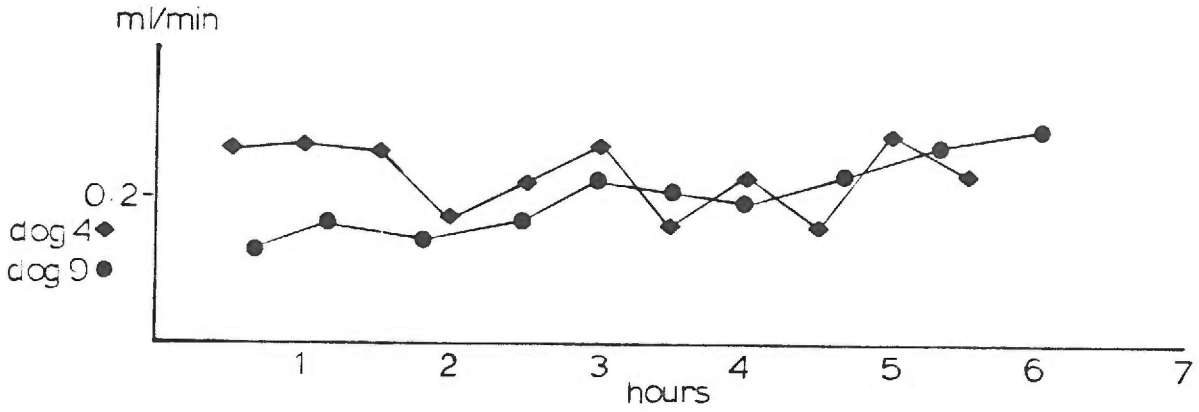
FIGURE 2 Urine flow is reported in ml/min. per 100 grams of kidney weight over the course of the experiment.

A = control dogs (#4 and #9) and 10 μg./kg./min. dopamine (#5, #6, #7)

B = 1.05 μg./kg./min. dopamine

FIGURE 2

URINE FLOW (A)



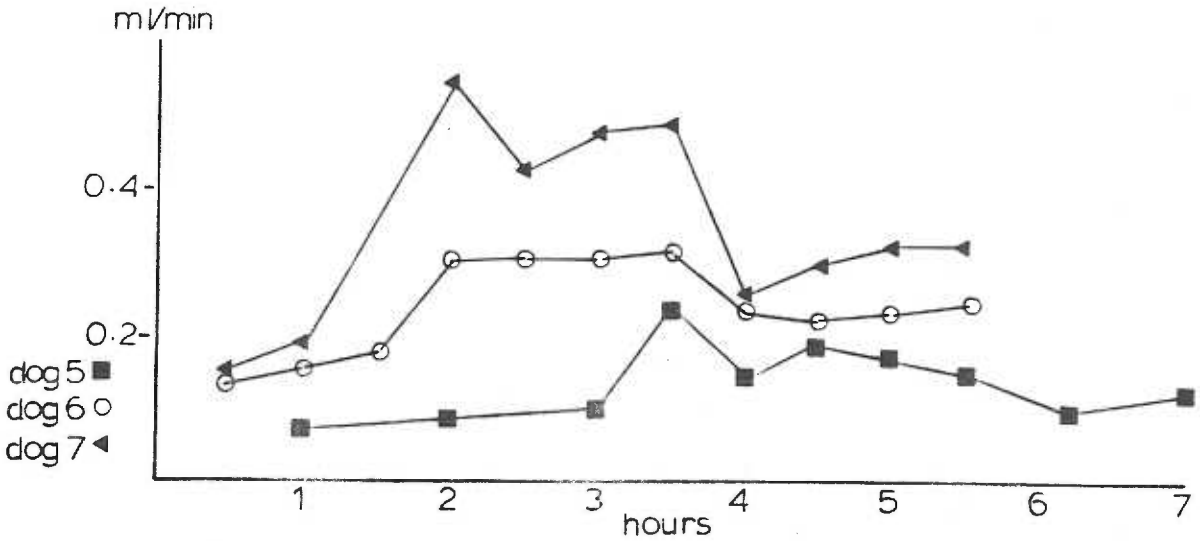
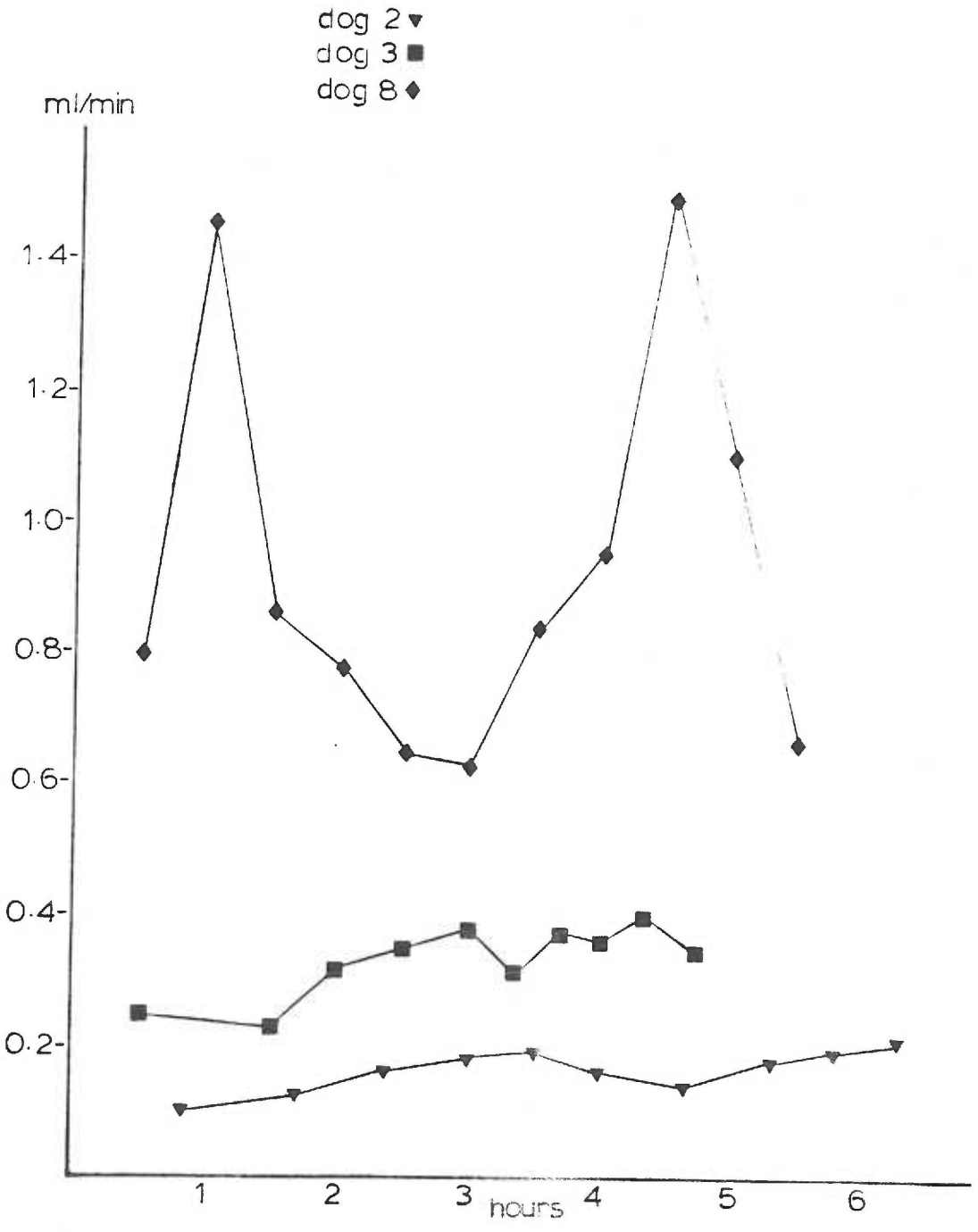


FIGURE 2 (continued)

URINE FLOW (B)



Glomerular Filtration Rate (GFR) (Refer to Figure 3)

Glomerular filtration rate increased for all animals over the course of the experiments. Mean values of GFR for control animals ranged from 26 ml./min. to 112.8 ml./min. per one hundred grams of kidney weight. Both control dogs had a rise of GFR during the period equivalent to dopamine infusion. In addition, GFR continued to rise in the post-infusion periods.

