CHEMICAL STUDIES OF THE BLOOD IN HIGH INTESTINAL OBSTRUCTION : *I. The Distribution of Phosphorus and Intracellular Changes*

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CHEMICAL STUDIES OF THE BLOOD IN HIGH INTESTINAL OBSTRUCTION[1]

I. The Distribution of Phosphorus and Intracellular Changes

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This paper presents an investigation of the effects of experimental high intestinal obstruction in dogs, with particular emphasis on changes observable in the distribution of phosphorus in the blood. The experiments were undertaken as a part of a series of studies of phosphorus distribution in the blood of man and experimental animals in a variety of pathologic conditions. In dogs, marked changes of the distribution of phosphorus were observed following pyloric and mid-duodenal obstruction; an attempt is here made to correlate these changes with the variations in the blood electrolytes commonly observed in intestinal obstruction.

That experimental high intestinal obstruction brings about or is accompanied by marked changes in the blood has been demonstrated in many investigative studies of this problem reported during the past few years. The more important of these changes are: (1) Concentration of the blood indicated by an increased relative cell volume, cell count and serum protein content. (2) Increased nonprotein nitrogen, mostly urea. (3) Low chloride, generally conceded to be due to losses of Cl by secretion into the stomach or the obstructed gut, whence it is vomited or fails to be reabsorbed. (4) High CO_2 content, increased in compensation for the loss of chloride except when there is an accumulation of acids (such as lactic), or when there is great loss of base as well as chloride in the vomitus. It was first pointed out by Gamble and Ross (1925) that the reduction of the ionic content of the body fluids by the losses of electrolytes (base as well as chloride) is the significant factor in the rapid general dehydration of the body that follows pyloric obstruction.

Reviews of the many investigations of intestinal obstruction may be found in the articles by Ellis (1922), Gatch, Trusler and Ayers (1927), Cooper (1928), McVicar and Weir (1929) and in three papers of a sym-

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posium on the subject by Foster (1928), Orr and Haden (1928), and McIver and Gamble (1928).

In the present study most attention is directed to the organic acid-soluble phosphorus of the blood, designated by Kay and Byrom (1927) "Ester-P." The nature of the compounds determined in this fraction is not well known, but they are generally believed to be mainly hexose- and glycerophosphoric acid esters (Goodwin and Robison (1924)). In a review of the literature on the distribution of phosphorus in the blood, Peters and Van Slyke (1931) have discussed the nature of the phosphoric acid esters and, pertinent to the problems to be discussed here, the rôle these compounds may play as non-diffusible anions in the cells. They state that part of the alkali in the cells is certainly neutralized by the organic compounds of phosphoric acid, and that there are approximately 30 mM. of organic phosphate present in the erythrocytes; also, that the available data indicate that the known phosphoric acid compounds of the cells bind about one equivalent of alkali per mol of phosphoric acid. Although the organic phosphorus compounds of the cells are known to be considerably altered (decreased as well as increased) in several different pathologic conditions, the significance of such changes for the acid-base equilibrium of the blood has not apparently been investigated to any considerable extent.

Many complete determinations of the electrolytes in the blood plasma or serum of man and experimental animals in normal and in various pathologic states have been reported, but few figures are available from which one may visualize the chemical structure of the cells in a manner at all comparable to that of the plasma. In the previous chemical studies of the blood in intestinal obstruction, attention has been directed almost exclusively to changes observed in the plasma, but Haden and Orr (1926) and White and Bridge (1927) demonstrated losses of chloride from various body tissues comparable to the losses of chloride from the blood. Hastings, Murray and Murray (1921) reported the results of three experiments in which they determined the Na, K, Mg, Cl and P in both serum and cells of the blood of dogs before and after pyloric obstruction. In those experiments they found losses of chloride from the blood cells parallel to the losses from the serum, and also slight increases of organic as well as inorganic phosphorus in both serum and cells. Except for the latter study, it appears that only the inorganic phosphates of the serum have been considered in relation to the acid-base equilibrium of the blood in intestinal obstruction.

DISCUSSION OF THE METHODS OF STUDY

In attempting to determine the chemical constituents of the blood cells there is a choice of direct analysis of the centrifuged cells or indirect calculation from measurements of the relative volume of the cells and analyses of the whole blood and plasma. Objections may be offered to either method. Long centrifuging is necessary to pack the cells completely (usually more than 30 minutes at 3500 rotations per minute) and even with the greatest care it is difficult to be sure that all the serum has been squeezed from between the cells. Accurate measurement of samples of the packed cells by pipette is difficult. During long centrifuging chemical changes may occur coincident with glycolysis —lactic acid formation, hydrolysis of organic phosphorus compounds, etc.,— which may lead to errors in some of the determinations. Hydrolysis of the phosphoric esters is considerably accelerated in blood samples from animals suffering such types of "intoxication" as those dealt with here; it is, therefore, necessary to make the determinations of the organic phosphorus fractions as quickly as possible after the blood is drawn. Against the indirect method of determining the cell constituents has been the lack of an accurate method for measurement of the relative volume of cells in the blood, but the accuracy of this measurement is considerably increased by the method used in this study. In control studies the indirect method gave closer checks in duplicate determinations of the cell contents than did the direct method. The values for the cells have been calculated by means of the formula:

$$\text{mM.}_{\text{cells}} = \frac{\text{mM.}_{\text{whole blood}} - (\text{mM.}_{\text{plasma}} \times \text{Volumes per cent}_{\text{plasma}})}{\text{Volumes per cent}_{\text{cells}}} \times 100$$

The numerical expression of the values for the cell constituents presents a problem somewhat different from that dealt with in the expression of similar values for the constituents of plasma or serum. When these chemical values are expressed in terms of units per volume (mgm. per 100 cc. or mM. per liter) such expressions may lead to confusion if changes in size of the cells reduce or increase considerably the number of cells which can be packed by centrifuging into a given volume. Mere shift of water due to osmotic changes in either cells or plasma may considerably change the figure for a given cell constituent in terms of units per volume, although the actual amount of that substance in the individual cell or its value in the whole blood remains unchanged. A shrinkage of the relative cell volume from 45 per cent to 42 per cent, without change in the number of cells per c.mm., will change the figure for hemoglobin in the packed cells from 35.5 grams per cent to 38.1 grams per cent—granted, of course, that in the meantime the hemoglobin is not actually altered.

