The Left Shifted Oxyhemoglobin Curve in Sepsis:

A Preventable Defect

GEORGE M. WATKINS, M.D.,*† ALVARO RABELO, M.D., LOUIS F. PLZAK, M.D., GEORGE F. SHELDON, M.D.‡

From the Department of Surgery, Peter Bent Brigham Hospital, and Harvard Medical School, Boston, Massachusetts

Nine patients with severe sepsis were studied to determine causes for any alterations in oxygen dissociation. Seven of the patients had oxyhemoglobin curves shifted to the left of expected and diminished DPG levels. These deficiences were not corrected in one case. The other eight patients survived or expired with normal to elevated P_{50T} and DPG levels. In this study, three factors occurring either individually, in concordance, or in sequence were present when P_{50T} was decreased. Correction of these deficiencies lead to normalization and, in one case, exceedingly high P_{50T} and DPG levels. Where hypophosphatemia, acidosis, and transfusion of DPG deficient blood were avoided, no such change occurred. Hypophosphatemia is a common occurrence in the seriously ill patient whether or not hyperalimentation is used and may occur in spite of phosphate supplementation. Blood transfusions with CPD as the preservative are effective in reducing the severity of this disorder by the addition of an inorganic phosphate load. Septic shock itself had no untoward effect on oxygen dissociation. This held true even in the terminal stages of the disease process.

THE AMOUNT OF OXYGEN consumed by the body equals the cardiac output multiplied by the difference in oxygen content between arterial and venous blood. Oxygen content of blood is determined largely by the amount of oxygen in combination with hemoglobin. The equilibrium of the reaction may be viewed as being determined by substrate availability (that is, oxygen tension in the plasma environment and hemoglobin concentration) and the "kinetics" of the reaction (position of the oxyhemoglobin curve). Because the curve is sigmoidal, percent hemoglobin saturation and oxygen content are essentially maximal at oxygen tensions of 75 mm Hg or above. Once this plateau is reached, increases in PO_2 and shifts in the position of the oxyhemoglobin curve have little effect on oxygen content. Venous blood normally has a PO_2 of 40 to 45 mm/Hg. This range falls on the "steep" or vertical portion of the oxyhemoglobin curve. Small changes in the position of the curve can cause large alterations in venous oxygen content at or below these partial pressures.

The organic phosphate 2,3-diphosphoglycerate (DPG) affects the position of the oxyhemoglobin curve (also designated as P_{50T} when measured at standard condition) by decreasing the affinity of hemoglobin for oxygen. Reports,[8,19,20] reviews,[14,22] and textbooks[18] have recently suggested that intraerythrocytic DPG deficiency is an integral part of the pathophysiology of sepsis and septic shock. It is the purpose of this paper to analyze the changes in oxygen dissociation observed in such states.

Materials and Methods

Nine patients with severe sepsis were studied. All of the group had high fever, tachycardia, tachypnea, leuko-

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* Current address: Department of Surgery, University of Connecticut Health Center, Two Holcomb Street, Hartford, Connecticut 06112.

† National Institutes of Health Post-Doctoral Fellows in Surgery.

cytosis, and potentially overwhelming infections. Six of the nine had one or more episodes of hypotension characterized by attending physicians as septic shock. Pathogenic organisms were cultured from the blood in five of the nine cases.

In addition to the usual clinical parameters, the following were determined: DPG (by a modification of Lowrey's Technique[15]), arterial pH, oxygen and carbon dioxide tension,* plus serum phosphate and hemoglobin levels.† The position of the oxyhemoglobin curve at standard conditions (P_{50_T}) was measured by tonometering blood in known concentrations of carbon dioxide and oxygen at 37C. Percent oxyhemoglobin saturation at three different oxygen tensions was determined by spectrophotometer.† The pH of each sample was measured and a P_{50_T} was established utilizing computer programs derived from the Severinghous nomogram and the Hill Equation.[24]

Results

Six of the nine patients died. Infection was established as the major contributor to the demise of the five who had postmortem examinations. Three patients survived. These three patients had no definite episodes of septic shock, but manifested the other signs of severe sepsis.

* Radiometer Acid-Base Analyzer PHM 71 Radiometer A/S Copenhagen, Denmark.

† I.L. 182 Co-oximeter, Instrumentation Laboratories, Lexington, Mass.

While seven of the nine patients had diminished P_{50_T} and DPG levels during a portion of their septic course, only one of the patients who expired had such defects at time of death.