Dogs receiving dopamine at a rate of 1.05 µg./kg./min. showed an increased GFR over that observed during control periods. Glomerular filtration rates ranged from 39.5 ml./min. to 60.8 ml./min. per one hundred grams of kidney weight. However, none of the changes were significantly different from control values.

Glomerular filtration rate increased significantly over control values in two dogs during dopamine infusion of 10 µg./kg./min. In all dogs receiving 10 µg./kg./min. post-infusion GFRs were greater than those observed during dopamine infusion. The changes were significant in one animal (#6).

Plasma Phosphate Concentrations (P_{PO_4})

In general plasma phosphate concentrations did not appear to change during the experiment (Figure 4). Phosphate concentrations ranged from 4.2 mg% to 9.0 mg% among the animals. However, statistical analysis shows that the P_{PO_4} increased significantly in all animals except dog #2. See Table I.

The control values for P_{PO_4} are consistent with plasma phosphate concentrations found in dogs housed in the animal care department of this institution.*

* Personal communication from the doctor of veterinary medicine.

FIGURE 3 Glomerular Filtration Rate is reported per 100 grams of kidney weight. Mean values are shown for each group of dogs.

A = control animals

B = 1.05 µg./kg./min. dopamine

C = 10 µg./kg./min. dopamine

The letters in the bars refer to control (C), infusion (D), and post-infusion (P) periods.

FIGURE 3

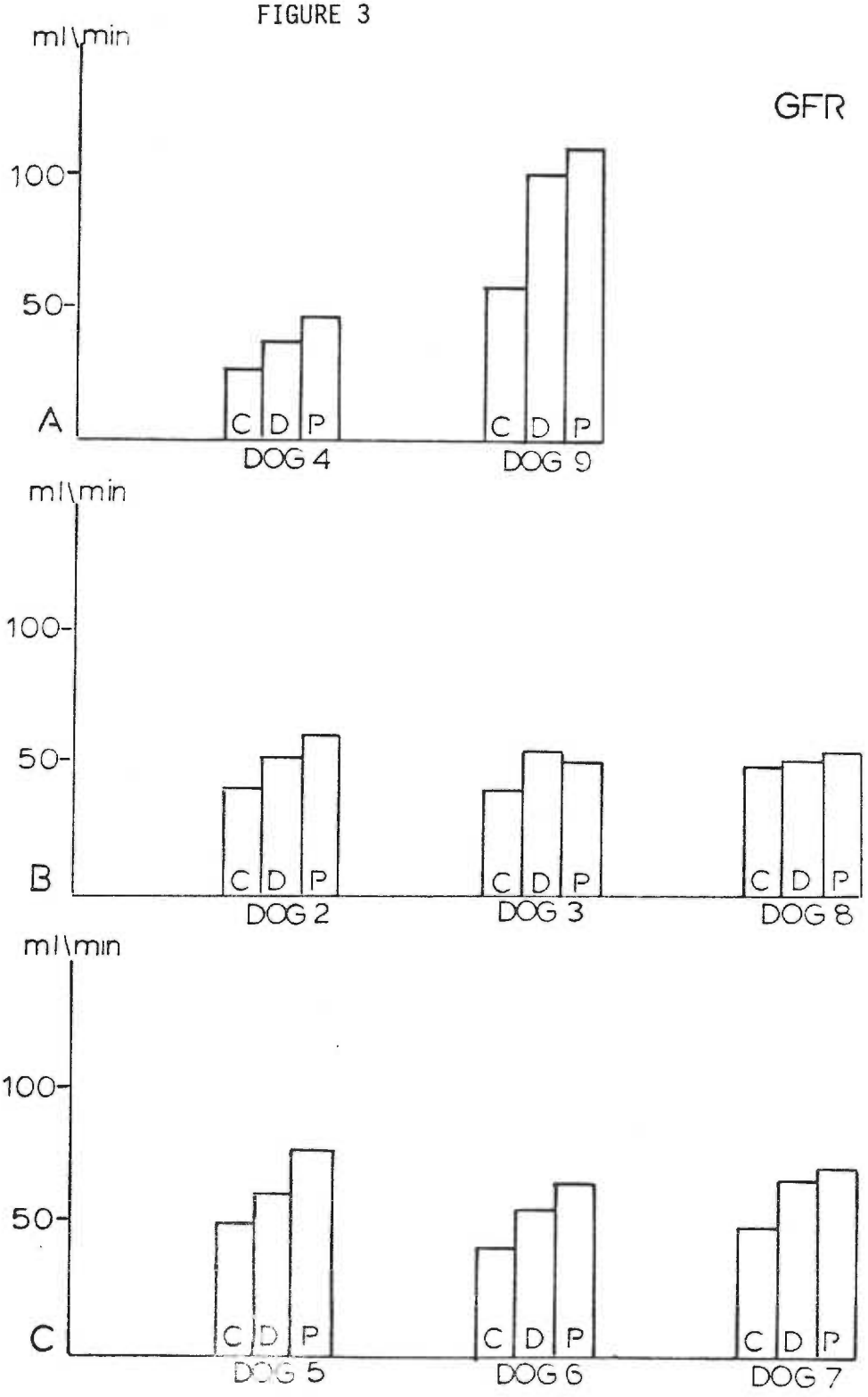
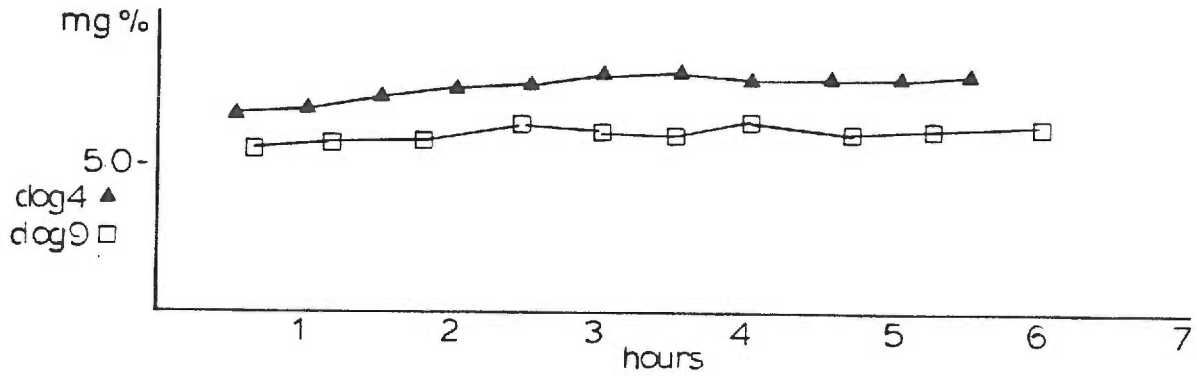
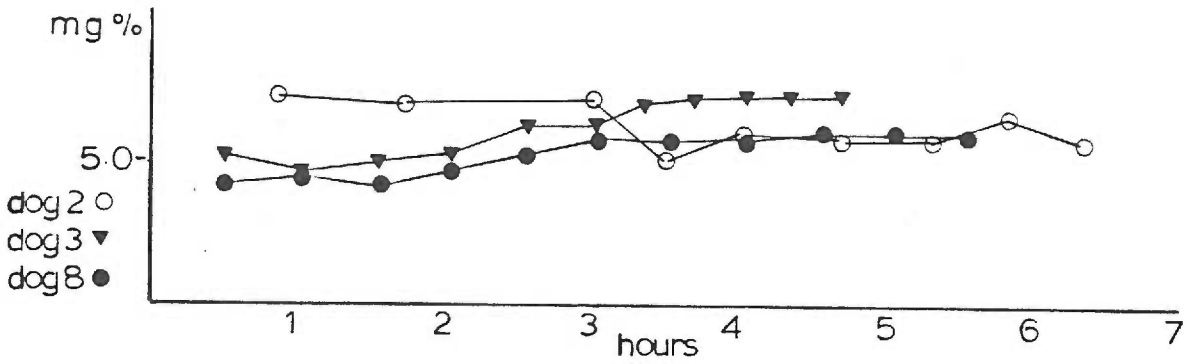