These considerations have led to the use of two ratios which give a measure of absolute changes (or constancy in some cases) within the cells which are independent of volume, size of cell, water loss and the like. The ratio, grams of hemoglobin in the whole blood divided by the red cell count in millions per c.mm., is designated "Hb : RBC count ratio" and represents the hemoglobin in grams $\times 10^{-6}$ per million red blood cells. The ratio, mgm. of organic acid-soluble P in the whole blood divided by the red cell count, is designated "Ester-P : RBC count ratio" and represents this phosphorus fraction in milligrams $\times 10^{-6}$ per million red blood cells.

Heparin was used as the anticoagulant for determining the relative cell volume, while potassium oxalate was used in the blood taken for chemical measurements. Oxalate causes some shrinkage of the cells, but it was here employed for the reasons that follow. Heparin contains some P and is, therefore, unsuitable for the P distribution determinations. Since a large number of analyses were done on each blood sample, economy of blood was necessary. The yield of serum from blood simply allowed to clot was so small in the concentrated blood samples taken late in the period of intoxication that the amounts of blood required to furnish sufficient serum thus were almost prohibitive. Defibrination usually caused some escape of ester-P from the cells; this was especially true when dealing with the pathologic blood samples. The values determined in the whole blood are, of course, unaffected by the anticoagulant and the error in the value calculated for the cells should be less than that suspected in the plasma. Since the P distribution and the intracellular changes were the principal objects of this study, the use of oxalate seemed to be justified.

METHODS

The dog's blood was taken usually from the femoral artery (sometimes from the left ventricle when the animal was in extremis) into a 50 or 100 cc. glass syringe containing paraffin oil. The blood was at once distributed to separate tubes, and treated as indicated below. The amounts of blood varied according to the needs of the number of analyses to be done, but the proportion of added anticoagulants was kept constant.

For the relative cell volume, cell counts and hemoglobin, 1 mgm. heparin to 2 cc. of blood.

For the whole blood CO_2, plasma CO_2 and plasma Cl, blood was delivered into a centrifuge tube containing dry potassium oxalate under oil (25 mgm. to 10 cc. of blood). 1.0 cc. of whole blood for the CO_2 determination was removed immediately by means of a special pipette described elsewhere (Guest (1931)), and after removal of the excess of oil the blood was centrifuged under solid paraffin to obtain plasma for the CO_2 and Cl determinations.

For the phosphorus partition in whole blood and plasma, the whole blood Cl, sugar and nonprotein nitrogen, 25 to 30 cc. of blood were added to 60 mgm. of potassium oxalate.

Relative cell volume was determined by a capillary tube method which will be described in detail later (Guest and Siler (unpublished)). After centrifugation of the blood at high speed, about 18,000 rotations per minute for 4 minutes, in small capillary tubes, the lengths of the columns of cells and plasma were read by means of a measuring microscope, from the base of the blood column to the top of the red cells, to the top of the white cells and to the top of the column of plasma. The relative volumes of cells designated "Total" and "RBC only" were calculated from these measurements. *Red cell counts* were made using 0.1 cc. of heparinized blood diluted in a 50 cc. volumetric flask filled with Hayem's solution. After shaking with glass beads, a drop was transferred from the depths of the flask to a counting chamber and the count made as usual. Counts were made in duplicate and usually agreed within less than 100,000/c.mm., closer checks being obtained by this method than by the use of the small hemocytometer pipette. *Erythrocyte size:* The red blood cell volume in 1 c.mm. of blood, divided by the red blood cell count, in millions per c.mm., gives the average erythrocyte size in terms of cubic microns. *Hemoglobin* was determined by the CO capacity method of Van Slyke and Hiller (1928), using 0.2 cc. of blood. The figures for the CO capacity in terms of volumes per cent were divided by the factor 1.34 to convert them to grams per cent of hemoglobin. The hemoglobin is expressed in three ways in the tables: (1) grams per 100 cc. of whole blood; (2) grams per 100 cc. of red cells; (3) "Hb : RBC count ratio" as described in the discussion of methods. *Serum protein* was determined by the Abbe refractometer, using the formula of Reiss (1903). In the blood samples late in the period of intoxication it is doubtful whether these values are of more than comparative value. *Sugar and* *nonprotein nitrogen:* The precipitation method of Folin (1930) was used for obtaining the filtrates without hemolysis of cells. Nonprotein nitrogen was determined by the method of Folin-Wu (1919); sugar by the method of Folin-Wu, as revised by Folin (1929). *Chloride* was determined by an unpublished method of Fiske, described briefly by Fiske and Sokhey (1925). *CO_2 content* was determined by the method of Van Slyke and Neill (1924), using the factors of Van Slyke and Sendroy (1927). *Phosphorus:* The method of Fiske and Subbarow (1925) was used for the determination of the inorganic, total acid-soluble and total P. For lipin P, the extraction method of Bloor (1918) was used, the P content of an aliquot of the alcohol-ether extract being determined after acid digestion by the Fiske-Subbarow method.

EXPERIMENTAL

Mongrel dogs of a variety of breeds were used. Blood samples were taken in most instances one or two days before operation, but in a few cases only an hour or so before. The obstructions were made in the mid-duodenum, just below the pancreatic and bile ducts, or at the pylorus. The duodenum or pylorus was divided and both stumps inverted with a double line of sutures. Beginning 12 to 24 hours after operation, in most cases the dogs were allowed water ad libitum to increase the vomiting. Without treatment they lived from 40 hours to five days, the majority dying at from 50 to 72 hours after operation. Dogs with mid-duodenal obstruction seemed to suffer a less rapid fall of the blood chlorides than those with pyloric obstruction, but otherwise there seemed to be little difference in the effects of the two operative procedures. Given salt solution parenterally, the obstructed dogs lived 12 to 20 days and appeared to be in good condition when they were sacrificed—except three that developed signs of distemper and were sacrificed early. Control experiments included the administration of glucose solution parenterally to dogs with pyloric obstruction (see Figure 4), and simple deprivation of food and water in dogs without operation (see Table 4 and Figure 5). The experiments selected for presentation here are typical and have all been repeated, some of them many times.

