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I hereby recommend that the thesis prepared under my supervision by S. Rapoport

entitled The Role of Acid-Soluble Phosphorus Compounds of the Red Blood Cells.

be accepted as fulfilling this part of the requirements for the degree of Ph. D.

Approved by:

George M. Guest

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THE ROLE OF ACID-SOLUBLE PHOSPHORUS COMPOUNDS IN THE RED BLOOD CELLS.

by

S. Rapoport

Presented by S. Rapoport to the Department of Biochemistry of the Graduate School, University of Cincinnati, in part fulfillment of the requirements for the degree Ph.D.

To be published under the joint authorship of G. M. Guest and S. Rapoport.

From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati

This discussion of the acid-soluble phosphorus compounds of the red blood cells concerns chiefly their importance in the acid-base equilibrium of the blood, and their probable role in the intermediate phosphorus metabolism of the body. It is based upon clinical and experimental studies of a variety of conditions where large changes in concentration of acid-soluble phosphorus in the blood cells are found. Examples from these studies are presented to demonstrate the changes which have been found associated with rickets, bilateral nephrectomy, alkalosis of pyloric obstruction, and different types of acidosis. As a basis for the interpretation of the findings in these conditions, current concepts of the glycolytic cycle in blood are summarized briefly; the reactions of the glycolytic process being responsible for the synthesis and hydrolysis of the organic phosphorus compounds with which these studies are concerned.

METHODS

Inorganic and organic acid-soluble P were determined by the method of Fiske and Subbarow (1), with minor modifications (2). Adenosinetriphosphate was determined by the method of Lohmann (3), the "pyrophosphate" fraction obtained by 7 minutes hydrolysis with boiling N/1 H_2SO_4 being multiplied by 1.5 to give the value designated "adenosinetriphosphate" (4). Diphosphoglycerate was determined by the method of Rapoport (5). Chlorides were determined either by the method of Van Slyke and Sendroy (6), or by the method of Sendroy (7). CO_2 was

determined by the method of Van Slyke and Neill, using the factors of Van Slyke and Sendroy (8). Total base (total inorganic cations) was determined by the method of Leva and Guest (9). pH was determined with the glass electrode. The per cent volume of cells in heparinized blood was determined by the method of Guest and Siler (10), and the concentrations of cellular constituents were calculated indirectly, by means of the cell volume, from values determined on whole blood and serum.

DISTRIBUTION OF THE ACID-SOLUBLE PHOSPHORUS FRACTIONS IN NORMAL HUMAN RED BLOOD CELLS

The following represent the usual ranges of concentrations of inorganic P and four fractions of the organic acid-soluble P found in normal human red blood cells:

Inorganic P: -usually between 1 and 3 mg. per 100 cc. These values tend to be higher in infants and young animals than in older subjects.

Organic acid-soluble P: -usually between 50 and 60 mg. per 100 cc., most often nearer 50 mg.

Adenosinetriphosphate P: -usually between 9 and 12 mg. per 100 cc., the average being near 10 mg.

Hexosephosphates: Estimates of hexosephosphates in the blood cells must be based upon indirect evidence, since these compounds have not yet been isolated in pure form from blood. Available data indicate, however, that both the monophosphate and diphosphate esters are present in the blood cells, and that these together normally account for approximately 15 mg. of phosphorus per 100 cc. of cells.

Diphosphoglycerate P: normally makes up about half of the organic acid-soluble P, i.e., from 25 to 30 mg. per 100 cc.

These figures are nearly the same for the cells of dogs, but are higher for the cells of rabbits and rats, the other species used in the experimental studies which are cited here.

THE GLYCOLYTIC CYCLE IN BLOOD.

The synthesis and hydrolysis of organic acid-soluble phosphorus compounds in the red blood cells are accomplished by reactions which form part of the glycolytic cycle in blood. In this, the glycolytic process has an important biologic function apart from the break-down of sugar: namely, the regulation of the concentration at which these phosphorus compounds are carried in the blood under different conditions.

The schematic representation of the glycolytic cycle in blood shown in Figure 1 is based upon theories of Embden, Meyerhof, Parnas, Dische and others. The diagram is designed to illustrate the principle

steps in blood glycolysis as they may be described according to current concepts, and to show some of the interrelationships that can be traced between the phosphorus compounds subsequently discussed.

Glucose is represented going into, and lactic acid out of, the cycle, while inorganic P may go in either direction. A reaction 1 between glucose and adenosinetriphosphate yields hexosediphosphate and adenylic acid. Hexosediphosphate is maintained in enzymatic equilibrium with two mols of triosephosphate. Reactions 2 between triosephosphate and pyruvic acid lead to the formation of phosphoglyceric acid and lactic acid. Phosphoglyceric acid is converted to phosphopyruvic acid, and this in turn reacts with adenylic acid in the resynthesis 3 of adenosinetriphosphate, thus completing the cycle. The pyruvic acid liberated in this reaction reacts anew with triosephosphate in the synthesis 2 of phosphoglyceric acid. Adenosinetriphosphate is also formed by direct phosphorylation 4 of adenylic acid from inorganic phosphate. This esterification 4 of inorganic P is closely linked with the reactions 2 between pyruvic acid and triosephosphate.

It is important to note here that all changes of organic acid-soluble phosphorus which are to be discussed hinge upon the last-named set of reactions. The synthesis of adenosinetriphosphate by direct phosphorylation of adenylic acid seems to be the only way in

which phosphorus can enter this cycle, and the only means by which the concentrations of the organic acid-soluble phosphorus compounds can be increased. The hydrolysis of adenosinetriphosphate seems to be at least the most important route by which the phosphorus compounds can be reduced, and phosphorus can leave the cycle.

The concentrations of organic acid-soluble phosphorus in the blood cells thus are necessarily subject to the influence of various factors affecting the glycolytic system. One important factor is the pH of the blood. In experiments with blood in vitro, it has been shown (4) that even slight shifts in pH of the blood from normal toward greater acidity or alkalinity respectively favor the decomposition or the synthesis of diphosphoglycerate in the cells. This effect is seen in various types of acidosis, where the concentration of diphosphoglycerate in the blood cells is reduced, and in alkalosis, where the diphosphoglycerate is increased. Another important factor influencing the concentration of acid-soluble phosphorus in the cells appears to be the supply of phosphorus available. In nephritis and other conditions with impairment or suppression of renal function, the inability to excrete waste endogenous phosphates leads to the accumulation of an excess of inorganic phosphates in the blood; with this the concentration of diphosphoglycerate in the cells is greatly increased. In rickets, on the other hand, with lack of phosphorus we find low concentrations of organic acid-soluble phosphorus in the cells accompanying the well-known decreases of inorganic phosphorus in both serum and cells. Examples of these conditions are presented in the following pages.