ml\min
GFR
100
50
A
C D P
DOG 4
DOG 9
ml\min
100
50
B
C D P
DOG 2
DOG 3
DOG 8
ml\min
100
50
C
C D P
DOG 5
DOG 6
DOG 7

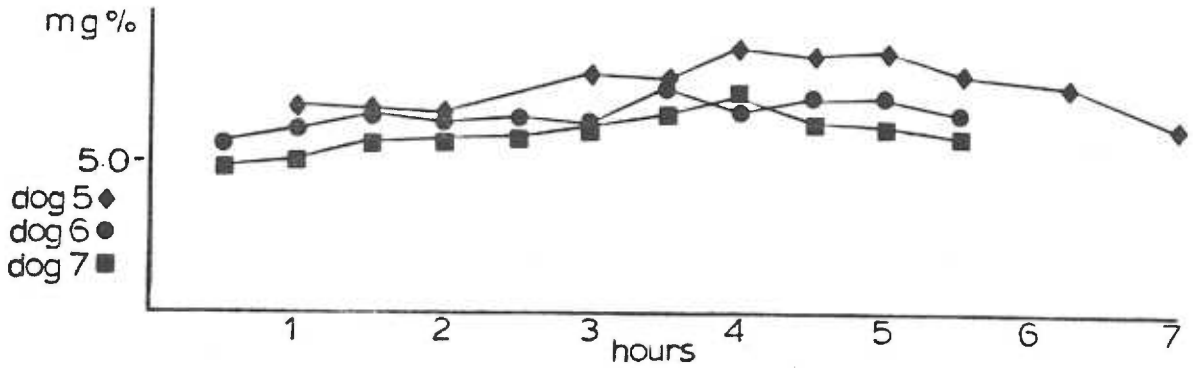
FIGURE 4 Plasma phosphate concentrations are reported in mg % for each group of dogs over the course of the experiment. Control animals (#4 and #9) are shown at top. Dogs receiving 1.05 µg./kg./min. are shown in the middle figure. The bottom figure shows dogs receiving 10 µg./kg./min.

FIGURE 4

P_{PO_4}







Filtered Load of Phosphate (L_{PO_4}) (Refer to Figure 5)

Control animals showed a rise in L_{PO_4} during infusion periods over control periods. The L_{PO_4} values continue to rise during the post-infusion periods. Values for L_{PO_4} during infusion were 155% and 189% of control values. During post-infusion periods, L_{PO_4} values were 190% and 205% of control values.

Filtered load of phosphate increased over control values during infusion of dopamine at 1.05 µg./kg./min. Values ranged from 122% to 175% of control levels during infusion. A variable response was observed during post-infusion periods. Two dogs showed a small rise in L_{PO_4} over infusion values. One animal had a small decline in L_{PO_4} from the infusion value, but remained above control levels.

Filtered load of phosphate increased over control values during infusion of dopamine at 10 µg./kg./min. This increment ranged from 150% to 262% of control. Post-infusion values ranged from 150% to 288% of control. Two animals showed a rise in post-infusion values. One animal showed no change in L_{PO_4} in post-infusion periods.

Phosphate Clearance (C_{PO_4}) (Refer to Figure 6)

In all animals C_{PO_4} increased during the course of the experiment. Control animals did not show a uniform change in C_{PO_4}. Clearance of phosphate increased in both dogs during the infusion period. However, in the post-infusion period one dog had a rise and one dog a decline in C_{PO_4} compared to the infusion period.

Clearance of phosphate increased in all dogs receiving 1.05 µg./kg./min. of dopamine. The increase ranged from 134% to 251% over control values. During the post-infusion period C_{PO_4} continued to increase over that observed with dopamine infusion and ranged from 183% to 371%

FIGURE 5 Filtered Load of Phosphate is reported as percent of control values for each group of dogs. Mean values for each period per 100 grams of kidney weight were used to compute values shown.

A = control dogs

B = 1.05 µg./kg./min. dopamine

C = 10 µg./kg./min. dopamine

The letters in the bars refer to control (C), infusion (D), and post-infusion (P) periods.