Phosphorus distribution in the blood of normal dogs. (*Table 1*)

In Table 1 are given the averages of the values for the distribution of phosphorus determined in the blood of 30 apparently normal dogs. The actual determinations are of the fractions designated (1) inorganic, (2) total acid-soluble, (4) total, and (6) alcohol-ether soluble. The values for (3) organic acid-soluble "Ester-P," (5) acid-insoluble and (7) non-lipin, acid-insoluble "Residual P," are respectively obtained by the differences 2–1, 4–2, and 5–6. The negative value of the (7) "Residual P" in the plasma is obtained so frequently that it may be suspected that the alcohol-ether extraction actually includes some

TABLE 1

The distribution of phosphorus in dog's blood. Averages of the values determined in 30 normal dogs

	Whole blood	Plasma	Cells
Relative cell volume, total............*volumes per cent*	49.5*		
RBC only......................*volumes per cent*	48.4		
RBC.......................*millions per c.mm.*	7.325		
Erythrocyte size...........................*cu. mu*	66.0		
Phosphorus distribution			
1. Inorganic NaH_2PO_4, Na_2HPO_4...............*mgm. per 100 cc.*	2.85	3.52	2.16
2. Total acid-soluble..........*mgm. per 100 cc.*	28.23	3.77	53.2
(2–1) = 3. Organic acid-soluble: "Ester-Phosphorus"............*mgm. per 100 cc.*	25.38	0.25	51.0
4. TOTAL...................*mgm. per 100 cc.*	43.40	15.68	71.7
(4–2) = 5. Acid-insoluble.............*mgm. per 100 cc.*	15.17	11.91	18.5
6. Alcohol-ether soluble: lipin, phosphatides............*mgm. per 100 cc.*	14.16†	12.2	16.7
(5–6) = 7. Non-lipin, acid-insoluble residual undetermined.....*mgm. per 100 cc.*	1.0	−0.3	1.8
Ester-P : RBC count ratio..........................	*3.46*		

* In a larger series of 52 dogs the average relative cell volume is lower, 46.4 per cent, but the average erythrocyte size is 66.1 cu. microns.

† Lipin phosphorus was determined in only 17 of these bloods.

phosphorus of non-lipin nature that is also extracted in the trichloracetic acid. The differences are so slight, however, that this error is negligible and for practical purposes the "Lipin" and "Acid-insoluble" fractions in the plasma may be considered identical. The larger value (7) in the whole blood is due perhaps mostly to nucleic acid in the leucocytes.

Mid-duodenal obstruction. Dog number 121. (*Table 2*)

In this table are shown the principal typical changes observable in the blood of a dog with experimental high intestinal obstruction. Increased concentration of the blood is indicated by the increased serum protein, cell volume and cell count. In this animal there was little change in the erythrocyte size, although in similar experiments there is usually observed a slight shrinkage. The nonprotein nitrogen was markedly increased. The plasma Cl fell and CO_2 content of the plasma rose. The total P of both plasma and cells increased markedly. Quantitatively, the greatest increase of the individual fractions of the total P was in the organic acid-soluble "Ester-P" of the cells. To make this immediately clearer the actual changes in the P distribution are shown in the lower part of the table by the differences between the values determined before and 48 hours after the operation. The next

TABLE 2

Dog number 121. Mid-duodenal obstruction. Allowed water ad lib. Weight 19.5 kilos. Lost 3.0 kilos. Died about 60 hours after operation

	Before operation			48 Hours after operation		
	Whole blood	Plasma	Cells	Whole blood	Plasma	Cells
Blood cells, total......*volumes per cent*	52.9			59.3		
RBC only........*volumes per cent*	51.5			56.3		
RBC..........*millions per c.mm.*	7.80			8.19		
Erythrocyte size..............*cu. mu*	66.0			68.7		
Protein.............*grams per 100 cc.*		7.7			10.5	
Nonprotein nitrogen...*mgm. per 100 cc.*	26.0			133.0		
Sugar..............*mgm. per 100 cc.*	91.0			155.0		
Chlorides...............*mM. per liter*		104.0			66.3	
CO_2 content............*mM. per liter*		24.3			42.6	
Phosphorus distribution						
1. Inorganic.........*mgm. per 100 cc.*	2.01	2.69	1.40	6.51	9.41	4.5
2. Total acid-soluble..*mgm. per 100 cc.*	26.7	2.72	48.0	50.0	10.58	77.0
3. Organic acid-soluble *mgm. per 100 cc.*	24.69	0.03	46.6	43.49	1.17	72.5
4. TOTAL...........*mgm. per 100 cc.*	42.1	13.0	68.0	70.5	26.7	100.5
5. Acid-insoluble......*mgm. per 100 cc.*	15.4	10.28	20.0	20.5	16.12	23.5
6. Lipin............*mgm. per 100 cc.*	13.2	9.6	16.4	15.4	16.1	14.9
7. Non-lipin, acid-insoluble.........*mgm. per 100 cc.*	2.2	0.68	3.6	5.1	0.02	8.6
Ester-P : RBC count ratio............	*3.16*			*5.31*		
Changes in phosphorus, after operation:						
1. Inorganic....................................				+ 4.5	+ 6.72	+ 3.1
3. Organic acid-soluble, ester-P....................				+18.8	+ 1.14	+25.9
4. TOTAL......................................				+28.4	+13.7	+32.5
5. Acid-insoluble................................				+ 5.1	+ 5.84	+ 3.5
6. Lipin..				+ 2.2	+ 6.5	− 1.5
7. Non-lipin, acid-insoluble				+ 2.9	− 0.66	+ 5.0

greatest change was in the increased inorganic P of the plasma. The changes in the acid-insoluble P were different in the plasma and in the cells: the lipin P was increased in the plasma, while the non-lipin, residual P was increased in the cells.