RICKETS

Figure 2 illustrates changes in the concentrations of different fractions of the acid-soluble phosphorus in the blood cells of rats found during the development of rickets induced by a high Ca low P diet, and during the healing of rickets induced by a single small dose of vitamin D. The data upon which this chart is based were derived from approximately 260 rats which were used in previously reported experiments (11).

The values indicated by the starting points of the curves are averages of those found in normal 50 gram rats. The dotted lines represent average values found in the bloods of normal rats, fed a normal diet, during the age period covered by the rickets experiments. Note that during the first few days of this age period the values for inorganic P and adenosinetriphosphate P increase, and that the values for organic acid-soluble P and diphosphoglycerate P decrease slowly; the latter values reaching constant levels after about 25 days.

The solid lines in the left half of Figure 2 represent averages of values found during the development of rickets, over a period of 25 days. The solid lines in the right half of the chart represent averages of values found in rats continued on the same rachitogenic diet, but given a single dose of 5 units irradiated ergosterol on the 21st day of the dietary period.

DEVELOPMENT: The development of rickets in these rats was associated with decreases in all fractions of the acid-soluble phosphorus. During the first 5 days the concentrations of inorganic P and adenosinetriphosphate P dropped abruptly to low levels and then remained constant through 25 days and longer. The organic acid-soluble P decreased progressively for about 20 days, but thereafter remained constant. The decrease in the organic acid-soluble P was accounted for almost entirely, after the first few days, in the diphosphoglycerate fraction which fell to about half its original concentration.

HEALING: The single dose of 5 units of vitamin D, given on the 21st day of rachitogenic diet, was just sufficient to induce ++++ healing in the epiphyses, as shown by line tests, but this dose was insufficient to restore the rachitic condition completely to normal. Following this dose of vitamin D, the first and most striking change was the sharp increase in concentration of diphosphoglycerate P in the cells - and with it the organic acid-soluble P. The diphosphoglycerate P increased to considerably above the expected normal level and then, about 7 days after the vitamin D was given, decreased again to slightly below that level. The adenosinetriphosphate remained practically unchanged for about 7 days and then increased; its increase coinciding with the secondary decrease of the diphosphoglycerate. During the first few days after the vitamin D was given, the inorganic P in the serum rose to about half its normal concentration, but fell again after 5 or

6 days. With this brief increase of the inorganic P in the serum, the concentration of inorganic P in the cells fell to zero, rising again only after about 7 days. All values were far below normal 22 days after the vitamin D was administered.

The time relationships of these changes must be considered to be only approximate, because of the variability found among different groups of animals. The sequence of the changes, however, deserves emphasis. During the development of rickets the inorganic P and adenosinetriphosphate P fell quickly to low concentrations, while the concentration of diphosphoglycerate decreased slowly over a longer period of time; with initiation of healing, the diphosphoglycerate increased first, the adenosinetriphosphate and inorganic P in the cells later. Recalling the reactions of the glycolytic cycle, the low concentrations of inorganic P and adenosinetriphosphate in the cells found immediately following the administration of vitamin D can be explained as probably due to the synthesis of phosphoglycerate proceeding faster than inorganic P was supplied to the system.

NEPHRECTOMY

In Figure 3 are represented chemical changes found in the blood of a dog during 7 days following the removal of both kidneys. The changes illustrated here are typical of those which we have found,

varying in magnitude, accompanying varying degrees of impairment or suppression of renal function in different clinical and experimental conditions.

Following the nephrectomy in this dog, the nonprotein nitrogen in the blood increased at a steady rate, the increase through the 7 days being represented by a practically straight line. The increase of inorganic P was almost as regular as that of the nonprotein nitrogen. The concentration of organic acid-soluble P in the cells increased from 50 to 97 mg. per 100 cc. This corresponds to findings reported by Ashley and Guest (12). Here, this increase is closely accounted for at each stage by changes in concentration of the diphosphoglycerate P which increased from 35 to 78 mg. per 100 cc.; the increases in OAS.P and DiPGl.P being respectively 47 and 43 mg. per 100 cc. It seems reasonable to believe that the increase of organic P in the cells under such conditions is mainly influenced by the excess of inorganic phosphates accumulating in the blood due to the failure of excretion of the waste endogenous phosphates.

During the 7 day period following the nephrectomy, the concentration of Cl in both serum and cells decreased markedly. Here we see a reciprocal relationship existing between changes in concentrations of diphosphoglycerate and of chloride in the cells, which may be seen again in other conditions illustrated in Figures 4 to 7.

PYLORIC OBSTRUCTION

Figure 4 illustrates changes found in the blood of a dog during 5 days following pyloric obstruction. Here again is demonstrated a striking reciprocal relationship between changes of chloride and diphosphoglycerate in the blood cells.

In this condition, as is well known, losses of chloride from the body by vomiting lead to progressive decreases of chloride in the blood, only partly compensated by increases of bicarbonate. The decreases of Cl in serum and cells, and increases of CO_2 in the serum, shown in Figure 4, are typical of findings commonly noted in this condition. In similar experiments, Guest and Andrus (13) previously found large increases in concentration of organic acid-soluble P in the blood cells, accompanying the decreases in concentration of Cl in the cells. Such changes again are demonstrated in Figure 4, and here the increases of organic acid-soluble P are closely accounted for at each stage by the parallel increases in the diphosphoglycerate.

In their earlier studies Guest and Andrus (13) also showed that in dogs with intestinal obstruction the parenteral administration of physiological salt solution prevented the increases of organic acid-soluble P in the blood cells, as well as preventing the decreases of chloride in the blood and the development of alkalosis.