FIGURE 5

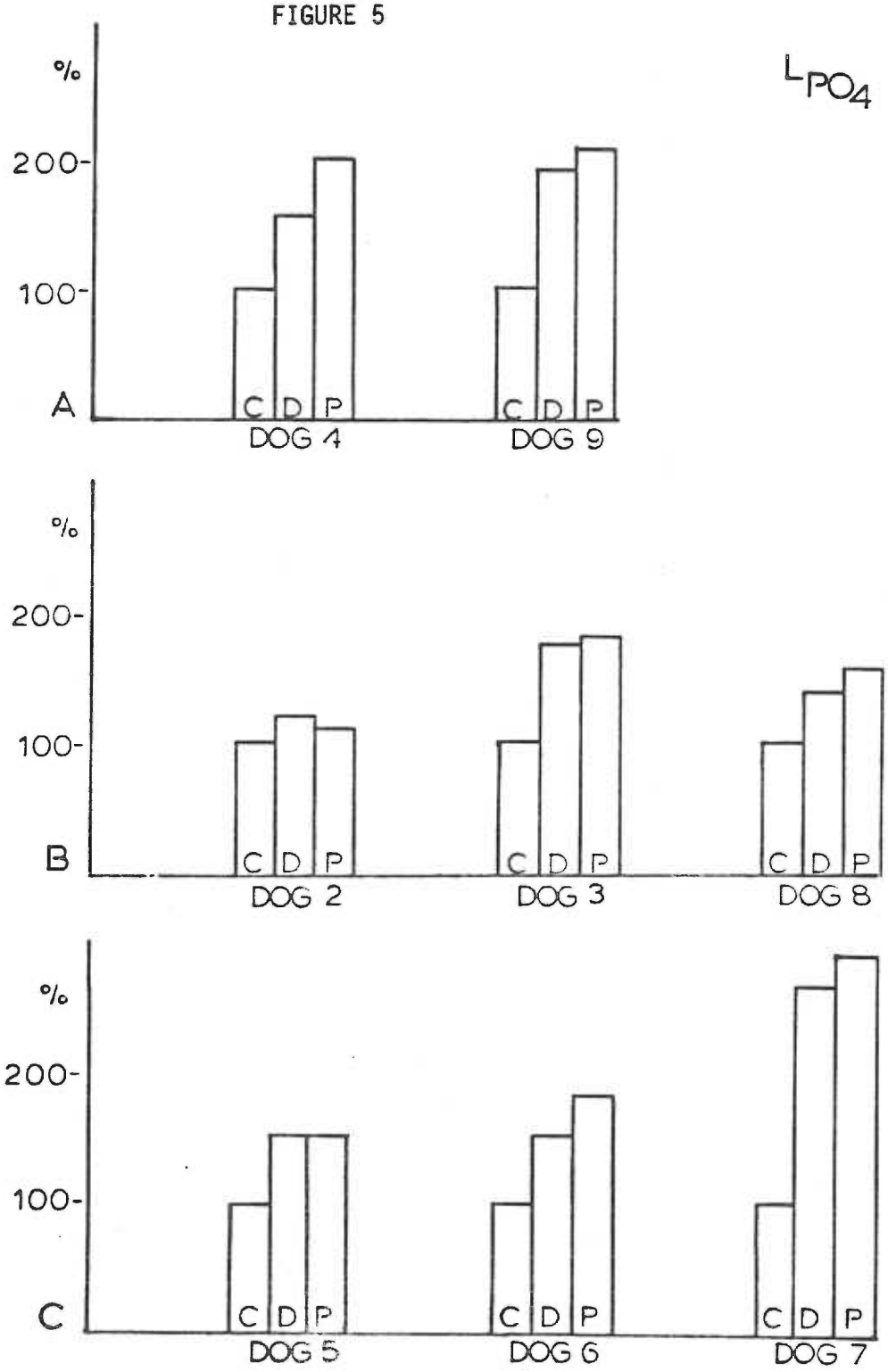

%
200-
100-
A
C D P
DOG 4
DOG 9
L PO4
%
200-
100-
B
C D P
DOG 2
DOG 3
DOG 8
%
200-
100-
C
C D P
DOG 5
DOG 6
DOG 7

FIGURE 6 Clearance of phosphate is reported as per cent of control values for each group of dogs. Mean values per 100 grams of kidney weight were used to compute values shown.

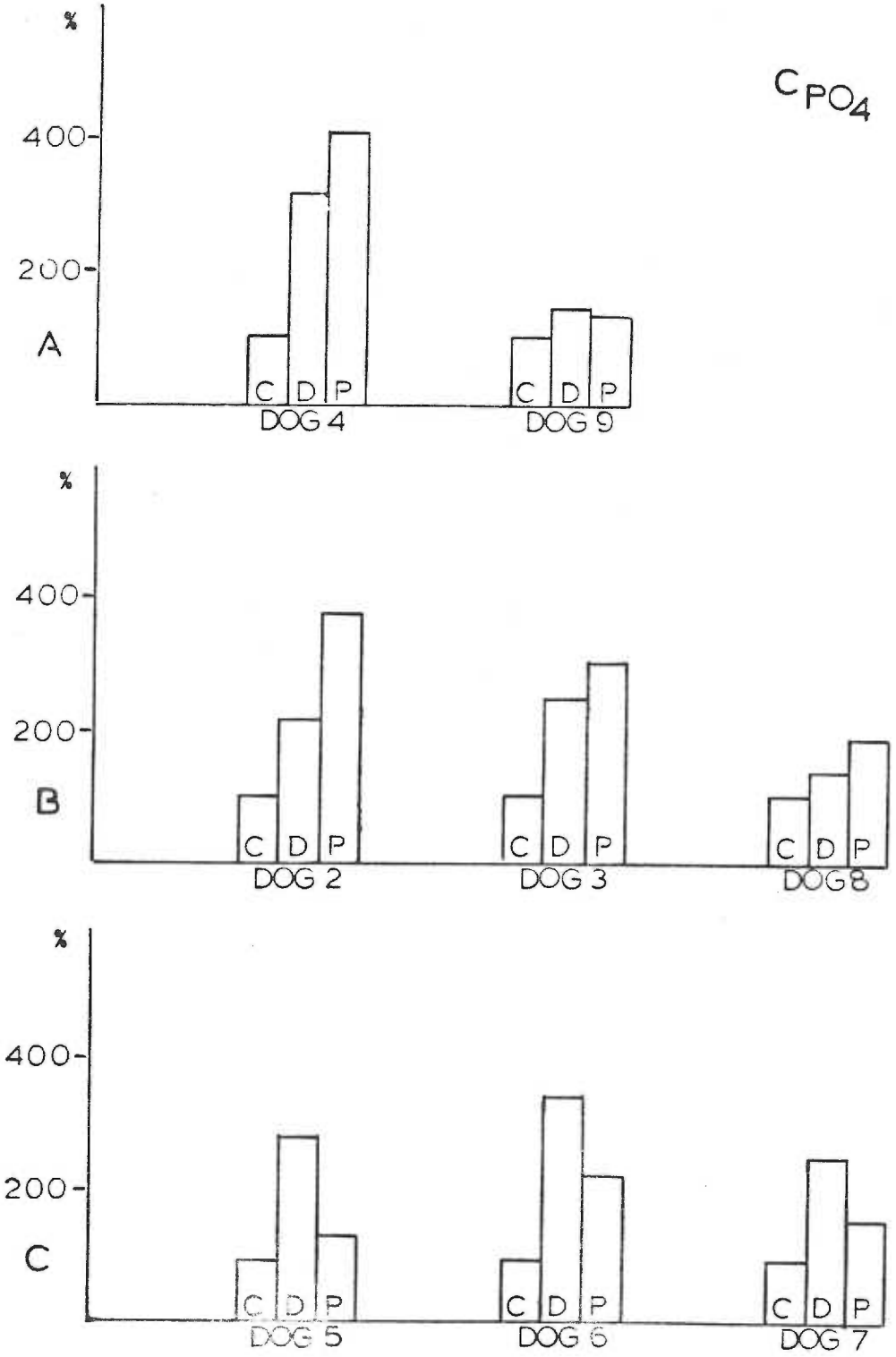
A = control animals

B = 1.05 µg./kg./min. dopamine

C = 10 µg./kg./min. dopamine

The letters in the bars refer to control (C), infusion (D), and post-infusion (P) periods.

FIGURE 6


%
400
200
A
C D P
DOG 4
DOG 9
C_{PO_4}
%
400
200
B
C D P
DOG 2
DOG 3
DOG 8
%
400
200
C
C D P
DOG 5
DOG 6
DOG 7

of control values.

A dopamine infusion of 10 µg./kg./min. was associated with a rise in C_{PO_4} in all dogs. Values during the dopamine infusion ranged from 257% to 354% over control values. Post-infusion values for C_{PO_4} declined from those obtained during drug infusion. However, C_{PO_4} did not return to control levels. Values for post-infusion C_{PO_4} ranged from 151% to 240% of control values.

Excretion Rate of Phosphate (Refer to Figures 7 and 8)

All animals showed a rise in phosphate excretion rate during the course of the experiment.

Reabsorption of Phosphate (T_{PO_4}) (Refer to Figure 9)

In the control animals phosphate reabsorption increased over control rates during infusion and post-infusion periods. Post-infusion values were greater than infusion values. Values of T_{PO_4} were increased during infusion to 131% and 197% of control. Post-infusion values increased to 169% and 233% of control.

Dogs given dopamine at a rate of 1.05 µg./kg./min. also showed a rise in T_{PO_4} over control levels. Values were 109% to 170% over control values. Post-infusion T_{PO_4} values ranged from 84% to 170% of control values. There was a decline in T_{PO_4} in two dogs (#2 and #8) and one dog (#3) had no change in T_{PO_4} in the post-infusion period.

Dogs receiving dopamine infusions at a rate of 10 µg./kg./min. had a pattern of change in T_{PO_4} similar to that shown by the control animals. There is a consistent rise in T_{PO_4} over control values during dopamine infusion. Post-infusion periods show a rise of T_{PO_4} over dopamine infusion periods.

FIGURE 7 Excretion rate of phosphate is shown as mg./min. per 100 grams of kidney weight over the course of the experiment.