Mid-duodenal obstruction. Dog number 60. (*Figure 1*)

This figure presents graphically the changes observed in the blood of a dog at 68 and 96 hours after mid-duodenal obstruction. The dog died 97 hours after operation. The principal changes were: (1) Nonprotein nitrogen, markedly increased. (2) Decrease of the Cl in both plasma and cells, with increase of the CO_2 content in both. The terminal fall in the CO_2, sometimes much greater than in this dog, is doubtless due to the accumulation of organic acids which is to be expected at this time, especially with a high nonprotein nitrogen value. (3)

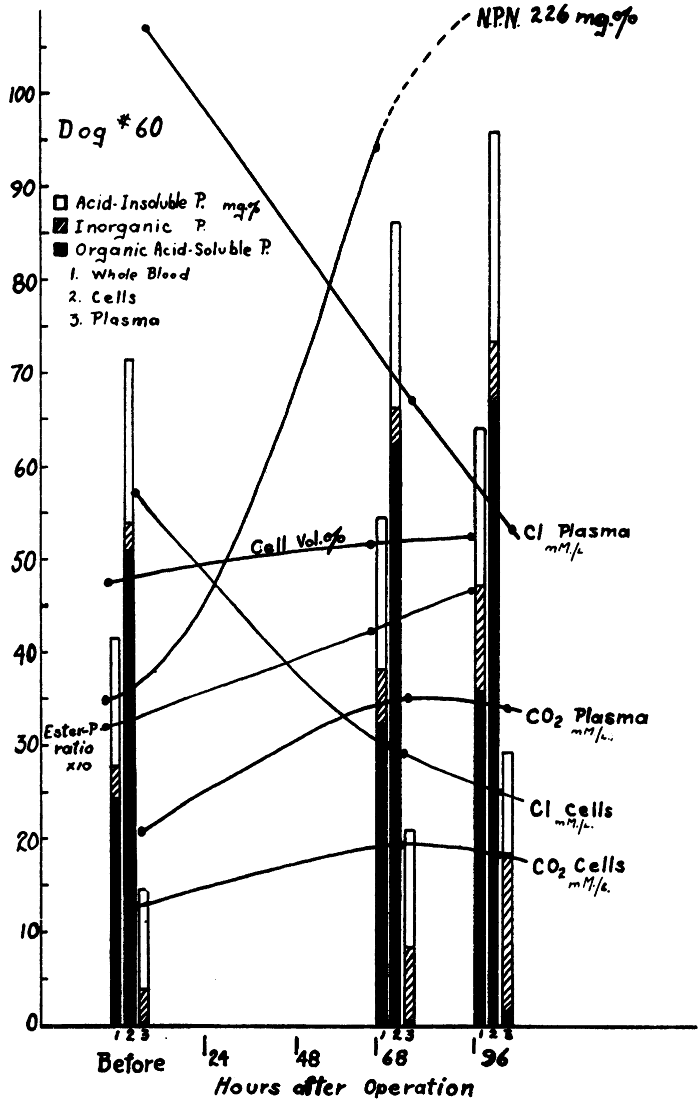

FIG. 1.[2] CHANGES IN THE BLOOD OF A DOG, FOLLOWING MID-DUODENAL OBSTRUCTION

[2] In these figures the columns 1, 2, 3 represent the total P as mgm. per cent in the whole blood, cells and plasma respectively. In the columns the solid, cross hatched and blank portions indicate respectively the organic acid-soluble "Ester-P," the inorganic and the acid-insoluble P. In Figure 4 the lipin P is also represented, at the top of the columns, by perpendicular lines within that portion representing the acid-insoluble P. The curves as drawn represent the chloride and CO_2 contents in terms of millimols per liter (mM./L.) and the nonprotein nitrogen as milligrams per 100 cc. (mg. per cent). The figures for the *Ester-P : RBC count ratio,* the *Hb : RBC count ratio* and the RBC count in millions per c.mm. have been multiplied by 10 for convenience in making a clearer chart.

Phosphorus distribution: The ester-P is increased within the cells, and the inorganic P in the plasma. The increases of the acid-insoluble P are slight in comparison with these two fractions. (4) The serum protein, not shown in the figure, was 7.4 grams per cent before the operation and increased in the two subsequent blood samples after operation to 10.1 grams per cent and 10.6 grams per cent respectively.

The changes observed in the blood following pyloric obstruction are similar to those shown occurring after mid-duodenal obstruction in these two experiments. A typical example of pyloric obstruction is displayed in Table 1 of the succeeding paper (Andrus, Guest, Gates and Ashley).

Mid-duodenal obstruction + salt solution. Dog number 78. (Figure 2)

The effectiveness of the parenteral administration of salt solution in prolonging life, in relieving dehydration and in combatting those changes of the blood Cl, CO_2, nonprotein nitrogen, etc., which are commonly observed in intestinal obstruction have been demonstrated many times. (See the reviews cited above.) The two experiments displayed in Figure 2 and Table 3 demonstrate that changes of the blood phosphorus after obstruction are also prevented, at least in great measure, by the administration of salt solution.

After mid-duodenal obstruction this dog received daily 40 cc. of 0.9 per cent NaCl solution per kilogram body weight, injected subcutaneously. The dog's weight fell progressively from 17.5 kgm. to 12.3 kgm. on the eleventh day and 12.0 kgm. on the sixteenth day after operation. During the first 4 days the blood Cl fell, but thereafter the saline injections brought the Cl in the cells to a normal value and the plasma Cl to 118 mM. per liter, considerably above its initial level. The CO_2 during these first 4 days rose, then returned to the initial level. With these changes in the Cl and CO_2, the organic acid-soluble ester-P, after increasing in the cells through the fourth day, returned to normal. In contrast to the cells, the plasma P showed a gradual decrease through the whole period of eleven days. The nonprotein nitrogen progressively decreased. At the sixteenth day, 5 days after the last blood sample was taken, the dog was developing what appeared to be signs of distemper and was sacrificed. The autopsy findings were, however, essentially normal.

Pyloric obstruction + salt solution: treatment interrupted after 18 days. Dog number 263. (Table 3)

After pyloric obstruction this dog was given 1000 to 1200 cc. of 0.9 per cent NaCl solution subcutaneously and intraperitoneally daily for 18 days; this represents more than 100 cc. per kilogram body weight.