Three conditions were found to be associated with depressed P_{50_T} and DPG levels; recent massive infusion of old blood, acidosis and hypophosphatemia. In some instances two or more were present at the same time or as part of the train of debilitating events in the course of a patient's illness. With the correction of these deficiencies, the position of the P_{50_T} returned to normal or supernormal levels and there was a concomitant rise in DPG levels. Toble 1 summarizes the patients' profiles.

Massive Transfusion of Old Blood

Three patients had massive transfusions. Acid-citrate-dextrose (ACD) was the blood preservative utilized during the early part of one of the patient's illness. After her admission to Peter Bent Brigham Hospital, the blood preservative was citrate-phosphate-dextrose (CPD), as was true with all other patients.

Case Reports

Patient 1, D.M.: This 14-year-old white female was admitted to another hospital. She underwent emergency subtotal colectomy for ulcerative colitis. Infection of the right abdominal wall was noted three days later. At that time, rectal bleeding necessitated an abdominal perineal resection. She continued to bleed from her incisions and multiple transfusions of ACD blood were given. She was transferred to Peter Bent Brigham Hospital two weeks after

TABLE 1. *Profiles of Septic Patients*

Patient	Age	Primary Diagnosis	Associated Conditions	Complications	Cause of Death	Decrease in pH	Decrease in PO_4	Decrease in DPG	Decrease in P_{50}	Significant Infusion of Stored Blood
D. M.	14	Ulcerative colitis	None	Clostridial wound infection	Clostridia	No	Yes	Yes	Yes	Yes
J. M.	19	Ulcerative colitis	None	Bilateral subdiaphragmatic abscesses	Survived	Yes	Yes	Yes	Yes	Yes
K. R.	75	Incarcerated hernia	Emphysema	Clostridial wound infection	Clostridial sepsis	Yes	No	Yes	Yes	No
T. H.	76	Prostatism	Bleeding stress ulcer	Septicemia, pancreatic and subdiaphragmatic abscesses	Survived	No	No	No	No	No
F. J.	15	Septic abortion	None	Infected hydrothroax renal failure	Sepsis	Yes	Yes	Yes	Yes	No
T. M.	76	Cerebellar tumor	Myocardial infarction	Perforated viscus	Peritonitis*	No	No	No	No	No
L. C.	29	Granulomatous colitis	Multiple small bowel fistulae	Intra-abdominal abscesses	Survived	No	Yes	Yes	Yes	No
E. C.	77	Ruptured aortic aneurysm	Severe coronary arteriosclerosis	Acute renal failure	Sepsis	No	Yes	Yes	Yes	No
J. Mc.	37	Gunshot wound to abdomen	None	Renal failure peritonitis	Sepsis	No	No	Yes	Yes	Yes

Normal pH was greater than 7.37. Normal PO_4 was 3–4.5 mg%. Hypophosphatemia was considered significant only if levels less than 2.7 mg% persisted for greater than three days. Normal DPG was 13 ± 1 μm/gm Hb. Normal P_{50} was 24.5 ± 3mm Hg.

* Clinical diagnosis. No postmortem examination performed.

her original operation. Admission pulse was 130 and BP was 80/60. The patient, obviously jaundiced, was bleeding from open abdominal and perineal wounds. No evidence of disseminated intravascular coagulation was found. Gram negative rods were discovered on smears of aspirates from the abdominal wall. With the use of hyperbaria, antibiotics and infusion of whole blood, the patient improved transiently. An exploratory laparotomy performed during a hyperbaric treatment revealed widespread pelvic and retroperitoneal inflammation. Four days after admission, massive pleural effusions necessitated the placement of bilateral tube thoracostomies. Blood cultures grew out multiple organisms including *Clostridium perfringens* and *Escherichia coli*. In spite of continuing infusions of fresh blood and fluids, the patient deteriorated further, developing high output renal failure, respiratory failure, and a small bowel fistula. She expired a month after admission. During the last two week period, multiple blood cultures grew out *Escherichia coli*. At postmortem examination, the patient had the additional findings of hepatic vein thrombosis and bilateral acute tubular necrosis.