AMMONIUM CHLORIDE ACIDOSIS

Figure 5 demonstrates changes found in blood cells and serum during the development of and recovery from ammonium chloride acidosis. This was a self-experiment by S. Rapoport, who took by mouth 25 gm. of ammonium chloride on two successive days, and 12 gm. the third day. (The NH_4Cl was taken in divided doses, in enteric coated tablets.) Immediately after the blood sample was taken at the end of the third day period, he took 30 grams of a mixture of sodium and potassium phosphate. During the three day period in which the NH_4Cl was taken, there was a marked increase of Cl in both serum and cells, the CO_2 content of the serum decreased nearly to half its initial level, and the pH decreased from 7.37 to 7.06. During the development of acidosis the concentration of organic acid-soluble P in the cells decreased from 48 to 32 mg. per 100 cc., and this change was almost exactly accounted for in the diphosphoglycerate fraction.

Similar decreases in concentration of organic acid-soluble P in the blood cells in ammonium chloride acidosis were first demonstrated by Haldane (14), also in a self-experiment. Haldane found that such acidosis as that described above, if left untreated, required 7 to 10 days for full recovery. This was found also by Rapoport (15) in a previous experiment when he first identified these changes of the organic acid-soluble P in the diphosphoglycerate fraction. Here,

however, as shown in Figure 5, the ingestion of the phosphate was followed by rapid recovery, partly demonstrable at 5 hours, and complete after 24 hours.

During the three day period of development of acidosis, the excretion of both P and Cl in the urine increased to more than double the normal rate. During the two days following ingestion of phosphate the urinary excretion of Cl decreased to considerably less than normal.

This and other observations have led to the clinical trial of phosphate solutions in the treatment of certain types of acidosis. One example, of an infant so treated, is cited in the next paragraph, and more data on this subject will be reported later.

ACIDOSIS OF GASTROENTERITIS

Figure 6 illustrates changes found in the blood of a one-month old infant with severe acidosis and dehydration, due to enteritis of undetermined etiology. The condition was attended with marked diarrhea, but no vomiting. Stool cultures were negative for dysentery bacilli. In the chart are represented data from blood analyses made daily or every second day during 11 days of the recovery period. The use of micro methods permitted these studies to be made on samples of 2 and 3 cc. of blood.

In the first blood samples examined, the CO_2 content of the serum was 3.5 m. eq. per l.; the Cl concentration was high in both serum and cells, the high Cl_c:Cl_s ratio indicating a very low pH; and the concentration of organic acid-soluble P in the cells was reduced to 30 mg. per 100 cc.

The curves demonstrate changes in concentrations of Cl in serum and cells, CO_2 in the serum, and organic acid-soluble P in the cells, found in the blood of this infant during 11 days. With adjustments of the CO_2 and Cl to normal concentrations, the concentration of organic acid-soluble P in the cells rose progressively, reaching a concentration higher than normal, and then returned to normal.

As treatment, the infant received salt and glucose solutions by continuous intravenous drip, and a blood transfusion on the third day. Sodium bicarbonate solution was given intravenously the first and second days in doses calculated to correct the acidosis, but only half the expected increase in serum CO_2 was obtained the first day. A phosphate solution was given by mouth the second and third days; this was stopped at the end of the third day when the organic acid-soluble P in the cells reached a normal concentration.

DIABETIC ACIDOSIS

Figure 7 illustrates blood changes found in the severe acidosis of diabetic coma, and during recovery from this condition. These observations were made on an adult patient of the medical service of the Cincinnati General Hospital, whom we were permitted to study by the courtesy of Dr. M. Blankenhorn and Dr. C. A. Mills. The patient had been in coma for several hours before she was brought to the hospital and the first observations were made.

In the first blood sample, drawn before treatment was started, the serum CO_2 content was 3.8 m. eq. per l., and the pH was 6.84; the concentration of Cl in the serum was 107 m. eq., and in the cells 80 m. eq. per l. The concentration of organic acid-soluble P in the cells was 22 mg. per 100 cc., and the diphosphoglycerate was too low for estimation - less than 3 mg. per 100 cc., probably zero.

Initial treatment consisted in the administration of insulin and the continuous intravenous injection of physiologic salt solution. After 3 hours, the pH of the blood serum was found unchanged, and the other values showed only slight changes. After 6 hours, when the findings on the second blood sample were noted, 30 gm. of sodium bicarbonate were given intravenously. Two hours after the sodium bicarbonate solution was administered, the serum CO_2 was found to be increased to 17 m. eq. per l., and the pH to 7.43. Twelve hours later (i.e., 22 hours after the first blood sample) the serum CO_2

content was found to be increased still further, to 37 m. eq. per l., and the pH to 7.65. Subsequently the CO_2 and pH values gradually fell to normal. The curves representing organic acid-soluble P and diphosphoglycerate concentrations in the cells follow courses parallel to each other, rising in the first 22 hours, then falling slightly at 48 hours, and thereafter gradually rising to normal levels. Clinically, the patient's condition appeared to be fairly well restored to normal after 48 hours, but even at the end of the fourth day the values for organic phosphorus in the blood cells were still abnormally low.

The changes of inorganic P and adenosinetriphosphate, closely parallel, deserve special attention. In the first 24 hours the concentration of inorganic P in the serum decreased to 0.6 mg. per 100 cc., and in the cells decreased to zero. (The curve INORG. P in Fig. 7 represents inorganic P in the whole blood.) During the same period the concentration of adenosinetriphosphate P in the cells decreased from 12 to 6.6 mg. per 100 cc. On the fourth day the concentration of inorganic P in the cells was still zero, and of adenosinetriphosphate P, only 4.8 mg. per 100 cc. On the seventh day, however, all values were normal. The decrease of inorganic P in the blood can be ascribed partly to the effects of insulin, partly to concomitant effects of correcting the acidosis - effects which favor the resynthesis of organic phosphorus compounds in tissues as well as in the blood cells.

During this period striking changes in the urinary excretion of phosphorus were found. During the first 4 hours the rate of phosphorus excretion in the urine was extremely high, but during the next 12 hours it decreased rapidly and in the second 24 hour period there was practically no phosphorus found in the urine. The urinary phosphorus excretion was negligible throughout the remainder of the 7 day period represented in the chart, but was normal on the tenth day.