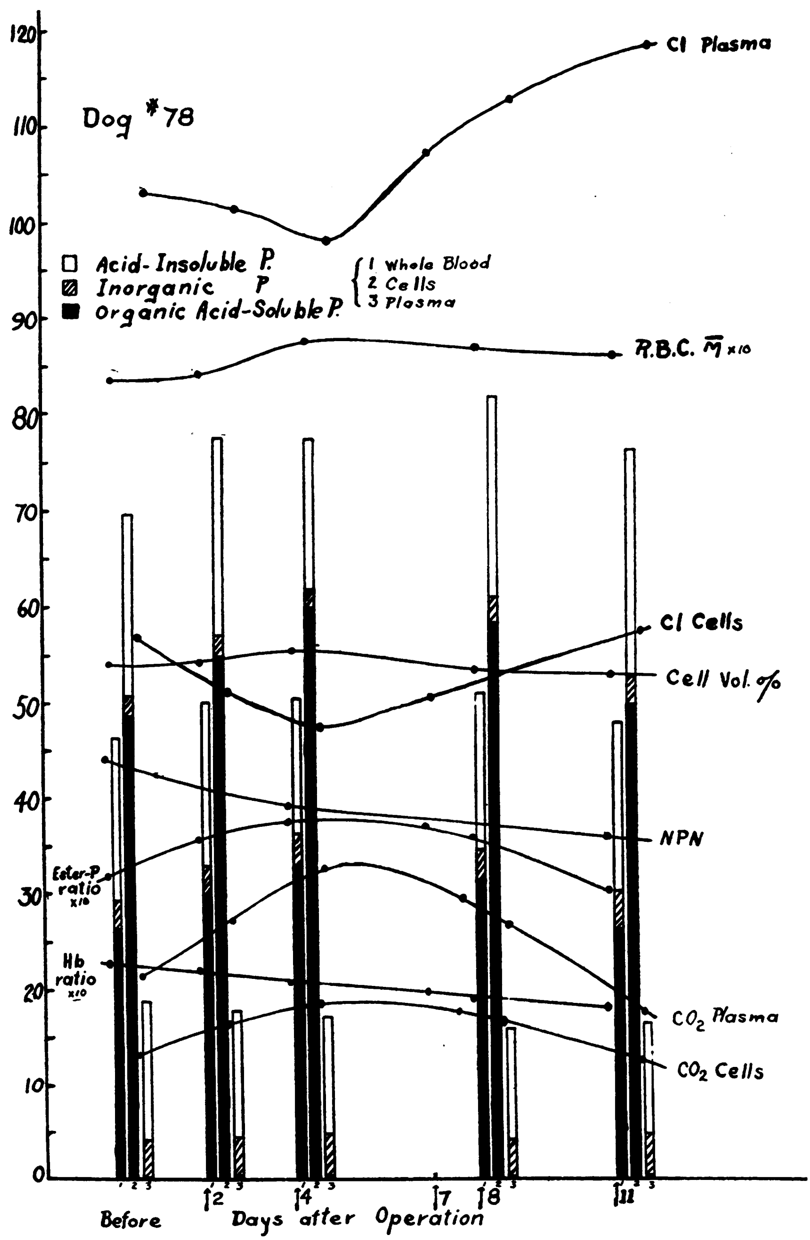

FIG. 2. MID-DUODENAL OBSTRUCTION + DAILY SUBCUTANEOUS INJECTIONS OF 0.9 PER CENT NACL SOLUTION

TABLE 3

Dog number 263

1. Normal.
2. Pyloric obstruction: Received daily subcutaneous injections of 1000–1200 cc. 0.9 per cent NaCl solution for 18 days. The dog appeared to be in good condition when the saline injections were stopped.
3. Last blood sample taken after three days without treatment. Death occurred about 4 hours after the blood sample was taken.

	1	2		3
		Pyloric obstruction		
	Before operation*	Salt solution daily		3 days no salt solution
Days after operation		11	18	21
Weight ... *kilos*	10.8	9.1	8.0	
Blood cells, total ... *volumes per cent*	47.8	45.4	41.8	47.2
RBC, only ... *volumes per cent*	47.3	44.2	40.7	45.8
RBC ... *millions per c.mm.*	7.00	6.82	6.42	7.91
Erythrocyte size ... *cu. mu*	67.5	64.7	63.3	57.9
Hemoglobin, whole blood ... *grams per 100 cc.*	16.67	16.32	14.61	17.50
Hemoglobin, cells ... *grams per 100 cc.*	35.2	36.9	35.9	38.2
Hb : RBC count ratio	*2.38*	*2.39*	*2.27*	*2.21*
Serum protein ... *grams per 100 cc.*	6.1	5.7	4.9	8.7
Nonprotein nitrogen ... *mgm. per 100 cc.*	23	19	20	111
Sugar ... *mgm. per 100 cc.*	91		67	82
CO_2 content, plasma ... *mM. per liter*	22.0		39.8	50.9
CO_2 content, cells ... *mM. per liter*	13.2		21.4	26.5
Chloride, plasma ... *mM. per liter*	110.5	101.0	97.5	65.0
Chloride, cells ... *mM. per liter*	60.3	56.9	42.5	17.3
Phosphorus distribution				
Whole Blood				
1. Inorganic ... *mgm. per 100 cc.*	3.65	4.0	3.79	7.96
2. Total acid-soluble ... *mgm. per 100 cc.*	27.0	30.4	30.5	47.8
3. Organic acid-soluble ... *mgm. per 100 cc.*	23.35	25.6	26.7	39.84
4. TOTAL ... *mgm. per 100 cc.*	43.5	43.7	42.3	67.2
5. Acid-insoluble ... *mgm. per 100 cc.*	16.5	13.3	11.8	19.4
Ester-P : RBC count ratio	*3.33*	*3.75*	*4.15*	*5.03*
Plasma				
1. Inorganic ... *mgm. per 100 cc.*	4.3		4.35	10.46
2. Total acid-soluble ... *mgm. per 100 cc.*	4.5		4.4	11.12
3. Organic acid-soluble ... *mgm. per 100 cc.*	0.2		0.05	0.66
4. TOTAL ... *mgm. per 100 cc.*	16.5	13.5	12.3	25.0
5. Acid-insoluble ... *mgm. per 100 cc.*	12.0		8.0	13.9
Cells				
1. Inorganic ... *mgm. per 100 cc.*	2.94		3.0	5.16
2. Total acid-soluble ... *mgm. per 100 cc.*	51.6		66.8	88.8
3. Organic acid-soluble ... *mgm. per 100 cc.*	48.6	56.0	63.8	83.6
4. TOTAL ... *mgm. per 100 cc.*	73.0	80.0	84.0	114.4
5. Acid-insoluble ... *mgm. per 100 cc.*	21.4		17.2	25.6

* Figures in this column are assembled from 3 separate preoperative blood samples.