Comment

This patient had recurrent episodes of septic shock and bacteremia. Prior to her transfer to Peter Bent Brigham Hospital, she received massive quantities of ACD blood stored longer than 14 days. On admission she also received two units of CPD blood of greater than 14 days' age. At this time she was hypophosphatemic and had depressed P_{50_T} and DPG levels. Both the ACD and CPD blood were of such age that the intra-erythrocytic organic phosphates had been depleted. The patient was no longer hypophosphatemic after receiving CPD blood and was able to regenerate organic phosphates. With the use of fresh blood thereafter, normal DPG and P_{50_T} levels were maintained to the time of the patient's death. Figure 1 illustrates the pertinent findings.

Patient 2, J.M.: This 19-year-old white male was admitted to another hospital after having diarrhea for five days. Six days later, a caecal perforation with pelvic abscess was treated by tube caecostomy and drainage. A fecal fistula developed through the operative wound on the sixth postoperative day. Subtotal colectomy and "oversewing" of jejunal and ileal fistulae were performed. Tissue diagnosis was ulcerative colitis. The patient was transferred to Peter Bent Brigham Hospital two weeks later. At admission pulse was 120; BP, 128/70; respiration, 30; and temperature, 100F. A culture from a jugular catheter tip grew out *Staphylococcus aureus*. Colistin and carbenicillin were given. Four days later, perirectal and bilateral subdiaphragmatic abscesses were drained. Multiple cultures of the fistulae, abscess cavities, urine and sputum grew out *Escherichia coli* and *Pseudomonas aeruginosa* over the next month. Throughout these four weeks his temperature remained between 100 and 104F, while the heart rate was consistently 120–160 beats/minute. Blood pressure varied between 110/70 and 140/90. White cell count ranged from 5,500 to 21,000. The patient had recurrent toxic hallucinations. Multiple transfusions, large quantities of intravenous fluid, and protein hydrolysate hyperalimentation (Aminosol-Abbott Laboratories, Needham Heights, Massachusetts) were given during the course of his illness. The patient gradually improved and was discharged four months after admission with a mucocutaneous rectal stump fistula and a well functioning ileostomy. Phosphate, pH, DPG, hemoglobin and P_{50_T} changes during this period are shown in Fig. 2.

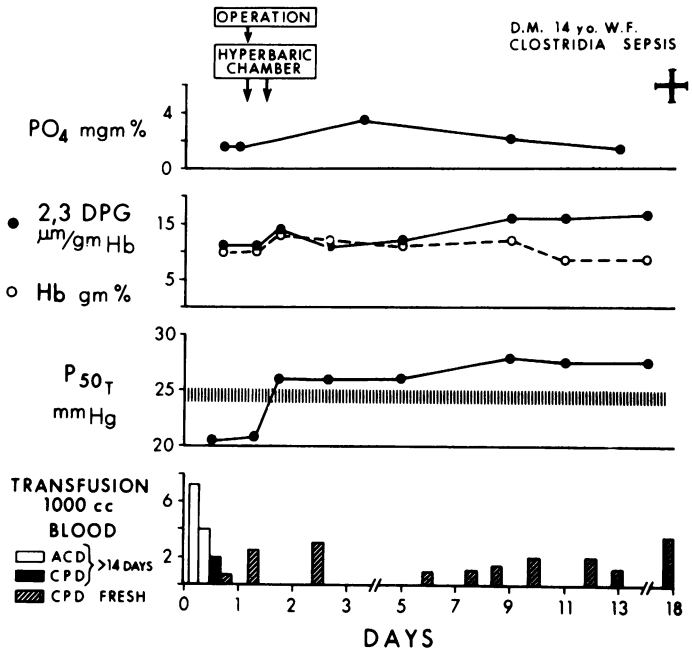

FIG. 1. The normal range (mean ± 2 standard deviations) of P_{50_T} in this and all other figures is shown by the bar with vertical stripes. pH in this patient was a constant 7.49 to 7.51 until the day of her death.

