under 4b were carried out. The isotope was infused for 5 hours. The plasma level of $Sr^{85}$ rose gradually and was 5.5% at 8 hours (column A): it declined thereafter at rates comparable to that of the rapid injection of $Sr^{85}$. Urinary strontium excretion increased gradually and was highest at 8 hours. The cumulative 24 hour urinary excretion was 13.8% (column A). The marked enhancement of strontium excretion by simultaneous but slow administration of the same dose of calcium, approximately 1 g $Ca^{++}$, infused for 1 hour preceding and for 5 hours simultaneously with $Sr^{85}$ is shown in column B. The cumulative 24 hour urinary strontium excretion rose to 23.4%.

These experiments illustrate that the major route of excretion of intravenously administered strontium is via the kidney; in 15 days as much as 57% was excreted in one patient. Calcium significantly increases renal clearance of strontium and thereby decreases the body load.

*Summary.* Radiostrontium metabolism following intravenous administration of tracer doses of strontium$^{85}$ was studied in 4 human subjects. Plasma levels, urinary and fecal strontium excretion were measured. The plasma levels declined rapidly. The main route of excretion is via the kidney. Rate of strontium excretion seems to depend upon the state of bone metabolism. It was low in Paget's disease and high in senile osteoporosis. Simultaneous administration of calcium gluconate significantly enhanced the strontium excretion.

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## Mechanical Fragility of Erythrocytes from Patients with Paroxysmal Hemoglobinuria.* (22198)

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It has been established that pH is an important factor in *in vitro* hemolysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria (PNH). *In vitro,* hemolysis of PNH erythrocytes in normal human serum is characterized by: 1) a hemolytic system which requires properdin, complement and $Mg^{++}$(4), all normal constituents of normal serum; 2) absence of abnormal hemolysins or agglutinins in patient's serum(2,3,4); 3) maximum hemolysis at pH 6.5-7.0; 4) minimal hemolysis in normal human serum outside pH range 6-8.

As pH of PNH blood is decreased from pH 7.8, and hemolysis becomes maximum, it is conceivable that the hemolytic system could weaken the erythrocyte cell wall in such manner that *in vivo* trauma would augment hemolysis. This question was submitted to test by determining mechanical fragility (MF) *in vitro,* of normal and PNH erythrocytes in active and inactive properdin systems at varied levels of pH.

*Materials and methods.* To determine the effect of decreased pH on MF, hydrochloric acid of N/1.2 to N/8 was used to obtain the desired pH of blood samples. To maintain this pH during rotation, imidazole in HCl (3.44 g imidazole/100 ml of 0.2 N HCl) was used as buffer because of its effectiveness when used in small quantities(7). Although this solution is hypertonic, it becomes approximately isotonic (isotonic equals 1.72 g/100 ml of 0.1 HCl), when it is diluted with equal volume of HCl used to alter pH. Defibrinated blood samples were obtained from 3 normal males, 3 normal females and 3 patients with PNH. Of the 3 PNH pa-

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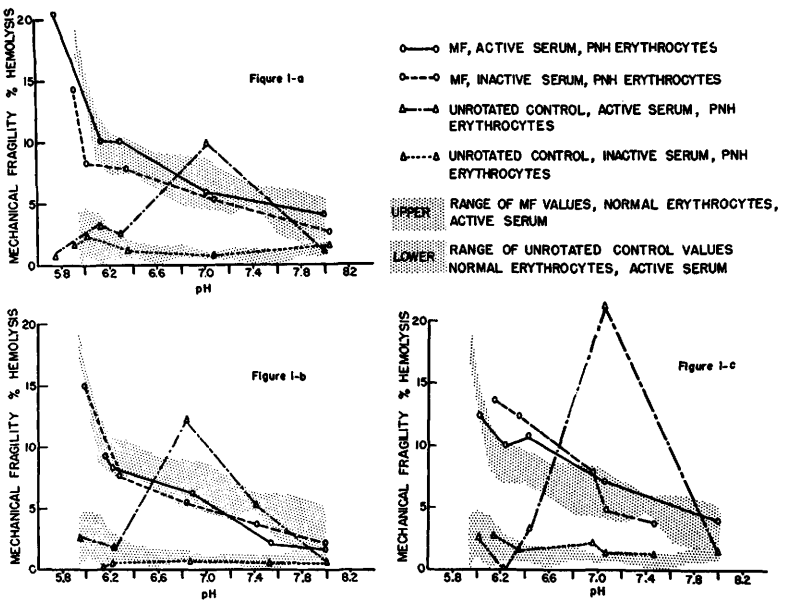