These changes in urinary phosphorus excretion, together with the fact that the concentration of organic acid-soluble phosphorus in the blood cells increased so slowly, suggest that the labile phosphorus reserves of the patient had been exhausted by increased excretion during the period of acidosis. During the period of recovery, the resynthesis of the organic phosphorus compounds in the blood and presumably also in the tissues kept the concentration of both inorganic P and adenosinetriphosphate P in the cells very low until the diphosphoglycerate was restored and a certain equilibrium was reached between supply and demand.

Further studies are being made to see whether under such conditions the administration of phosphate will hasten recovery from diabetic acidosis and hasten the restoration of the organic phosphorus compounds in the blood cells to normal.

ELECTROLYTE EQUILIBRIUM OF THE BLOOD CELLS.

The data presented in Tables 1 and 2 show the close agreement that may be obtained between the total anions and total inorganic cations of blood cells when the organic phosphorus compounds are taken into account. The anion equivalencies of diphosphoglycerate in these blood samples were calculated from existing data (16) on the titration curve of this substance. Under "other organic P" is included the organic acid-soluble phosphorus not determined as diphosphoglycerate. Values assigned to this unidentified fraction were based on the titration curve of adenosinetriphosphate (17). While this fraction is known to include other compounds (mainly hexosephosphates), the error introduced by assuming it to be all adenosinetriphosphate is small. The equivalency of hemoglobin was calculated according to data of Hastings and co-workers (18) on dog hemoglobin, and data of Adair (19) on human hemoglobin. The bicarbonate values were corrected for carbamate CO_2 (20) and for free H_2CO_3.

In the two columns of Table 1 are listed the concentrations of anions and inorganic cations (total base) in the cells of blood samples drawn from a dog before and 2 days after pyloric obstruction. The changes found in the second sample are similar to those illustrated in Figure 3. The sums of the anions in each column differ from the values for the inorganic cations by only ± 3 per cent, and the diphosphoglycerate is shown to be the most important anion compensating for the decrease of chloride in the cells.

In Table 2 are listed the concentrations of anions and cations in the cells of three of the blood samples drawn from the diabetic patient represented in Figure 8. The blood samples were drawn respectively before treatment was started, 1 day later and 7 days later (the first, fourth and seventh samples shown in Figure 8.) In calculating the equivalencies of the ketone acids, it was assumed that B-hydroxybutyric acid made up 65 per cent of the total ketones determined in the first sample, and 75 per cent of the ketones in the second sample. In calculating the anion equivalency of the hemoglobin in all samples (venous blood) it was assumed that the oxygen saturation was 50 per cent. The oxygen content of the blood as drawn was not determined in these samples, as it was in the samples represented in Table 1. The saturation was probably less than 50 per cent in the first sample, and more in the others. While these assumptions introduce some uncertainties, such calculations adequately serve the purpose of the present discussion. In this example of diabetic acidosis, the decrease in concentration of diphosphoglycerate is seen to be the most important adjustment compensating for the increases of chloride and other anions in the blood cells. The values for total base, low in the first sample, and high after recovery, are typical of values which we have found in other cases. These data will be reported more fully in later communications.

DISCUSSION

These observations emphasize the importance of several functions of the organic acid-soluble phosphorus compounds of the red blood cells. Their participation in carbohydrate metabolism has been described by various investigators who have studied their role in the glycolytic process. Less attention, however, has been paid to other functions of these compounds which are suggested more or less clearly by the results of the studies here reported.

That these compounds constitute a phosphorus reserve of considerable importance is suggested especially by the observations on rickets and acidosis. In rats given the high Ca low P diet, the concentration of organic acid-soluble P in the blood cells decreased gradually during the ~~evolution~~ period of a phosphorus deficiency ~~in the body~~ and ~~the~~ development ~~of~~ the rachit~~o~~genic condition. The findings in acidosis suggest that the well-known increase of phosphorus excretion in the urine in this condition comes partly from the diphosphoglycerate of the blood cells. The slow return to normal concentration of these ~~o~~ organic acid-soluble phosphorus compounds during recovery from diabetic acidosis, where this reserve and probably also that in other tissues was exhausted, has been noted in the description of Figure 8. In these conditions, therefore, the concentration of diphosphoglycerate in the blood cells appears to be an index of the state of the body with regard to total amounts of labile phosphorus available.

Great lability of this "reserve" store of organic acid-soluble phosphorus compounds in the blood cells in abnormal circumstances is easily demonstrable in conditions such as those which have been cited here. This was also previously demonstrated in experiments dealing with the effects of large doses of irradiated ergosterol in rabbits (2) and in studies of the decomposition of diphosphoglycerate in acidified blood in vitro (4). Evidence that the organic acid-soluble phosphorus compounds of the blood cells are normally in a state of flux, continuously decomposed and resynthesized, has been reported recently by Hevesy (21) who studied the fate of radioactive phosphorus taken up by normal blood cells. In the normal human, and in normal rabbits, radioactive phosphorus injected intravenously was rapidly transferred from the plasma to the organic phosphorus compounds of the blood cells. Two hours after the injection, a considerable part of the organic phosphorus in the blood cells was found to be replaced by the "labelled" phosphorus. Such findings suggest that there is a continuous passage of inorganic phosphorus to and fro between cells and plasma, and that in the cells there occurs a continuous esterification of inorganic P and hydrolysis of organic P compounds. Hevesy's data indicate that through such processes the organic phosphorus compounds in the cells may be completely "rejuvenated" (i.e., decomposed and resynthesized) in 24 hours or less.

Along with their role as a labile phosphorus "reserve", the organic acid-soluble phosphorus compounds probably serve as intermediary transport substances for the transfer of phosphorus to

serve various needs of the body. The fact that the increased excretion of phosphorus in the urine in acidosis is accompanied by a decrease in the concentration of diphosphoglycerate in the blood cells suggests that this substance may act as a transport substance for phosphorus destined to be excreted by the kidneys. The fact that the concentration of diphosphoglycerate in the blood cells increases following suppression of renal function also may be cited as indirect evidence that such a function is served by this substance. The fact that the concentrations of organic phosphorus compounds in the blood cells of rats on a rachitogenic diet were closely related to the processes of development and healing of rickets may be placed in the same category of indirect evidence that these substances serve as transport substances, in this case to transfer phosphorus needed in ossification. Robison and co-workers (22) have suggested that phosphorus utilized in bone passes through an intermediate esterification before it is assimilated by ossifying cartilage. An intermediate esterification of phosphorus in the blood also may serve in the transport of phosphorus destined for bone formation.