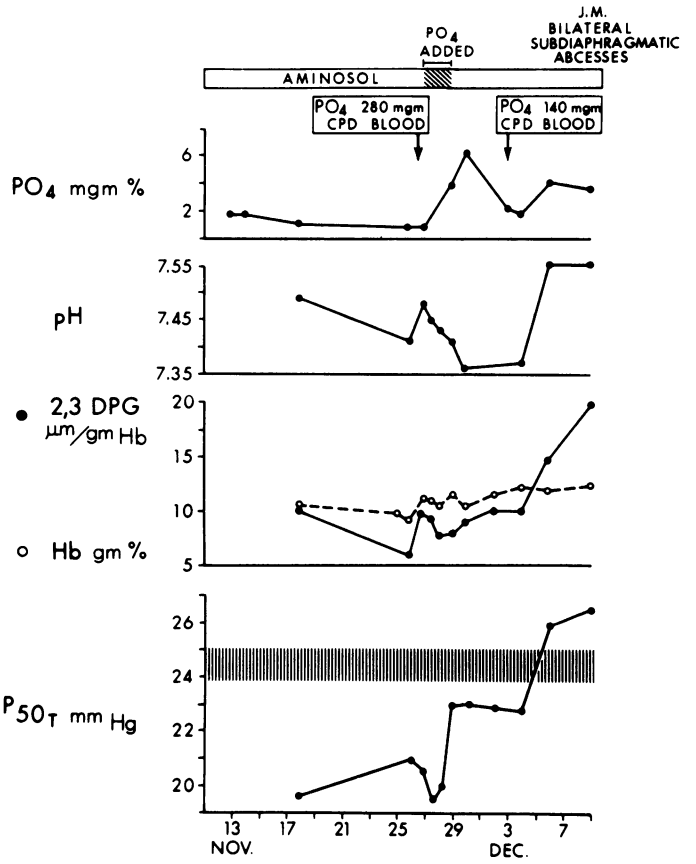

FIG. 2. Patient 2: Phosphate, pH, DPG, hemoglobin and P_{50_T} changes.

Comment

This patient had prolonged severe sepsis. The decreased P_{50T} was originally the result of phosphate deficiency after hyperalimentation with solutions containing no phosphate. Infusion of 400 cc of DPG deficient, CPD stored red cells in this 40 kg, immobilized, anemic patient represented at least a 25% dilution of his red cell mass. Thus, P_{50T} and DPG levels fell initially. Only after the serum phosphate rose, (because of the addition of inorganic phosphate contained in CPD blood), P_{50T} and DPG levels could increase. With continued hyperalimentation, phosphate levels fell again, and the same effects of CPD blood were noted with the second transfusion. The second transfusion consisted of three units of packed red cells, with the total inorganic phosphate infused per unit amounting to only ⅓ of that in a unit of blood. The lack of further elevation of P_{50T} during the period between transfusions was probably due to the mild acidosis present. For the remainder of his course, phosphate was added to the hyperalimentation solution. Figure 2 depicts these changes.

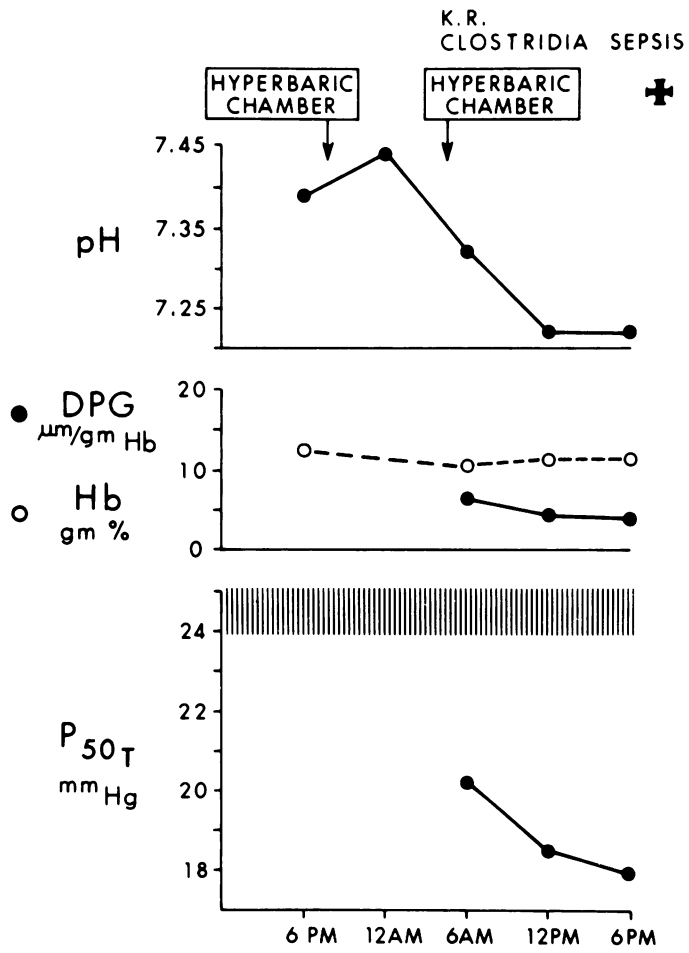

FIG. 3. Admission serum phosphate level was 3.6 mg% in this patient. No phosphate level was obtained the next day.