FIG. 1. Results of mechanical fragility studies done at various values of pH on blood of 3 patients with paroxysmal nocturnal hemoglobinuria (Fig. 1-a, 1-b, and 1-c correspond to Cases I, II, and III respectively) and 6 normal subjects.

tients, Cases I and III (Fig. 1-a and 1-c) had approximately 50% transfused cells at time of observation. The erythrocyte population of Case II (Fig. 1-b) contained no transfused cells at time of observation. Each sample was adjusted to 35% hematocrit and divided into aliquots (1.5 ml). The aliquots obtained from each PNH sample were divided into 2 groups, one group was designated the active group and the other inactive. Serum from each aliquot was removed and placed into corresponding tubes. Serums of inactive aliquots were heated in 60°C waterbath for 30 minutes to inactivate complement, thus destroying the activity of PNH hemolytic system, while the hemolytic system of active serum was allowed to remain intact. From the serum of each aliquot, 0.2 of the total volume was removed. This 0.2 volume was replaced by and thoroughly mixed with 0.1 volume of HCl and 0.1 volume of imidazole buffer to obtain the desired pH. The erythrocytes were resuspended in the acidified buffered serum and thoroughly mixed. A pH was determined on each sample in a Beckman pH meter (Model G) before and after the MF determination, the average of the two being used for interpretation of results. The MF of each aliquot was determined according to the method of Shen, Castle, and Fleming (9) as modified by Ham(1).

*Results.* Range of values of MF for normal erythrocytes in the pH range 5.95-8.0 is given in Table I and represented by the upper shaded area in Fig. 1. Range of percent hemolysis occurring in unrotated samples of normal erythrocytes subjected to a similar pH range is given in Table I and represented by the lower shaded area in Fig. 1. Normal values represented in Table I and on Fig. 1 were obtained by extrapolation of maximum and minimum values of MF at every 0.2 pH unit from a composite graph containing results of studies on 6 normal subjects.

Results of studies done on three PNH patients are presented in Fig. 1-a, 1-b, and 1-c. The curves labeled unrotated control, active serum, PNH erythrocytes in these Figures demonstrates the hemolysis of PNH erythrocytes in acidified serum. This observation indicates that the properdin system is intact in samples with active (unheated) serum. Correction for hemolysis in the rotated active sample attributable to the PNH mechanism

TABLE I. Range of Values of MF of Normal Erythrocytes at Various Values of pH.

| pH | No. of observations | Mechanical fragility Hemolysis in % Range | Avg | Unrotated control Hemolysis in % Range | Avg |
|---|---|---|---|---|---|
| 5.95 | 3 | 16.8 -19.2 | 17.5 | 0 -4.3 | 1.8 |
| 6.0 | 4 | 14.0 -15.9 | 14.6 | 1.0-5.6 | 1.9 |
| 6.1 | 4 | 8.3 -12.2 | 9.8 | 0 -4.1 | 1.4 |
| 6.2 | 6 | 7.1 -10.4 | 9.1 | .5-2.8 | 1.2 |
| 6.4 | 6 | 7.1 - 9.8 | 8.5 | .5-1.8 | .9 |
| 6.6 | 6 | 5.7 - 8.8 | 7.6 | 0 -1.5 | 1.0 |
| 6.8 | 6 | 4.6 - 8.4 | 6.5 | .6-1.5 | 1.1 |
| 7.0 | 6 | 4.3 - 8.2 | 6.4 | .4- .9 | .7 |
| 7.2 | 6 | 3.8 - 7.0 | 5.6 | .4-1.2 | .8 |
| 7.4 | 6 | 4.6 - 6.0 | 5.2 | 0 -1.1 | .8 |
| 7.6 | 6 | 2.1 - 5.9 | 4.4 | 0 -1.1 | .8 |
| 7.8 | 5 | 1.6 - 5.6 | 3.7 | .6-1.6 | 1.0 |
| 8.0 | 4 | 1.70- 5.0 | 3.7 | .6-1.6 | 1.3 |

was done by subtraction of unrotated active control value from the active rotated sample to obtain per-cent hemolysis attributable to mechanical trauma.

The reproductibility of the technic was tested by dividing a sample of normal blood into 7 aliquots and determining the MF of each separately after adding the same amount of acid and buffer. The range of pH values of the 7 aliquots was 6.9 to 7.05 and the range of MF values was 5.2-8.5%.

Morphologic evaluation of cell shape was done by examination of Wright stained erythrocytes. Spherocytes and budded forms were seen at pH 6 in both PNH and normal erythrocytes.