A = control animals (bottom figure)

B = 1.05 μg./kg./min. dopamine (middle figure)

C = 10 μg./kg./min. dopamine (top figure)

FIGURE 7

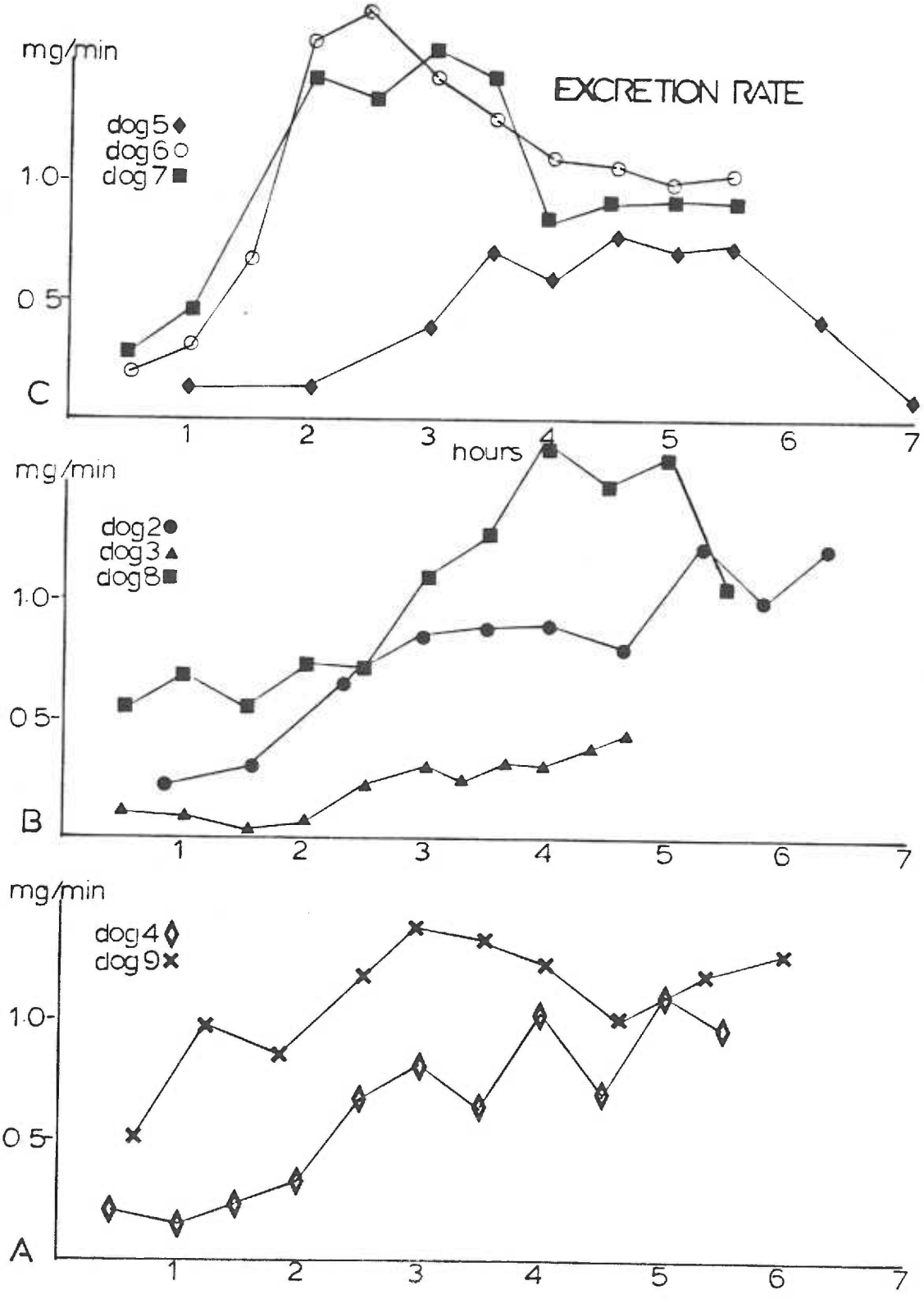

mg/min
EXCRETION RATE
dog5◆
dog6○
dog7■
1·0-
0·5-
C
1
2
3
4
5
6
7
hours
mg/min
dog2●
dog3▲
dog8■
1·0-
0·5-
B
mg/min
dog4◊
dog9×
1·0-
0·5-
A

FIGURE 8 Excretion rate of phosphate is shown as percent of control values. Mean values for each period per 100 grams of kidney weight were used to compute values shown.

A = control animals

B = 1.05 µg./kg./min. dopamine

C = 10 µg./kg./min. dopamine

The letters in the bars refer to control (C), infusion (D), and post-infusion (P) periods.