Acidosis

Three patients had respiratory or metabolic acidosis associated with a decreased P_{50T}. Two of the three patients had evidence of both septic shock and bacteremia. In one case, correction of the acidosis and associated phosphate deficiency resulted in increased DPG and P_{50T} levels. The patient survived. In the second instance the defects were corrected, but the patient expired. The third case represented acidosis without hypophosphatemia.

Case Report

Patient 3. K.R., a 65-year-old white male, was admitted to another hospital with abdominal pain. At laparotomy an incarcerated, non-stangulated inguinal hernia was found and reduced. Three days later the patient developed fever, hematuria and icterus. He was transferred to Peter Bent Brigham Hospital. On admission, pulse was 120; blood pressure 70/40; respiration, 16; temperature, 102F. The patient was icteric, agitated, and restless. Brawny edema was present over much of the trunk; crepitus was noted around the abdominal and groin incisions. The patient also had right lower lobe pneumonia. He was started on cephalothin and tetracycline intravenously, plus blood and plasma infusions. Hyperbaric therapy was begun. The next day, in spite of continuing massive infusions of plasma and isuprel and further hyperbaric treatment, his blood pressure continued to drop. He expired at 7:00 p.m. Cultures of tissue aspirates grew out *Clostridium perfringens* and blood cultures grew out diphtheroids. Postmortem examination confirmed the clinical impression of massive *Clostridium perfringens* infection. The pertinent findings in this case are shown in Fig. 3.

Comment

This patient had decreased P_{50T} and DPG levels when first studied. As acidosis increased, both deficiencies became more prominent. No abnormalities of serum phosphate were noted.

Below a pH of 7.35, DPG production is inhibited progressively as hydrogen ion concentration rises. The depressed DPG levels seen were probably a result of acidosis and not any specific effect of sepsis. It can be overcome by coexisting hyperphosphatemia.[1] This patient was the only one in the series who died with P_{50T} depression.

Hypophosphatemia

During septic periods, five patients had hypophosphatemia associated with abnormalities of DPG and P_{50T}. Two of these patients were receiving intravenous hyperalimentation solutions containing no phosphate. When hypophosphatemia was obviated (with an additional correction of acidosis in one case) P_{50T} and DPG levels were normalized or increased. One patient had hypophosphatemia and a depressed P_{50T} without obvious cause when first studied. She was not on hyperalimentation or phosphate binders. One patient was treated with hemo-

dialysis and aluminum hydroxide gel by mouth concomitantly. He also developed hypophosphatemia while not on hyperalimentation.

Case Reports

Patient 4. E.C., a 76-year-old white male, with a history of previous myocordial infarction, entered another hospital with a one day history of back pain and weakness. After emergency resection of a ruptured abdominal aortic aneurysm, the patient developed acute renal failure. On the first postoperative day, he had bloody diarrhea. Emergency laparotomy revealed no evidence of intestine with compromised viability. A tracheostomy was performed because of recurrent atelectasis. He was transferred to Peter Bent Brigham Hospital two days later. On admission pulse was 72; blood pressure, 160/80; and temperature, 98F. The patient was alert, cooperative, and had slight peripheral cyanosis. BUN was 98; white blood count was 7,200. He was placed on hemodialysis and aluminum hydroxide by mouth. An atrial flutter with a slow ventricular rate required the insertion of a demand pacemaker. His temperatures ranged between 102 and 105F. Multiple sputum and urine cultures grew out *Pseudomonas aeruginosa,* Bacteroides species and *Klebsiella pneumonia.* Kanamycin was given intermittently to maintain adequate blood levels. Massive infusion of blood and plasma substitutes were required to combat the recurrent episodes of hypotension occurring during the remainder of the patient's course. Hyperalimentation with protein hydrolysate (Amigen-Travenol Laboratories, Edison, New Jersey) was begun one month after admission. Aluminum hydroxide was stopped shortly thereafter. Ten days later, an isuprel infusion was required to maintain blood pressure, and blood cultures grew out Pseudomonas aeruginosa. The patient had a transient cardiac arrest after two more days and expired a few hours later.