*Discussion.* It has been postulated that increased MF of erythrocytes may be associated with increased spheroidicity or firm agglutination of red blood cells(10). Increased spheroidicity was observed at pH 6, but no agglutination occurred in the studies. Spheroidicity may be explained as follows: With a decrease in intracellular pH, the amphoteric hemoglobin molecule shifts toward its undissociated state, each hemoglobin molecule releasing a maximum of 10 $K^+$. Since each $K^+$ acts as an osmotic particle, the osmotic gradient into the cell is increased. This is further augmented by the passage of $HCO_3^-$ and $Cl^-$ into the cell to neutralize the excess of impermeable $K^+$. Osmotic equilibrium is maintained by the passage of $H_2O$ from the serum into cells thus increasing cell volume (8).

It has been proposed that erythrocytes of congenital spherocytosis, sickle cell anemia and acquired hemolytic anemia may be conditioned in the spleen in such manner as to increase both osmotic and mechanical fragility(5). Perhaps pH is one factor in the "conditioning" process taking place in the spleen. Studies of pH in splenic circulation are not at present available. However, Watson and Paine conclude that red cells in the splenic vein are slightly more spheroidal than cells in the splenic artery(6).

*Summary.* 1. As pH of blood samples from 6 normal subjects was decreased, susceptibility of normal erythrocytes to mechanical trauma or their mechanical fragility was increased. 2. MF determinations on PNH and normal erythrocytes were done at various values of pH including the range for optimum complement activity in normal serum and it was found that mechanical trauma did not contribute to hemolysis of normal or PNH erythrocytes even though the PNH hemolytic mechanism was active. 3. It is postulated that increased red cell volume due to a shift of $Cl^-$, $HCO_3^-$ and $H_2O$ into the erythrocyte, may be the mchanism of increasing spheroidicity of the erythrocyte and the MF as a function of decreasing values of pH.

*Conclusion.* 1. MF of erythrocytes of the PNH patient in an active properdin system does not differ from MF of the same erythrocytes in an inactive properdin-complement system, nor does the MF of these erythrocytes differ from that of normal erythrocytes at values of pH between 8.0 and 5.9. 2. The hemolytic system of PNH, which requires properdin, complement and $Mg^{++}$, does not enhance susceptibility of PNH erythrocyte to hemolysis by mechanical trauma *in vitro.* Hemolysis of PNH and normal erythrocytes attributable to mechanical trauma increases progressively as pH of blood decreases until a critical point (pH 6.0-6.2) where MF rises rapidlv.

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## Metabolism of Radioactive Para-Aminobenzoic Acid in an *Escherichia coli* Mutant.* (22199)

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While metabolism of para-aminobenzoate (PAB) has been extensively investigated in the mammalian body(1,2), its function and biochemical transformations in microorganisms are still obscure. Conversion of PAB into folic acid and the citrovorum factor occurs in some organisms(3,4) although there is some question whether these are the active forms of PAB. A number of other routes of metabolism have been reported. Davis(5,6) has implicated PAB in the metabolism by *Escherichia coli* of tryptophane, tyrosine and other aromatic compounds, as well as vit. $B_{12}$. Ratner *et al.*(7) isolated a glutamic acid peptide of PAB from yeast, and Flynn *et al.*(8) have found in 2 strains of streptococci an antibiotic containing PAB and cytosine among its components. Sloane *et al.*(9) identified aniline and p-aminophenol as degradation products in *Mycobacterium smegmatis.* The present studies had their inception in the isolation by Lampen, Roepke, and Jones(10) of an X-ray mutant of *E. coli* which required exogenous PAB for growth but apparently did not utilize it in producing folic acid. Since the wild strain of this organism normally has no requirement for preformed PAB, it seemed of interest to investigate the fate of this compound labeled with $C^{14}$ in such a mutant.

*Materials and methods.* PAB used was labeled in the carboxyl group with carbon 14 and had a specific activity of 0.037 mC/mg.‡ The mutant of *E. coli* (ATCC No. 9723a) was grown on basal medium modified from that of Cohen and Arbogast(11) by use of 0.4% glucose. The medium of Lampen *et al.*(10) contained asparagine as well, but this substance was not necessary for maximum growth, and was omitted to facilitate isolations. Organisms were grown in 3 liter batches with aeration for 24 hours at PAB concentration of 0.01 μg/ml. Experiments in which PAB level was 0.05 μg/ml gave similar results. Bacterial suspensions were filtered on Buechner funnel using Celite as filter aid. The filtrate contained less than 20% of total radioactivity. The cells, plus filter-aid, were suspended in water and made up to 60% acetone-water (v/v) by addition of acetone. The residue was filtered off, re-extracted, and extracts, containing approximately half the total cell radioactivity, were combined. Subsequent extractions of cells with hot acetone, chloroform,

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