During these 18 days the plasma Cl fell from 110.5 to 97.5 mM., and the Cl of the cells fell from 60.3 to 42.5 mM., indicating that the salt given was not adequate to keep the blood chlorides at their initial level. The dog, however, appeared to be in good condition, and when the saline injections were stopped on the 18th day after operation the blood nonprotein nitrogen was not increased. A blood sample was taken 3 days after the last injection of salt solution and the dog died about 4 hours after this sample was taken. The nonprotein nitrogen in this last blood sample was 111 mgm. per 100 cc. The chlorides had decreased to 65.0 mM. in the plasma and to 17.3 mM. in the cells, while the CO_2 was increased in both plasma and cells. During the first 18 days the plasma total P diminished; in the cells the organic acid-soluble "Ester-P" fraction increased slowly from 48.6 to 63.8 mgm. per 100 cc. After the abrupt interruption of the saline injections the ester-P in the cells rose in 3 days to 83.6 mgm. per 100 cc., while the total P of the cells reached 114.4 mgm. per 100 cc. In the first 18 days the red blood cell count fell somewhat, but rose in the last 3 days. The serum protein had fallen to 4.9 grams per cent on the 18th day, but in the last sample had increased to 8.7 grams per cent. The *Hb : RBC count ratio* changed very little during this whole period; an indication that in this important characteristic the red cells had not changed. Contrast with this the increasing *Ester-P : RBC count ratio.*

Pyloric obstruction + glucose solution. Dog number 298.
(Figure 3)

It has been argued that the increase of inorganic phosphate in the blood in intestinal obstruction is due to a failure of renal excretion of waste endogenous phosphates (see Discussion). The experiment shown in Figure 3 was performed to see whether the promotion of marked diuresis by the parenteral administration of water in the form of glucose solution would prevent the increase of phosphates in the blood.

After pyloric obstruction this dog received daily injections of 5 per cent glucose solution subcutaneously and intraperitoneally. The animal lived $6\frac{1}{2}$ days, a longer time than any of the dogs lived that did not receive salt solution. The body weight fell from 19.7 to 18.0 kilograms. The blood nonprotein nitrogen increased slowly, if compared with the nonprotein nitrogen increase in the untreated dogs with pyloric obstruction. The erythrocyte size and hemoglobin content of the cells remained remarkably constant, although there was a considerable decrease (6,700,000 to 5,375,000 per c.mm.) in the red blood cell count. The serum protein was 7.9 grams per cent before operation and increased to 8.3 and 8.5 grams per cent on the fourth and sixth days respectively. By the columns in the figure, representing the P distribution on the third and fifth days after operation, it may be seen that the increase

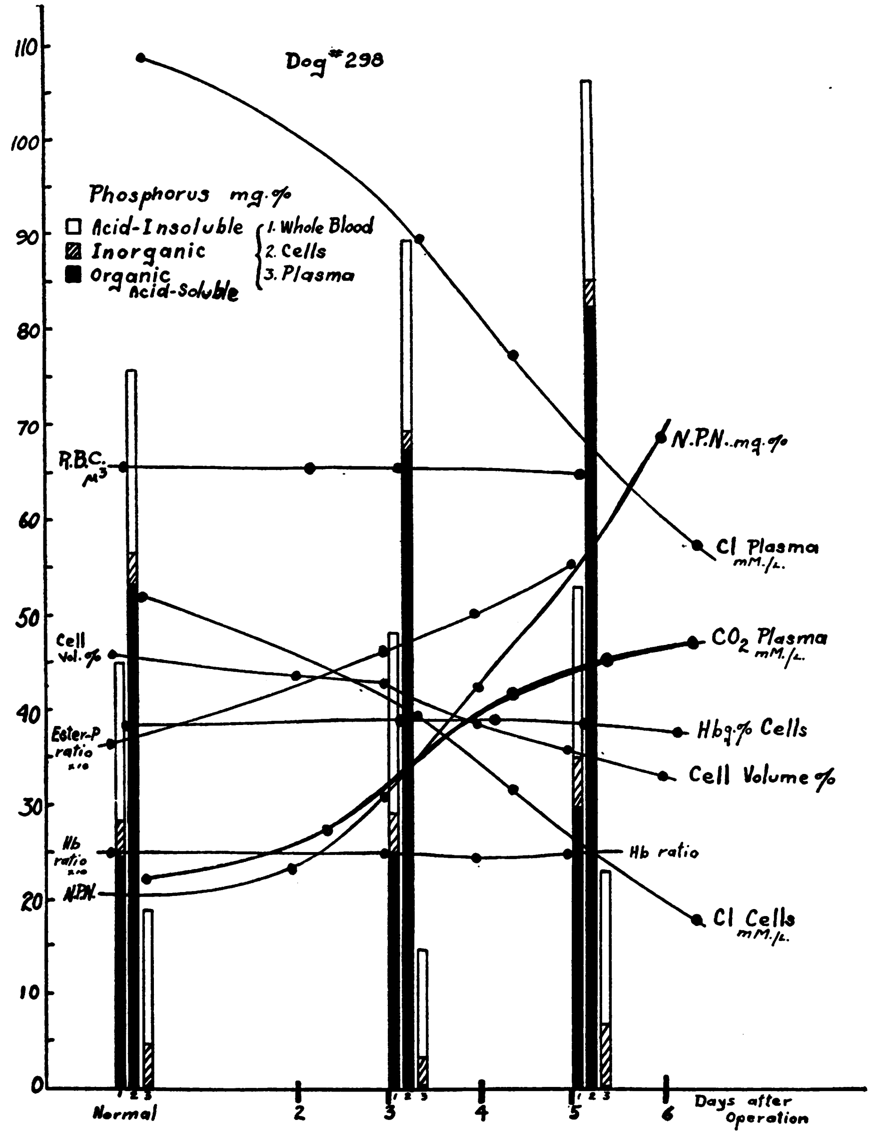

FIG. 3. PYLORIC OBSTRUCTIONS + DAILY SUBCUTANEOUS AND INTRAPERITONEAL INJECTIONS OF 5 PER CENT GLUCOSE SOLUTION

of the ester-P in the cells, as well as the losses of chloride from both plasma and cells, occurred exactly as observed in the obstructed dogs left without treatment.

Effects of deprivation of food and water. Dog number 69. (Table 4) Dog number 278. (Figure 4)

The two control experiments, shown in Table 4 and Figure 4, are cited to show that in normal dogs simple deprivation of food and water even after 16 and 18 days did not bring about any such remarkable changes of the phosphorus d: :ribution in the blood as are observed in the dogs with intestinal obs ruction.