Three dye dilution cardiac outputs were performed during the patient's hospital course. His heart rate was between 100 and 110 during the time the cardiac outputs were performed, with no evidence of hypovolemia or hypotension. The flow recorded was never above 3.5 liters per minute. At postmortem examination, the lower lobe of the right lung had multiple microabscesses. Acute pericholangitis, centrilobular hepatic necrosis, acute tubular necrosis, two old myocardial infarctions, and a severely compromised coronary circulation were also found. The parathyroid glands were normal. Figure 4. depicts the pertinent changes.

Comment

This patient died after recurrent episodes of septic shock and bacteremia. He was anemic and could not elevate cardiac output because of myocardial disease. In either instance both DPG and P_{50T} levels usually are elevated well above normal. Hypophosphatemia prevented this compensation. The original phosphate deficiency probably was induced by the combination of "phosphate binders" by mouth and dialysis. After correction of the initial defect, the patient was maintained on hemodialysis and a hyperalimentation regimen which included phosphate. Hypophosphatemia did not occur. Multiple transfusions of CPD blood were given. Although unknown factors may also have prevented hypophosphatemia, the number of CPD transfusions helped maintain inorganic phosphate levels. As hyperphosphatemia occurred, DPG and P_{50T} levels continue to rise. The terminal P_{50T} and DPG levels were the highest ever recorded in this laboratory.

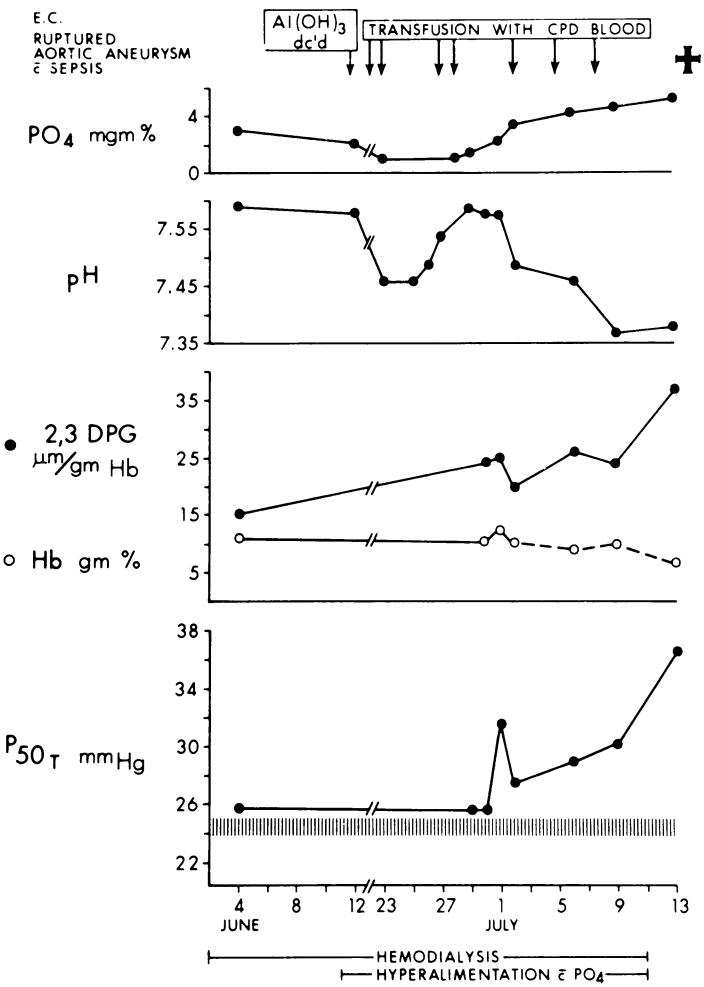

Fig. 4. Patient 4.