FIGURE 8

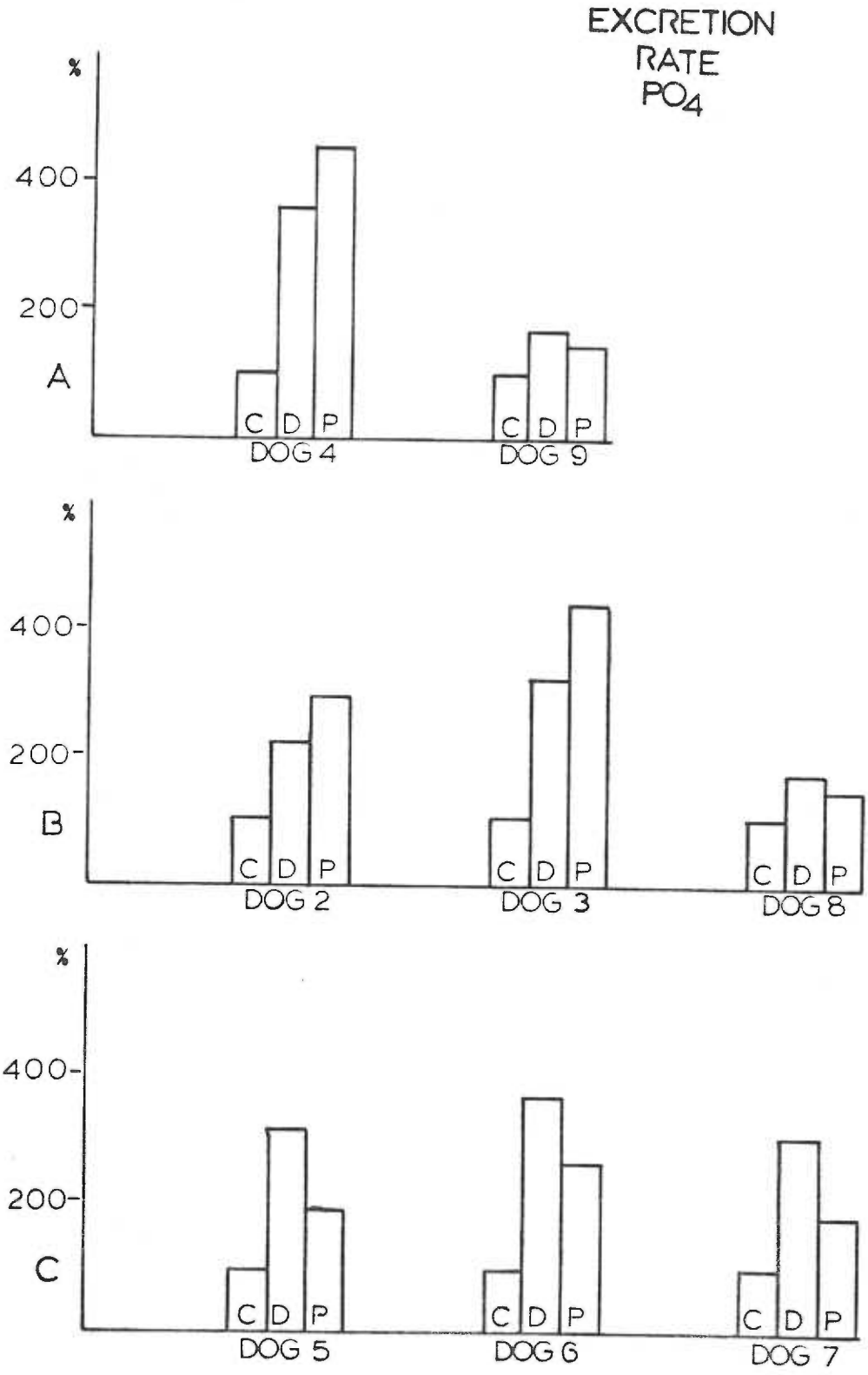

EXCRETION
RATE
PO4
%
400
200
A
C D P
DOG 4
C D P
DOG 9
%
400
200
B
C D P
DOG 2
C D P
DOG 3
C D P
DOG 8
%
400
200
C
C D P
DOG 5
C D P
DOG 6
C D P
DOG 7

FIGURE 9 Reabsorptive rate of phosphate is shown as per cent of control values. Mean values for each period per 100 grams of kidney weight were used to compute values shown. The discontinuous bar for #7 post-infusion (P) represents a value above limits of the graph (323%).

A = control animals

B = 1.05 µg./kg./min. dopamine

C = 10 µg./kg./min. dopamine

The letters in the bars refer to control (C), infusion (D), and post-infusion (P) periods.

FIGURE 9

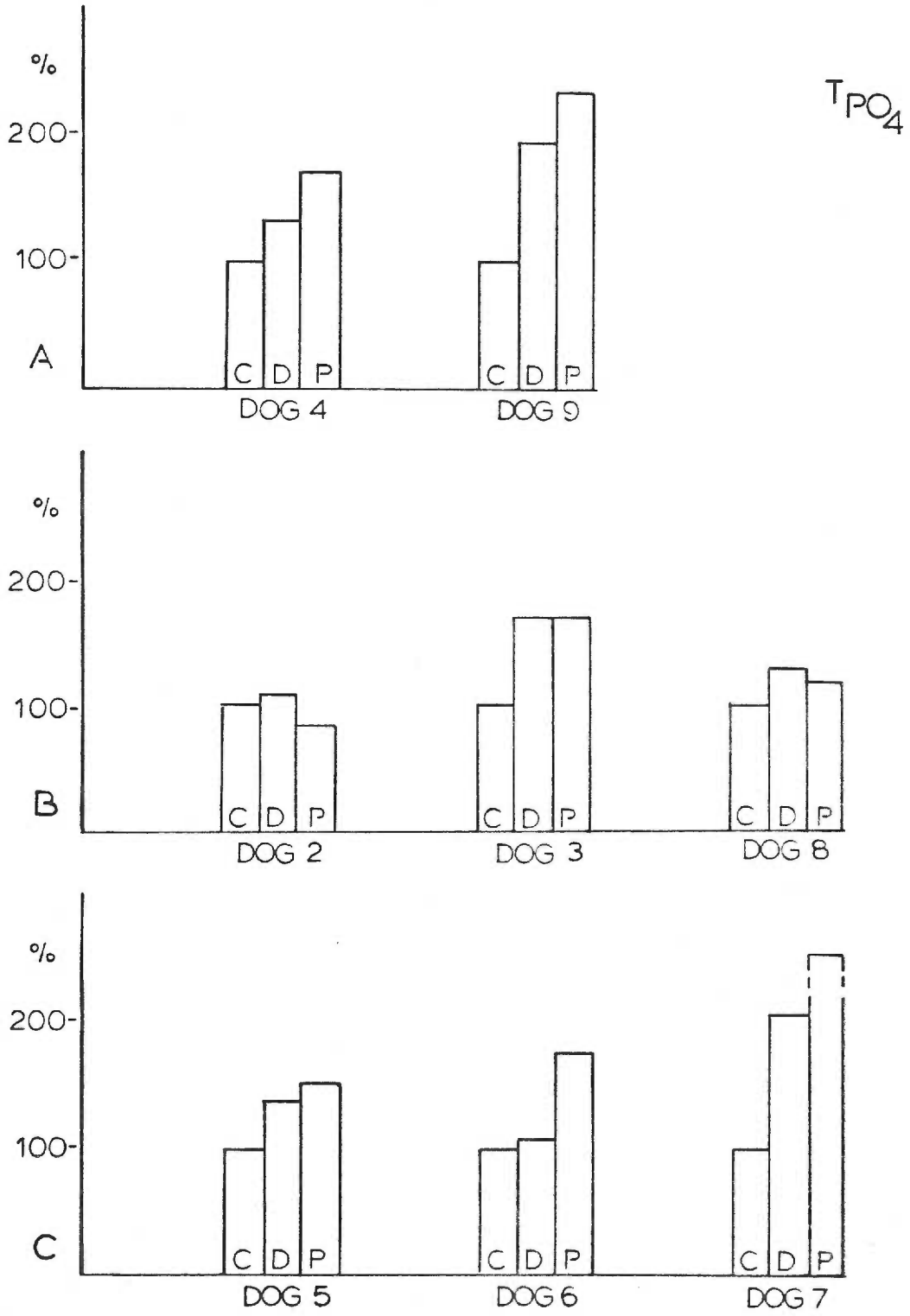

%
200-
100-
A
C D P
DOG 4
DOG 9
T_{PO_4}
%
200-
100-
B
C D P
DOG 2
DOG 3
DOG 8
%
200-
100-
C
C D P
DOG 5
DOG 6
DOG 7

FIGURE 10 Fractional Excretion of Phosphate. Mean values per 100 grams of kidney weight are shown for each period during the experiment.