The dog number 69 (Tab'e 4), deprived of food and water for 16 days, showed rather remarkable constancy in the blood chemical values. Note that the increasing chloride and serum protein indicate a con-

TABLE 4

Dog number 69. Deprived of food and water

	Before	Days of starvation			
		2	6	11	16
Weight . . . *kilos*	12.5	12.0	10.5	9.5	8.6
Blood cells, total . . . *volumes per cent*	54.0	56.0	55.4	56.9	48.2
RBC . . . *millions per c.mm.*	8.19	8.23	8.80	8.97	8.26
Hemoglobin, whole blood . *grams per 100 cc.*			19.25	18.24	16.94
Hb : RBC count ratio . . .			*2.18*	*2.03*	*2.05*
Serum protein . . . *grams per 100 cc.*	6.40	8.49	7.97	9.54	9.83
Nonprotein nitrogen . . . *mgm. per 100 cc.*	31.0	39	39	30	120
Sugar . . . *mgm. per 100 cc.*	98	100	129	96	129
CO_2 content, plasma . . . *mM. per liter*	22.48	22.29	21.68	23.87	17.9
CO_2 content, cells . . . *mM. per liter*	14.4	13.8	14.6	17.0	12.3
Chloride, plasma . . . *mM. per liter*	107	112.0	119.0	123.0	132.7
Chloride, cells . . . *mM. per liter*	56.1	64.6	61.4	68.5	69.8
Phosphorus distribution					
Whole blood					
1. Inorganic . . . *mgm. per 100 cc.*	2.29	2.91	3.02	3.45	8.4
2. Total acid-soluble . . . *mgm. per 100 cc.*	26.9	27.6	30.1	30.3	33.3
3. Organic acid-soluble . . . *mgm. per 100 cc.*	24.6	24.7	27.1	26.8	24.9
4. TOTAL . . . *mgm. per 100 cc.*	38.9	42.8	45.4	45.5	49.4
5. Acid-insoluble . . . *mgm. per 100 cc.*	12.0	15.2	15.3	15.2	16.1
Ester-P : RBC count ratio . . .	*3.00*	*3.00*	*3.08*	*2.98*	*3.01*
Plasma					
1. Inorganic . . . *mgm. per 100 cc.*	2.87	3.81	3.71	4.06	10.4
2. Total acid-soluble . . . *mgm. per 100 cc.*	3.06	4.08	4.00	4.66	10.8
3. Organic acid-soluble . . . *mgm. per 100 cc.*	0.2	0.3	0.3	0.6	0.4
4. TOTAL . . . *mgm. per 100 cc.*	10.15	15.6	15.5	13.5	21.7
5. Acid-insoluble . . . *mgm. per 100 cc.*	7.1	11.5	11.5	8.8	10.9
Cells					
1. Inorganic . . . *mgm. per 100 cc.*	1.80	2.20	2.46	2.99	6.25
2. Total acid-soluble . . . *mgm. per 100 cc.*	47.2	46.1	51.1	49.7	57.4
3. Organic acid-soluble . . . *mgm. per 100 cc.*	45.4	43.9	48.6	46.7	51.2
4. TOTAL . . . *mgm. per 100 cc.*	63.4	64.2	69.5	69.7	79.1
5. Acid-insoluble . . . *mgm. per 100 cc.*	16.2	18.1	18.4	20.0	21.7

siderable concentration of the blood. In the last sample the nonprotein nitrogen is high, and the inorganic P is considerably elevated, but even in this sample the changes in the phosphorus are almost negligible except for the inorganic fraction.

In the dog number 278 (Figure 4), deprived of food and water, there was a considerable decrease in the total phosphorus of the blood cells in the first six days, without any appreciable change in the phosphorus of the plasma. Subsequently there was a slow increase of the total cell phosphorus, but in the whole period of 18 days these changes are practically limited to the organic acid-soluble fraction. Note the progressive steady increase of the plasma chloride to 129 mM. per liter. On the 18th day the blood nonprotein nitrogen was high, and the dog was found dead on the morning of the 20th day.

DISCUSSION

The increases of inorganic phosphates in the blood in intestinal obstruction have been explained by the hypothesis, offered by Atchley and Benedict (1927), that the kidneys fail to excrete the endogenous waste phosphates. Wakeman, Peters and Lee (1931) found that in pyloric obstruction the concentration of P in the urine was very high, but it is perhaps possible that even a partial "retention" might result in the accumulation of phosphates in the blood since under these conditions there is excessive tissue catabolism which makes necessary a greatly increased excretion of waste phosphates. Such failure of excretion might be due to a diminution of kidney function (McQuarrie and Whipple (1919)) associated with the toxic nephritis described by Brown, Eusterman, Hartman and Rowntree (1923) as part of the intoxication that accompanies intestinal obstruction, or it might be due simply to lack of water, the result of dehydration brought about by vomiting. The increase of the nonprotein nitrogen in the blood might be explained on either basis, or both, and the increased nonprotein nitrogen figure may be accepted as an indication of a failure of excretion of various endogenous waste substances. The increase of ester-P in the cells seems not so easily explainable, but here again the diminished excretory function of the kidneys must be considered in view of the work of Kay (1926) and of Eichholtz, Robison and Brull (1925) which indicated that the inorganic phosphates of the urine may be derived from the ester-P of the blood by enzyme hydrolysis of these esters as the blood passes through the kidneys. Thus, failure of renal function might possibly include suppression of such enzymatic processes and, therefore, allow the accumulation of organic as well as inorganic phosphates in the blood. This subject will be further discussed in a later paper in which will be reported studies—similar to those reported here—of experimental nephritis and the effects of bilateral ligation of the ureters in dogs.