Patient 5. F.J., a 15-year-old white female, was transferred from a pediatrics hospital. Past history was unremarkable. One week prior to admission she attempted an abortion using a "sterile catheter." Two days later she developed chills, fever, vaginal bleeding, and hypotension. Admitting diagnosis was septic shock secondary to septic abortion. She was treated with penicillin, steroids, digitalis, and plasma expanders. She was transferred to Peter Bent Brigham Hospital five days after her original admission. At admission, pulse was 170; BP, 150/70; temperature 96.5F. A grade II/IV systolic murmur, no bowel sounds, and a large amount of vaginal debris were positive physical findings. White cell count was 12,100, SGOT was 63, and LDH was 432. Cephalothin and kanamycin were given. Dilatation and curettage was performed. She developed increasing fever, anemia, respiratory insufficiency, renal failure and hypertension. Blood cultures grew out Bacteroides species. Antibiotic therapy was changed to chloramphenicol, but she continued to have bacteremia with hypotension. Hemodialysis, steroids, digitoxin and hyperalimentation with Amigen were added to the regimen. Blood cultures began to grow out *Proteus vulgaris* two weeks later. Transient cardiac arrest occurred on this date. The patient expired 24 hours

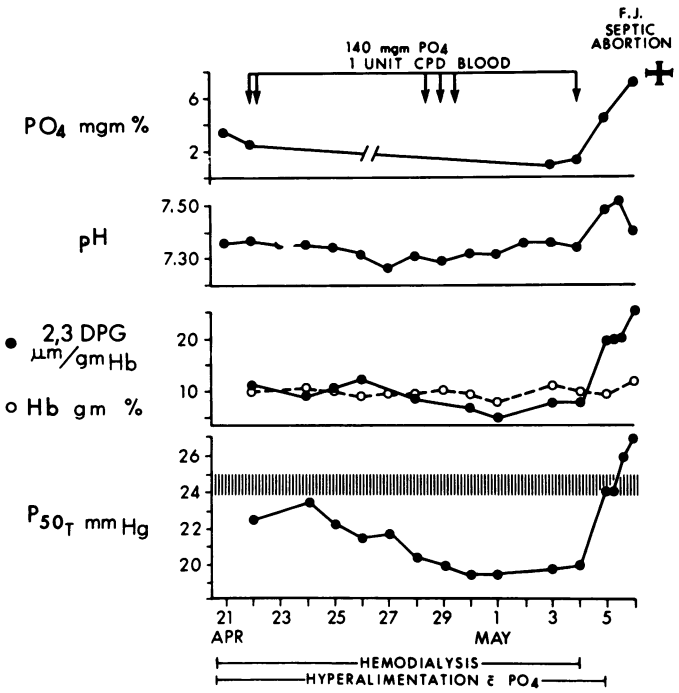

Fig. 5.

later. At postmortem examination bilateral necrotizing bronchiopneumonia with pulmonary abscess and empyema formation was found, as well as hepatic infarction and hepatic vein thrombosis. Pertinent findings are shown in Fig. 5.

Comment

This patient had repeated episodes of septic shock with multiple organisms being cultured from her blood. Her DPG and P_{50T} depletion was primarily due to hypophosphatemia and to a lesser extent, mild acidosis. The phosphate deficiency existed in spite of hyperalimentation with standard amounts of phosphate and multiple blood transfusions of CPD blood containing 140 mgm of phosphate per unit of blood. The combination of increased phosphate use in metabolic pathways plus hemodialysis, a procedure which lowers serum phosphate levels, probably accounts for the electrolyte abnormalities. After cardiac arrest, hyperalimentation was stopped. Initially, the patient developed metabolic alkalosis and mild hyperphosphatemia. P_{50T} and DPG levels rapidly rose. Later, pH dropped to 7.40, but serum phosphate rose to 6.0 mgm%. The continued increase in P_{50T} during the final stages was due to hyperphosphatemia.

No Abnormalities

Two patients had no abnormalities of serum phosphate, P_{50T}, or DPG. One died and one recovered. Neither massive transfusions of blood nor acidosis occurred during these patient's courses.

Discussion

Besides organic phosphate levels,[3,7] and the Bohr effect,[5] there are other modifiers of hemoglobin affinity for oxygen.[2,12,28] The importance of these factors needs further clarification. In acute alkalosis, P_{50} is lowered *in vivo*. Oxygen delivery is kept constant by a decrease in mixed venous oxygen tension.[28] The same mechanism obtains in moderate anemia (6.5 to 10 gms% of Hb),[29] in cases of myocardial dysfunction[30] and, acutely, after large losses of intravascular volume.[16] In states of hypoxia[13] and increased energy demands,[9] decreased venous oxygen tension plays a significant role in maintenance of oxygen delivery. DPG deficiency appears to be offset by this same mechanism of increased extraction.[6] Three major causes of DPG deficiency known to occur *in vivo* are hypophosphatemia,[23] acidosis,[1] and transfusion of large quantities of stored blood.[27] As a preservative for blood,[26] ACD has been the subject of increasing criticism. DPG and ATP levels are markedly reduced by the fifth day of storage. When CPD is used as a preservative, organic phosphates do not deteriorate until the seventh to tenth day.[4]