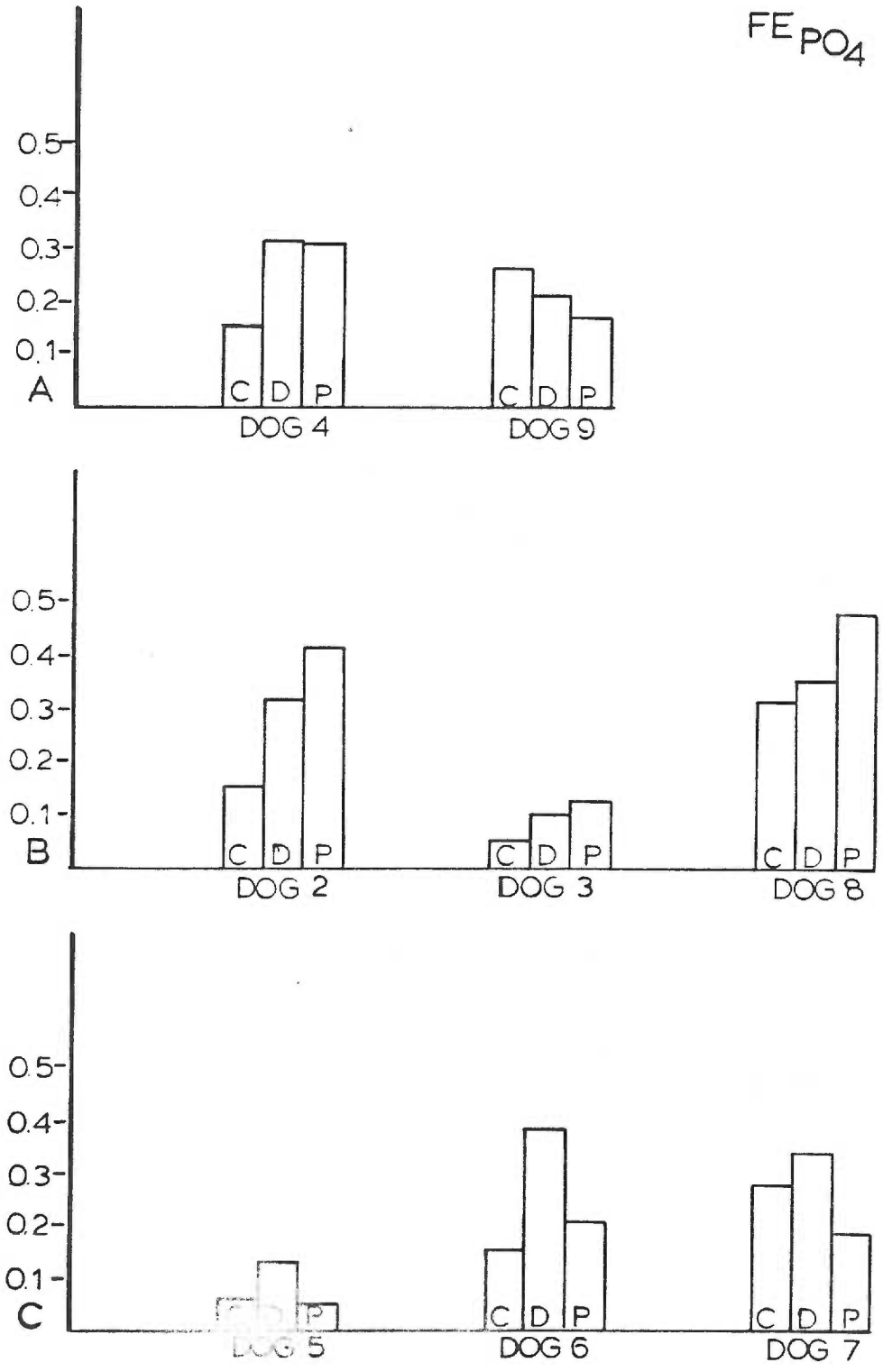
A = control animals

B = 1.05 µg./kg./min. dopamine

C = 10 µg./kg./min. dopamine

The letters in the bars refer to control (C), infusion (D), and post-infusion (P) periods.

FIGURE 10


FE PO4
0.5
0.4
0.3
0.2
0.1
A
C D P
DOG 4
DOG 9
B
DOG 2
DOG 3
DOG 8
C
DOG 5
DOG 6
DOG 7

Fractional Excretion of Phosphate (FE_{PO_4}) (Refer to Figure 10)

Control animals showed a variable FE_{PO_4}. One animal had an increase of FE_{PO_4} during the infusion period. Post-infusion values were similar to infusion values. The second control dog had a decline in FE_{PO_4} throughout the experiment. However, this is not a significant reduction.

With dopamine infused at low doses (1.05 μg./kg./min.) FE_{PO_4} increased over control values in all dogs. Post-infusion FE_{PO_4} increased in all dogs over dopamine infusion values. However, these increments are not statistically different from infusion values.

A variable response in FE_{PO_4} was also noted in dogs given dopamine at a rate of 10 μg./kg./min. All dogs showed a significant rise in FE_{PO_4} over control values when dopamine is infused. In two dogs post-infusion FE_{PO_4} declined significantly from drug infusion values.

Discussion

In general the results obtained from this study are different from those reported by Cuche, et al. (1976). This is especially true for fractional excretion of phosphate and plasma phosphate concentrations.

Fractional excretion of phosphate was increased significantly during and after dopamine infusion in the study reported by Cuche, et al. (1976). However, in this study the pattern of change in fractional excretion was variable and depended on the dose of dopamine (Figure 10).

The site of dopamine infusion was different in this experiment from the study by Cuche, et al. (1976). In the study reported here dopamine was infused in the femoral vein. Cuche, et al. (1976) infused dopamine into one renal artery (1.05 µg./kg./min.). Regardless of the site of infusion the effects of dopamine on the kidney should be the same for comparable doses. In this study dosages of dopamine entering the renal artery were lower than those used by Cuche, et al. (1976). These lower doses more closely mimic the clinical use of the drug. The dose of dopamine given via the renal artery by Cuche, et al. (1976) is approximately 30 to 50 times the amount the kidney would receive if standard intravenous clinical doses were given.

In this study, the dogs receiving a dopamine infusion of 10 µg./kg./min. had a change in fractional excretion comparable to the experiments reported by Cuche, et al. (1976). The fractional excretion rose during the infusion period and decreased after the infusion period. The post-infusion value though decreased over the infusion period is greater than the control value (Figure 10). The dogs receiving dopamine at a rate of 1.05 µg./kg./min. showed an increase in values during both infusion and post-infusion periods over those obtained during control periods. Thus dose may be an important factor in determining the changes in fractional excretion of phosphate with dopamine infusion.

Plasma phosphate concentrations did not change significantly in the experiment reported by Cuche, et al. (1976). However, in the study reported here the plasma phosphate concentrations increased

significantly in all but one dog (Table 1 and Figure 4). The plasma phosphate concentrations rose in the control animals as well as those receiving dopamine.

The concentration of phosphate in the plasma at any point in time depends on the balance between input or mobilization of phosphate from storage, absorption, and output of phosphate by the kidney and gastrointestinal tract. If plasma phosphate increases, then the increment is due to increased input relative to output.

The plasma phosphate concentration in this study may have increased as a result of parathormone stimulation (input from storage). Sodium excretion increases as a result of infusion of dopamine (Davis, et al., 1968; MacDonald, et al., 1974; McNay, et al., 1963) and saline volume expansion (Steele, 1970). Urinary sodium excretion results in an obligatory increased urinary calcium loss (Schneider, et al., 1973). It is possible that loss of calcium results from the volume induced natriuresis which could then act as a stimulus for increased parathormone secretion. Parathormone is associated with decreased sodium reabsorption in the proxmal tubule and increased phosphate excretion. In addition, phosphaturia is also associated with saline infusion (Schneider, et al., 1973).

Phosphate excretion rate and clearance of phosphate increased in all animals during the infusion period (Figure 6, 7, 8). This increase in urinary phosphate excretion may be due to an increased plasma phosphate concentration or an increased filtered load. The length of the experiments was too short to determine the duration of the phosphaturia.

Glomerular filtration rate did not change significantly during the course of the experiment reported by Cuche, et al. (1976). In the present study GFR increased over control values and remained elevated over control values during the post-infusion period (Table 1 and Figure 3). In the report by Cuche, et al. (1976) the GFR returned to control values after infusion of dopamine was discontinued.

An increase in GFR (Figure 3) combined with an increasing plasma phosphate concentration causes an increase in filtered load of phosphate. This increase in filtered load may account for the increase in phosphate excretion. In addition, the rate of reabsorption of phosphate also increased. However, this increment in reabsorptive rate did not match the increment in filtered load, hence phosphate excretion rate increased.

The reabsorptive rate of phosphate continued to increase over the course of the experiment in all dogs except numbers 2 and 8. Filtered load of phosphate also increased over the course of the experiment in all but one animal (#2). As filtered load of phosphate increased the rate of reabsorption of phosphate increased indicating that a maximum rate of transport for phosphate was not reached in these experiments. This conclusion is supported by the fact that the ratio of filtered load to rate of reabsorption was always less than 2 (Table 1). Therefore the transport maximum for phosphate was not reached (Pitts and Alexander, 1944).

Since the control animals as well as the animals which received dopamine had increased phosphaturia over the course of the experiment it is difficult to accept the hypothesis that dopamine causes phosphaturia. The report by Cuche, et al. (1976) did not contain results in

control animals (dogs receiving no dopamine).