The importance of organic acid-soluble phosphorus compounds as non-diffusible anions in the blood cells has been the subject of much conjecture. In 1923, when Van Slyke, Wu and McLean (23) summed up the then determinable anions and cations in horse blood cells and

found a considerable anion deficit, they suggested that this deficit probably lay in the unidentified phosphate esters. Others also have found variable anion deficits in similar studies of human blood cells in different conditions. The data presented here in Tables1 and 2 confirm the surmise of Van Slyke, Wu and McLean, and show that the most important of the phosphate esters, as an anion in the cells, is the diphosphoglycerate. The anion equivalency of diphosphoglycerate in human and dog blood cells is normally around 30 m. eq. per kg. of water; in pathologic conditions its concentration may decrease nearly to zero or increase to more than double. Through these changes, the diphosphoglycerate compensates for wide alterations in concentration of other anions and plays an important part in maintaining a state of equilibrium between the cations and anions of thecells.

The influence of diphosphoglycerate, as a non-diffusible anion in the red cells, upon the Donnan equilibrium will be treated elsewhere. Suffice to report here, the theoretical ratios for the distribution of the diffusible ions Cl^-, HCO_3^-, and H^+, calculated from observed concentrations of diphosphoglycerate and hemoglobin in the cells, agree closely with ratios actually observed in normal states and in the conditions of acidosis and alkalosis here described.

Again it should be noted that all such changes of diphosphoglycerate depend upon reactions of the glycolytic cycle, and upon various factors which influence those reactions. These findings thus bring the glycolytic process - an enzymatic system - into close relationship with other mechanisms concerned in adjustments of the acid-base equilibrium of the blood.

CONCLUSION

The organic acid-soluble phosphorus compounds of the red blood cells constitute a labile phosphorus reserve of considerable consequence, serving diverse functions. Readily synthesized and decomposed in the blood through reactions of the glycolytic cycle, these compounds participate in carbohydrate metabolism and also in the transfer of phosphorus in the body for other anabolic and catabolic processes. As non-diffusible anions in the cells, they play an important part in the maintenance of the acid-base equilibrium of the blood. The most important of these compounds, both in its normal concentration and in the magnitude of its changes in pathologic conditions, is the diphosphoglycerate. The concentration of diphosphoglycerate in the blood cells is <u>increased</u> following impairment or suppression of renal function and after pyloric obstruction; <u>decreased</u> in rickets, ammonium chloride acidosis, acidosis in infants with gastroenteritis, and in diabetic acidosis.

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BIBLIOGRAPHY

1. Fiske, C.H. and Subbarow, Y., Colorimetric determination of phosphorus, J. Biol. Chem., 1925, 66, 375.

2. Guest, G.M. and Rapoport, S., Effects of overdosage of irradiated ergosterol in rabbits: changes of diphosphoglyceric acid in the blood cells, J. Biol. Chem., 1938, 124, 599.

3. Lohmann, K., Über das Vorkommen und den Umsatz von Pyrophosphat in Zellen: Nachweis und Isolierung des Pyrophosphats, Biochem. Ztschr. 1928, 202, 466.

4. Rapoport, S., and Guest, G.M., J. Biol. Chem. (in press).

5. Rapoport, S., Über die Bestimmung der Glycerinsäure in freier und veresterter Form, Biochem. Ztschr. 1937, 289, 406.

6. Van Slyke, D.D., Determination of chlorides in blood and tissues, J. Biol. Chem. 1923, 58, 523.

7. Sendroy, Julius, Jr., Microdetermination of chloride in biological fluids, with solid silver iodate, II. Titrimetric analysis, J. Biol. Chem. 1937, 120, 405.

8. Peters, J.P. and Van Slyke, D.D., Quantitative clinical chemistry, Williams & Wilkins, 1931, V.2, 283.

9. Leva, Ernest and Guest, G.M., (unpublished data).

10. Guest, G.M. and Siler, V.E., Centrifuge method for determination of volume of cells in blood, J. Lab. & Clin. Med. 1934, 19, 757.

11. Rapoport, S. and Guest, G.M., Changes of organic acid-soluble phosphorus, diphosphoglycerate, adenosinetriphosphate, and inorganic phosphorus in the blood cells of rats during the development and healing of rickets, J. Biol. Chem. 1938, 126, 749.

12. Ashley, A. and Guest, G.M., Distribution of blood phosphorus after suppression of renal function, J. Clin. Invest. 1934, 13, 219.

13. Guest, G.M. and Andrus, W. De W., Chemical studies of blood in high intestinal obstruction; distribution of phosphorus and intracellular changes, J. Clin. Invest. 1932, 11, 455.

14. Haldane, J.B.S., Wiggleworth, V.B., and Woodrow, C.E., Effect of reaction changes on human inorganic metabolism, Proc. Roy. Soc., Lond., 1924 – 25, s.B., 96, 1.

15. Rapoport, S., Über Phosphorglycerinsäure als Transportsubstanz des Blutphosphors und ihr Verhalten bei experimenteller Ammonchloridazidose, Biochem. Zschr. 1937, 289, 411, 416.

16. a. Rubin, S.H., Determination of the buffer alkali in cutaneous blood, J. Biol. Chem. 1938, 126, 679.

b. Kiessling, W., Über die Titrationskurven einiger 3-Kohlenstoff-Phosphorsäuren ester und der Inosinpyrophosphorsäure, Biochem. Ztschr. 1934, 273, 103.

17. Lohmann, K., Untersuchungen zur Konstitution der Adenylpyrophosphorsäure. Biochem. Ztschr. 1932, 254, 381.

18. Hastings, A.B., Van Slyke, D.D., Neill, J.M., Heidelberger, M. and Harington, D.R., Studies of gas and electrolyte equilibria in blood. V. Acid properties of reduced and oxygenated hemoglobin, J. Biol. Chem. 1924, 60, 89.