Another benefit of CPD preserved blood was apparent in the patients with hypophosphatemia. The effective inorganic phosphate in a unit of ACD preserved blood is that originally contained by the blood; approximately 20 mgm. While plasma inorganic phosphate levels in stored blood rise with length of storage, the phosphate increase is that produced by the degradation of organic phosphates. An equivalent amount of inorganic phosphate must be used to replete intracellular organic phosphates after transfusion of such blood. The inorganic phosphate in CPD preservative adds 140 mgm to body stores whenever a unit of CPD blood is infused. The normal 70 kg male has a total body water of 39,000 cc.[21] While one unit of ACD blood will have no effect on total body phosphate, one unit of CPD blood would theoretically increase inorganic phosphate concentration by approximately 0.4 mgm%. If total body water were of lesser magnitude, the increase would be more pronounced.

While the direct effect of acidosis *in vivo* offsets to a great degree the P_{50} changes due to inhibition of DPG, a sudden shift to alkalosis in such a situation would produce a double deficit in oxygen transport. The depression of P_{50} by alkalosis is additive with the depression due to decreased DPG. Patients with acute respiratory insufficiency often exhibit such changes of pH during treatment. Acidotic patients who suffer cardiac arrest often become acutely alkalotic due to hyperpnea and sodium bicarbonate infusion.

Alkalosis, under normal circumstances, rapidly depresses P_{50} *in vivo*, but more slowly stimulates DPG synthesis, elevating P_{50T} in both adaptation to altitude[17]

and to anemia,[29] maintaining the dissociation of oxygen at near constant levels *in vivo*. This paradoxical occurrence probably maintains homeostasis by an increase in enzymatic activity, thus increasing DPG synthesis. In acidosis the same paradox holds true, but in an opposite direction (acidosis inhibits DPG production, decreasing P_{50_T} while acidosis itself has a direct effect of increasing P_{50} *in vivo*).

If phosphate deficiency occurs with alkalosis, the DPG changes and Bohr effects become additive because DPG cannot be produced. A much worse situation occurs than in isolated acute alkolosis where DPG values may be normal. On the contrary, where acidosis and hyperphosphatemia co-exist, the P_{50} *in vivo* may actually be far to the right of the P_{50_T}

The roles of acidosis and old blood were not surprising. The high rate of occurrence of hypophosphatemia was not anticipated. The hypophosphatemia occurred in four situations: a regimen of hyperalimentation without phosphate; hyperalimentation and hemodialysis; hemodialysis and use of phosphate binders; a case in which none of these were present.

A discussion of the many causes of hypophosphatemia is beyond the scope of this paper. However, on one ward randomly picked for study, 35% of the patients were hypophosphatemic at some time during hospitalization. While it is not suggested that all these patients had DPG and P_{50_T} depressions, we are in an era in which determination of inorganic phosphate levels must become as routine as other laboratory tests. Especially in the critically ill patient receiving hyperalimentation therapy, we can no longer depend upon small amounts of phosphate being infused and occasional serum phosphate levels determined as has been the standard.[10]

Relatively minor changes in venous oxygen tension can ordinarily offset the P_{50_T} changes.[28] But at some point cardiac output must also increase.[21] The venous oxygen tension reserve is compromised already when anemia, hypovolemia or increased energy demands are present either singularly or in combination. Where compensation cannot be further made, lactic acidosis appears.[11] The small changes are best described by noting that a 2 mm shift in the oxyhemoglobin curve position or a 2 mm change in venous oxygen-tension at the normal value of mixed venous blood are equivalent to 1.12 liters of cardiac output change.[29]

In the individual with severe sepsis or septic shock, the additional insult of a shift in P_{50_T}, although these shifts are small in quantity, must be met by relatively large increases in cardiac output once the venous oxygen tension reaches a critical level. The subsequent acidosis occurring when both mechanisms are exhausted will cause decreased oxygen affinity, temporarily offsetting the untoward P_{50_T} changes *in vivo*. However, such acidosis could set off a cascading effect of further inhibition of DPG production with continued *in vivo* lowering of the P_{50} causing still further increases of lactic acid production.

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