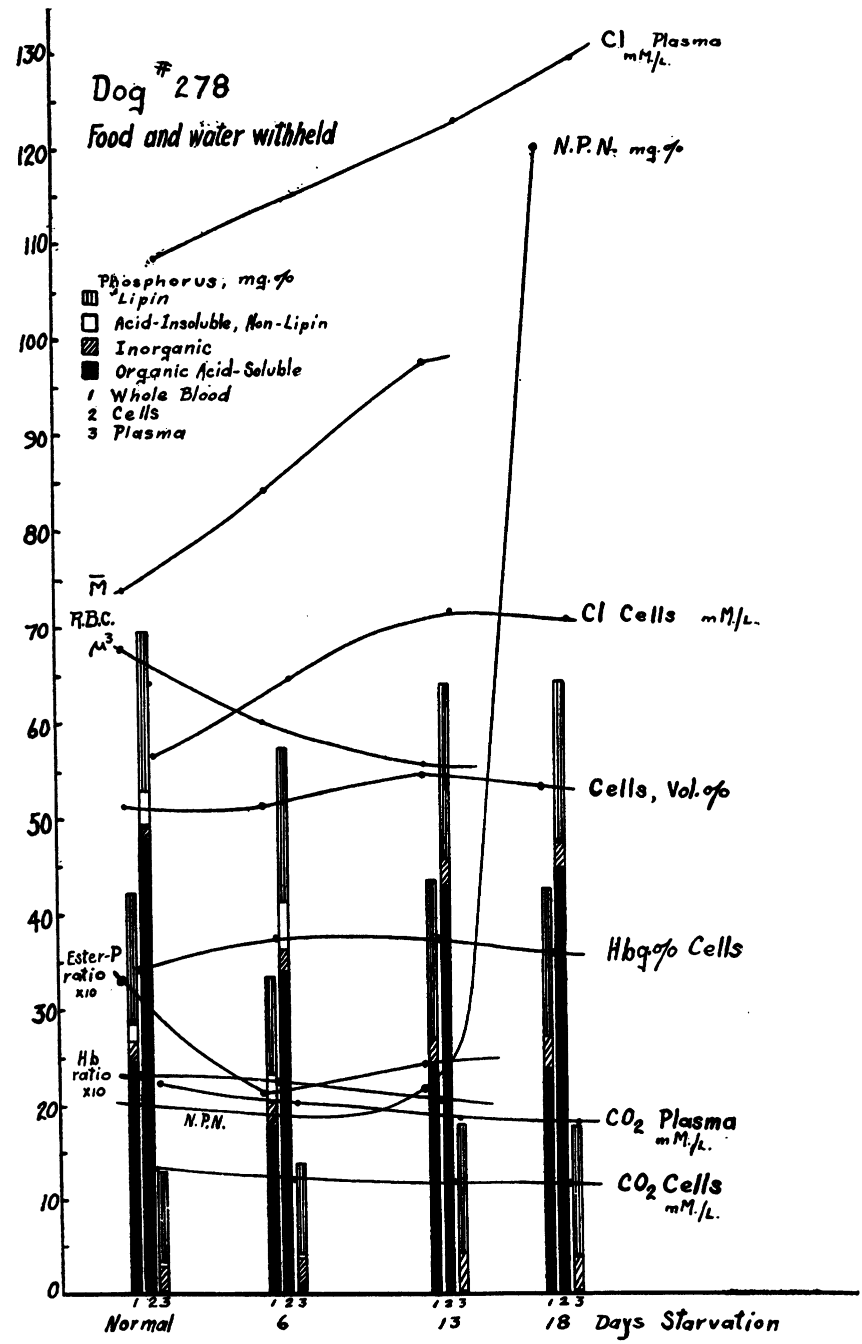

FIG. 4. CHANGES IN THE BLOOD OF A DOG DEPRIVED OF FOOD AND WATER

However, even granting that diminished renal function may be partly responsible for an accumulation of phosphates in the blood, the mechanism of the increase of ester-P in the blood cells remains obscure. The phosphorus compounds determined in this fraction are extremely labile, various factors decreasing and increasing their concentration. Haldane, Wigglesworth and Woodrow (1924) and Kay (1924) have demonstrated that chloride acidosis (induced by the feeding of NH_4Cl) brings about a great reduction of the ester-P of the blood cells. The mechanism of this effect has not been completely explained but in view of the evidence cited in the introduction, that the phosphoric esters are bound to alkali in the cells, it seems possible that the excess of Cl′ ions in such a state of acidosis is capable of displacing the ester-P from this alkali. Following this argument, if Cl′ is lost from the cells the ester-P may be retained by the alkali from which Cl′ was lost much as HCO_3' is known to be retained by the alkali of the blood from which Cl is lost. At any rate, in these dogs with intestinal obstruction there is observed greater parallelism between the losses of Cl from the cells and the increases of the ester-P in the cells than between any of the other chemical changes.

In the two experiments shown in Figure 2 and Table 3, the administration of salt solution parenterally prevented an accumulation of the nonprotein nitrogen in the blood and there was no appreciable change in the inorganic P; yet there was an increase of the ester-P paralleling closely the losses of Cl from the cells. Unless the excretion of waste phosphates is quite different from the excretion of waste nitrogenous products, it would appear that at least in these experiments the accumulation of phosphorus was not due to failing renal function. In the two dogs deprived of food and water (Table 4 and Figure 4) the terminal increases of the nonprotein nitrogen in the blood before death were not accompanied by any significant increase of the ester-P of the blood, even when in one dog (number 69) the inorganic P of the plasma was increased to 10.4 mgm. per 100 cc.

SUMMARY

Following experimental pyloric and mid-duodenal obstruction in dogs, marked changes in the distribution of phosphorus in the blood have been observed. The phosphorus was partitioned as the following fractions in the whole blood, plasma and cells: Inorganic; acid-soluble; organic acid-soluble or "Ester-P"; acid-insoluble; alcohol-ether-soluble or lipin-P; total phosphorus. The most important changes were marked increases in the fraction designated "Ester-P" which has an average normal value of about 50 mgm. per 100 cc. in the cells and only 0.3 mgm. per 100 cc. in the plasma. The increases of the ester-P were much greater than the changes in any of the other phosphorus fractions of the cells or plasma.

Changes in chloride and CO_2 content of both plasma and cells were compared with concomitant changes in the distribution of phosphorus. In all the experiments there was a close correlation between the progressive losses of chloride from the blood cells and the increases of organic acid-soluble phosphorus. It seems likely that as the organic phosphorus compounds increased they were bound to the alkali in the cells from which Cl′ was lost.

The parenteral administration of NaCl solution to obstructed dogs prevented the increases of organic acid-soluble phosphorus in the blood cells to about the same degree that it prevented the losses of chloride from the blood cells.

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