19. Adair, G.S., The hemoglobin system. V. Relation of hemoglobin and bases, J. Biol. Chem. 1925, 63, 517.

20. a. Ferguson, J.K.W., Carbamino compounds of CO_2 with human hemoglobin and their role in transport of CO_2, J. Physiol. 1936, 88, 40.

b. Stadie, W.C., and O'Brien, Helen, The carbamate equilibrium. II. Equilibrium of oxyhemoglobin and reduced hemoglobin, J. Biol. Chem. 1937, 117, 439.

c. Dill, D.B., Edwards, H.T. and Consolazio, W.V., Blood as physicochemical system; man at rest, J. Biol. Chem. 1937, 118, 635.

21. Hevesy, G., The application of isotropic indicators in biological research, Enzymologia 1938, 5, 138.

22. a. Robison, R., and Rosenheim, A.H., Calcification of hypertrophic cartilage in vitro, Biochem. J. 1934, 28, 684.

b. Robison, R., MacLeod, M. and Rosenheim, A.H., Possible significance of hexosephosphoric esters in ossification; calcifica-

tion in vitro, Biochem. J. 1930, 24, 1927.

23. Van Slyke, D.D., Wu, H., and McLean, F.C., Studies of gas and electrolyte equilibria in the blood. V. Factors controlling the electrolyte and water distribution in the blood, J. Biol. Chem. 1923, 56, 765.

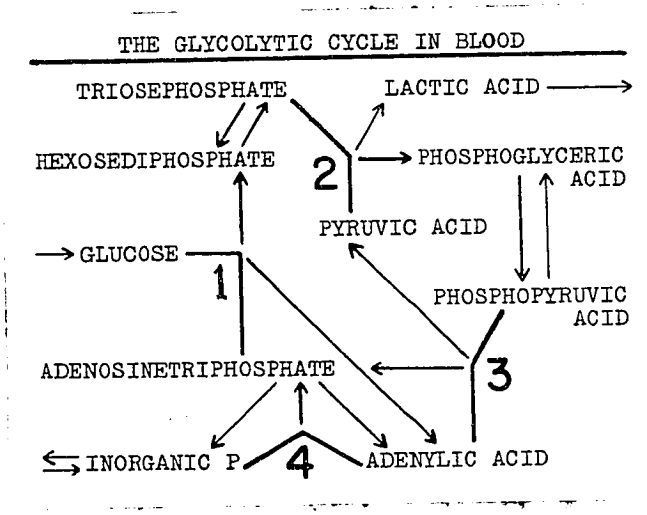

Fig. 1.

The glycolytic cycle in blood; a diagram based upon current theories of Meyerhof, Parnas, Dische and others.

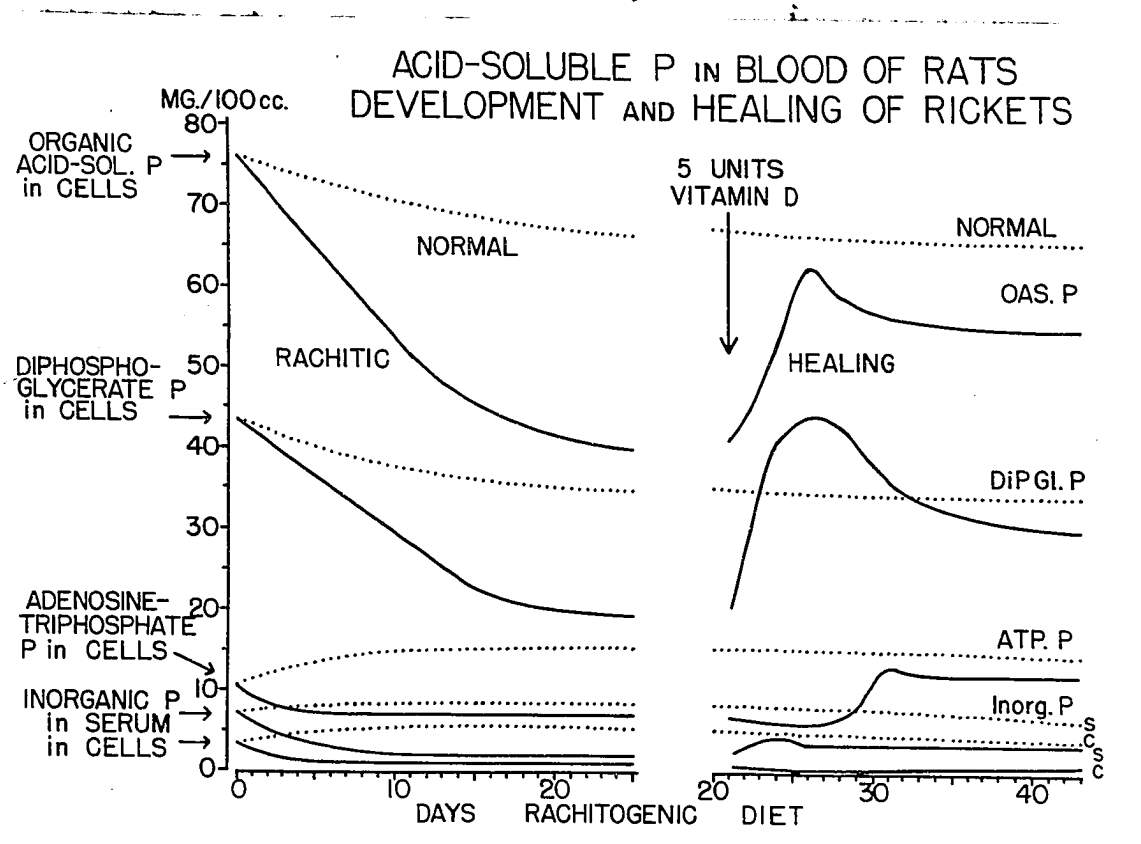

Fig. 2.

Changes of acid-soluble P in the blood of rats during the development and healing of rickets (continuous lines) and in the blood of normal rats (dotted lines) during the same age period.