Dogs have a wide variability in urinary phosphate excretion during the day as well as with prolonged anesthesia. Perhaps this variability in canine phosphate excretion accounts for the increases observed in this experiment as well as those reported by Cuche, et al. (1976). Dogs, in fact, may not be a good model for experiments involving endogenous phosphate excretion because of the wide variability observed.

Since rapid extracellular fluid volume expansion has been reported to cause phosphaturia and lower Tm for phosphate in man and dogs (Steele, 1970), it is important that the degree of extracellular volume expansion be ascertained. If the fluid infusion used in this study had been normal saline, the dogs' extracellular volume would have been expanded by a mean value of 17% (SD $\pm$ 5%). This calculation does not include insensible loss. However, the majority of the fluid given was 5% dextrose with 0.2% sodium chloride at a rate of 2 ml./min. While this was not a rapid infusion nor was it entirely normal saline, volume expansion may still have caused increased phosphate excretion in these experiments.

CHAPTER IV

Summary, Conclusions, and Suggestions for Further Study

In this study six dogs were given dopamine to assess the effects of this drug on urine and plasma phosphate concentrations. Three dogs were given 1.05 µg./kg./min., three dogs were given 10 µg./kg./min., and two dogs were given only saline with no dopamine.

An increased urine phosphate concentration was evident in all experimental animals including controls. This increased concentration of phosphate in the urine was evident before and after the administration of dopamine. The duration of this increased urine phosphate concentration could not be determined over the course of these experiments.

Plasma phosphate concentrations were increased slightly in all experiments except one.

The mechanisms involved in the increased concentrations of urine and plasma phosphate with this experiment are not known. Possible explanations are:

1. Parathormone may have been stimulated to increase urinary phosphate concentration and mobilize phosphate from storage to increase plasma phosphate concentrations.

2. Fluid infusion as an artifact of the method may have caused urinary phosphate concentrations to increase.

3. Dopamine induced natriuresis may be associated with phosphaturia.

In further studies the effect of dopamine could be determined on urine and plasma concentrations of phosphate, sodium, calcium, and PTH after phosphate loading of the experimental animal.

Patients receiving dopamine in clinical situations could be evaluated for evidence of phosphaturia. Serum phosphate, calcium and PTH concentrations should be examined.

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APPENDIX A

Chemical Methods

Inorganic Phosphate in Plasma and Urine

Procedure: Plasma (1:5.5 dilution)

1. Place 1.0 ml. plasma in a clean test tube
2. Add 2.5 ml. deionized water
3. Add 2.0 ml. 20% trichloroacetic acid, stopper and mix by shaking
4. Centrifuge at full speed for 10 minutes
5. Aspirate supernatant with a Pasteur pipette and place in a clean test tube
6. Again centrifuge the clear supernatant at full speed for 10 minutes to remove any white precipitated protein
7. From this clear supernatant, pipette 2 ml. into a glass test tube in duplicate
8. Add 3 ml. deionized water
9. Add 1 ml. Acid Molybdate Solution (Ammonium molybdate 1.25 gm./100 ml. in 2.5 N sulfuric acid)
10. Add 0.25 ml. Fiske-SubbaRow Reducer (Sigma)
11. Mix by shaking. Allow to stand for 5 minutes for color development
12. Read within 10 minutes of color development on a spectrophotometer at 660 nm absorbance. The blank is set at zero.

Urine

1. Urine at 1:100 or 1:150 dilution with deionized water can be used.
2. Proceed as with plasma beginning at step 7.

Inorganic Phosphate (continued)

Standards

1. Place 5 ml. of each standard solution in a separate test tube. For blank use 5 ml. deionized water. All standards are prepared in duplicate
2. Add 1.0 ml. Acid Molybdate Solution
3. Add 0.25 ml. Fiske and SubbaRow Reducer (Sigma)
4. Mix by shaking, allow to stand 5 minutes for color development
5. Set blank at zero at 660 nm absorbance on a spectrophotometer. Read standards within 10 minutes of color development
6. Read urine and plasma results from standard curve, then multiply by dilution factor

Principle of Procedure

The Fiske and SubbaRow method of inorganic phophate determination was introduced in 1925. Since that time modifications of the method have been made to overcome various technical problems. One of the advantages of the method described is the linearity of the standard curve throughout the clinically significant range.

Plasma is treated with trichloroacetic acid to remove protein and lipid phosphorus. When the acid molybdate solution is added to the supernatant a phosphomolybdate forms. A reducing agent (a mixture of sodium bisulfite, sodium sulfite, and 1-amino-2-napththol-4-sulfonic acid) is added to the phosphomolybdate and forms a phosphomolybdenum blue complex. The phosphate concentration is proportional to the intensity of the color development (Sigma Chemical Company, Tietz).

Inorganic Phosphate (continued)

Since plasma inorganic phosphate values decline with time as they are allowed to remain in contact with red blood cells, the specimens must be immediately centrifuged and the plasma separated (Carothers, Kurtz, and Lemann, 1976). Once the plasma is separated, the phosphate is stable under refrigeration for one week (Sigma Chemical Company).

Phosphate detergents used in washing glassware may react in the analysis. Glassware must be thoroughly rinsed with deionized water.

APPENDIX B

Inulin Determination (Method of Heyrovsky)

Procedure for Plasma (1:4 dilution)

1. Pipette 1 ml. of plasma into a test tube
2. Add 3 ml. of 20% trichloroacetic acid, cover, and mix by shaking
3. Allow to stand for 5 minutes, then centrifuge at full speed for 10 minutes
4. Place 1 ml. of the clear supernatant in a clean test tube. All samples are done in duplicate
5. Add 0.2 ml. of indole-3-acetic acid (500 mg. in 100 ml. 95% ethanol)
6. Add 7 ml. of 37% hydrochloric acid, cover, mix by shaking
7. Place in a 37^{o}C water bath for exactly one hour
8. Remove from water bath, cool under running tap water
9. Read in a spectrophotometer at 528 nm absorbance. Set blank at zero

Procedure for Urine

Urine at 1:100 or 1:150 dilution with deionized water may be used. Treat as for plasma beginning at step 4.

Standards

Standards at 1,2,5,7.5, and 10 mg./100 ml. solutions are made by diluting the inulin stock standard solution (50 mg./100 ml.) with deionized water. Duplicate samples are treated as for plasma beginning at step 4.

Inulin Determination (continued)

Principle

The treatment of inulin with indole-3-acetic acid and concentrated hydrochloric acid at a controlled temperature results in the color development. The combination of heat and acid hydrolyze inulin to fructose to form a purple complex (Sunderman and Sunderman, 1969).

The range of sensitivity of this method is 0.01 to 0.1 mg./ml.

AN ABSTRACT OF THE THESIS OF

BARBARA S. LENFESTY

For the MASTER OF NURSING

Date Receiving the Degree: June 8, 1979

Title: Changes in Plasma and Urine Phosphate Concentrations Induced by Dopamine

Approved: [redacted]
Jack L. Keyes, RN.D. Thesis Advisor

In this study eight dogs were given dopamine to assess the effects of this drug on urine and plasma phosphate concentrations. Three dogs were given 1.05 µg./kg./min., three dogs were given 10 µg./kg./min., and two dogs were given only saline with no dopamine.

An increased urine phosphate concentration was evident in all experimental animals including controls. The increased concentration of phosphate in the urine was evident before and after the administration of dopamine. The duration of this increased urine phosphate concentration could not be determined over the course of these experiments.

Plasma phosphate concentrations were increased slightly in all experiments but one.

It cannot be concluded from this experiment that dopamine causes an increase in urinary phosphate excretion.