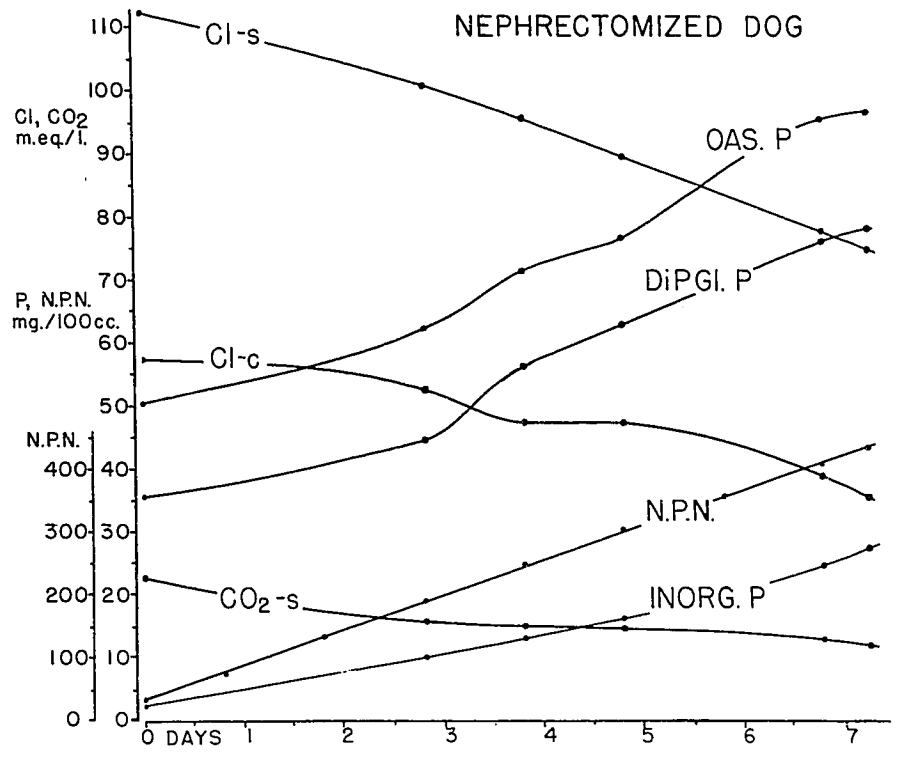

Fig. 3.

Changes in the blood of a dog following the removal of both kidneys.

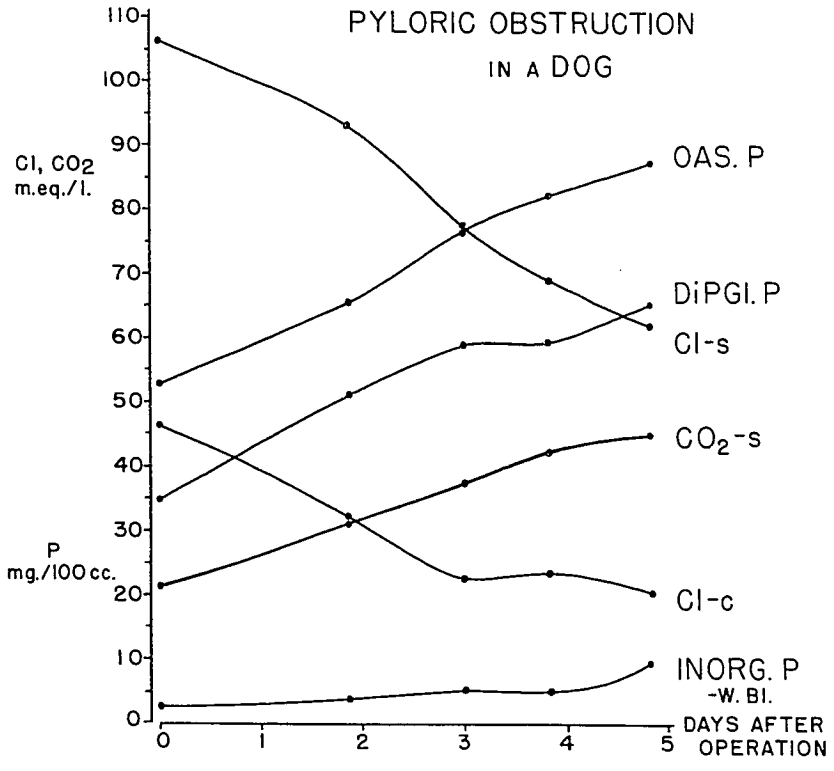

Fig. 4.

Changes in the blood of a dog following pyloric obstruction.

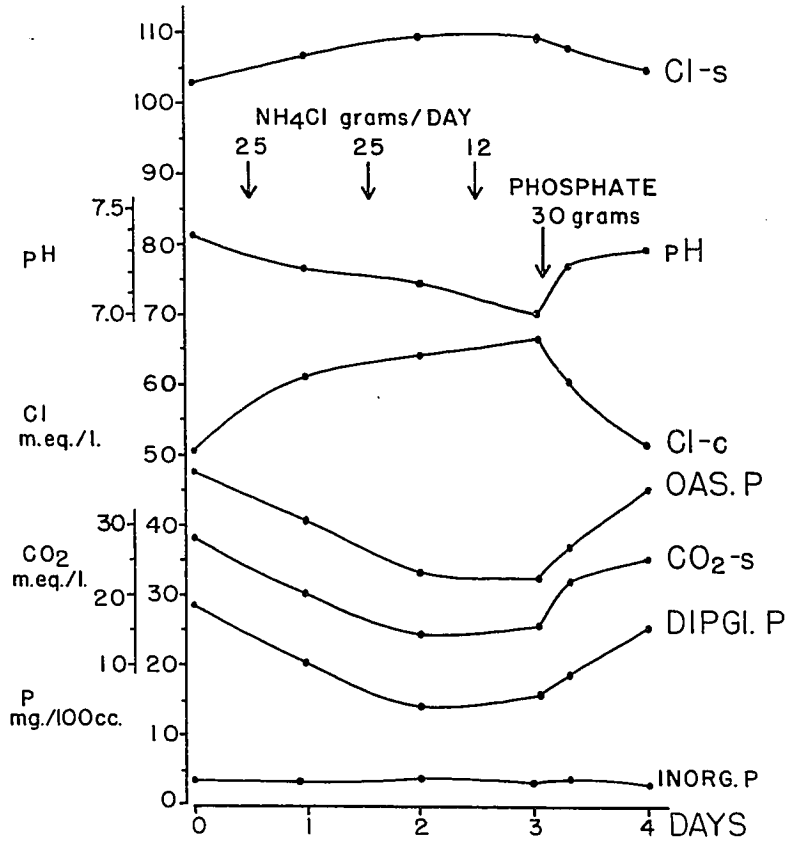

Fig. 5.

Changes in the blood of a man during the development of acidosis induced by the ingestion of ammonium chloride, and during recovery following the ingestion of sodium and potassium phosphate.

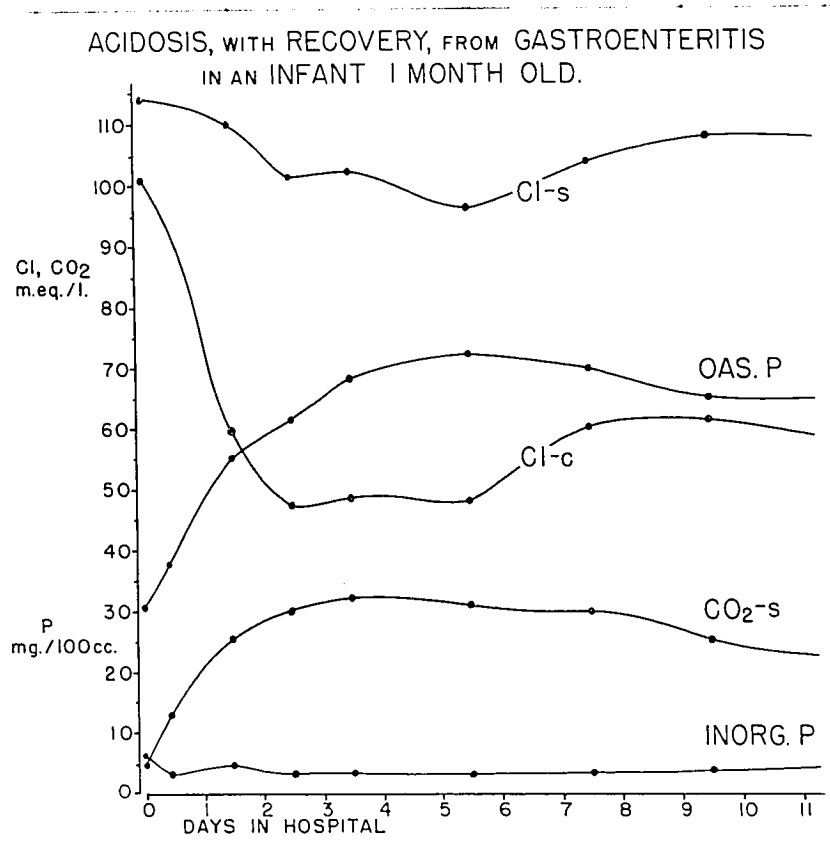

Fig. 6.

Blood changes in an infant during recovery from severe acidosis due to gastroenteritis.

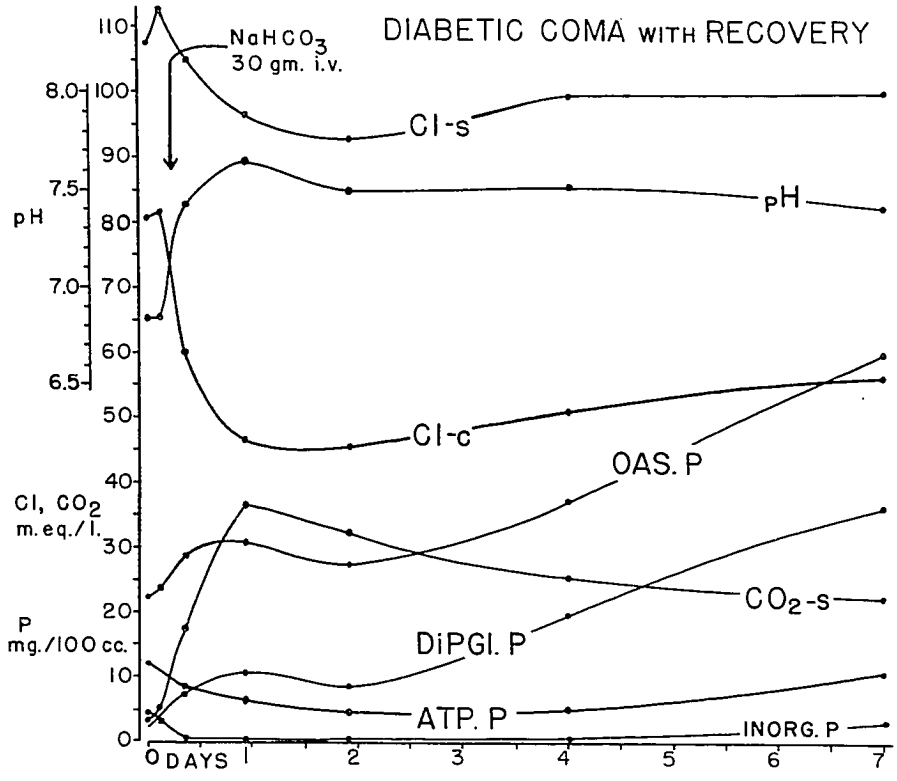

Fig. 7.

Blood changes in an adult woman during recovery from the severe acidosis of diabetic coma.

TABLE 1.

CONCENTRATIONS OF ANIONS AND CATIONS IN BLOOD CELLS OF A DOG, BEFORE AND AFTER PYLORIC OBSTRUCTION

	Before	2 Days After
	m.eq. per kg. water	
Chloride	65.3	23.5
Bicarbonate	16.8	22.0
Hemoglobin	41.8	37.5
Inorganic P	1.2	4.5
Diphosphoglycerate	33.5	65.4
Other Organic P	9.4	11.4
Sum Anions	168.0	164.3
Inorganic Cations	163.6	169.2

TABLE 2.

ANIONS AND CATIONS OF BLOOD CELLS IN DIABETIC ACIDOSIS AND DURING RECOVERY.

*	In Coma	Recovery	
		1 day	7 days
	m. eq. per kg. water		
Chloride	114.7	65.6	83.3
Bicarbonate	4.7	30.1	16.9
Hemoglobin	4.9	51.2	33.6
Inorganic P	2.4	0	2.0
Diphosphoglycerate	± 0	10.4	36.5
Other Organic P	11.0	14.2	15.7
Ketone Acids	±11.6	± 1.1	0
Sum of Anions	149.5	172.6	188.0
Inorganic Cations	140.1	175.3	189.0

* These blood samples are the first, fourth and seventh (last) represented in Figure 8.