THE ACUTE EFFECT OF ALKALOSIS ON PLASMA INORGANIC PHOSPHATE CONCENTRATION

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The fall in concentration of plasma inorganic phosphate during respiratory alkalosis is a well-documented phenomenon[1-6] that has received little publicity. Apart from the biological interest attached to this occurrence, it has obvious clinical importance in the interpretation of the results of plasma-phosphate determinations. In this paper we report the results of experiments which confirm the inverse relationship between blood pH and plasma inorganic phosphate concentration, and which suggest a possible explanation.

METHODS

Young adult males who were either in good health or recovering from unrelated illnesses were used for the experiments *in vivo*. They fasted for 12 hours before the experiments. Blood samples were taken by venipuncture for analysis before, during and after a period of alkalosis. In 9 cases the alkalosis was induced by voluntary hyperventilation for 20 minutes, and in a 10th by the rapid intravenous infusion (over 7½ minutes) of 200 mEq. of sodium bicarbonate. In all instances a positive Chovstek's sign could be elicited and in most cases the subjective symptoms of tetany (paraesthesiae and light-headedness) were present. Blood for serum-phosphate and total serum-CO_2 determination was taken with anaerobic precautions, and centrifuged immediately under liquid paraffin. The serum inorganic phosphate was determined by the method of Fiske and Subba Rau[7] and total serum CO_2 by the method of van Slyke and Neill.[8] Whole-blood pH was determined colorimetrically on capillary blood by the method of Hastings and Sendroy.[9] Capillary blood was milked out of a pricked finger into a small sealed funnel, containing liquid paraffin and dry potassium oxalate (3 mg.), and sodium fluoride (1 mg.) adjusted to pH 7·0. The blood was mixed with the anticoagulant by stirring with a glass rod. 0·5 ml. of blood was sufficient for each pH determination.

The *in vitro* experiments were conducted in the following manner: Blood was taken by sterile venipuncture and defibrinated by stirring with glass beads for 10 minutes and filtering through loosely packed cottonwool. The defibrinated blood was divided into an appropriate number of aliquots for each experiment and centrifuged. The plasma and buffy layer were discarded and the red cells washed twice by resuspension in 2 volumes of ice-cold 0·154M NaCl and centrifuging. The washed cells were finally resuspended in 1·5 times their volume of a medium of the following composition — sodium 145 mEq./l., potassium 5·0 mEq./l., calcium 2·0 mEq./l., sodium phosphate 1·5 mM/l., bicarbonate + chloride 152 mEq./l., and glucose 15 mM/l. The medium was equilibrated with 7% CO_2 - 93% O_2 before use. The pH of the medium was adjusted by varying its concentration of bicarbonate. The concentration of bicarbonate necessary to give any desired pH was determined approximately by calculation from the Henderson-Hesselbach equation, using a pK′ of 6·1 for the first proton of H_2CO_3 and the equation $H_2CO_3(\mu M/ml.) = 0{\cdot}03.pCO_2(mm.Hg)$. On the basis of preliminary experiments, slightly more bicarbonate than the calculated amount was added to the medium to allow for the lactic acid produced by red-cell metabolism during the first 45 minutes of incubation. The pH values given in the text refer to the mean pH after 45 minutes' incubation.

4·0 ml. of the final red-cell — medium suspension were pipetted into 25 ml. Erlenmeyer flasks and gassed in series with the 7% CO_2 - 93% O_2 mixture for the first 15 minutes of incubation, after which they were stoppered. The flasks were incubated at 37°C. in a Dubnoff shaking metabolic incubator for 90 minutes. The concentration of inorganic phosphate in the medium of the red-cell — medium suspension was determined before and after incubation. The haematocrit of the suspension was determined before incubation. Red-cell phosphate uptake in μM phosphate per ml. of RBCs per 90 minutes was calculated as follows:

$$\text{RBC phosphate uptake} = \frac{(1 - Hct)(P_0 - P_{90})}{Hct} \mu M/ml.\ RBCs$$

per 90 minutes.

Where Hct = haematocrit as a fraction of 1·0.

P_0 = medium inorganic phosphate concentration before incubation.

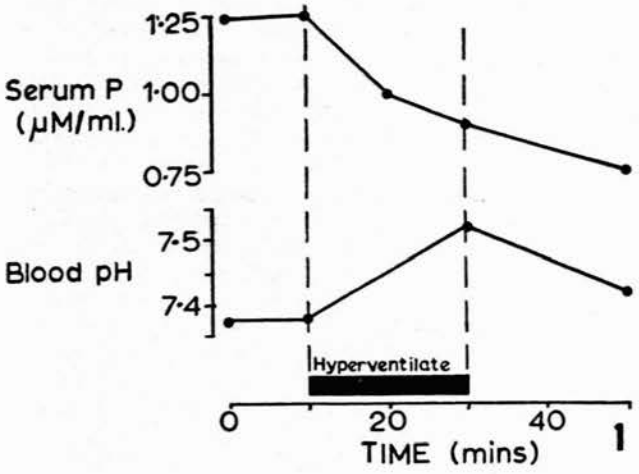
P_{90} = medium inorganic phosphate concentration after 90 minutes' incubation.

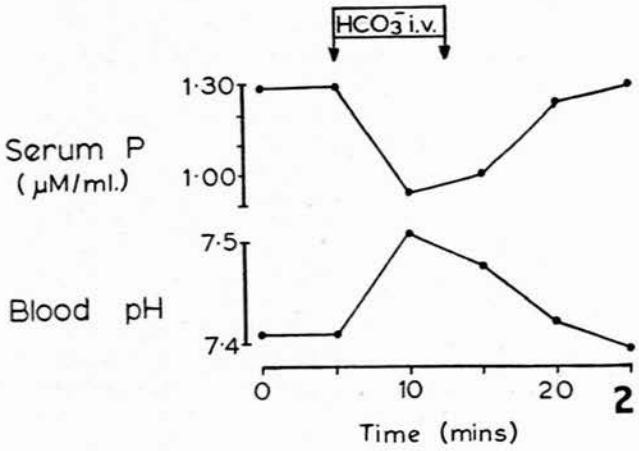
RESULTS

In Vivo Experiments

In each of the 9 subjects who hyperventilated voluntarily there was a rise in capillary blood pH and a fall in serum inorganic phosphate concentration. A typical experiment is shown in Fig. 1. The serum inorganic phosphate concentration, with one exception, continued to fall for 10 - 20 minutes after the subject had stopped hyperventilating, despite the invariable return of blood pH to normal within 10 minutes. The serum inorganic phosphate concentration fell to between 60 and 80% of the resting value.

The experiment summarized in Fig. 2 indicates quite clearly that it is the rise in blood pH, rather than the





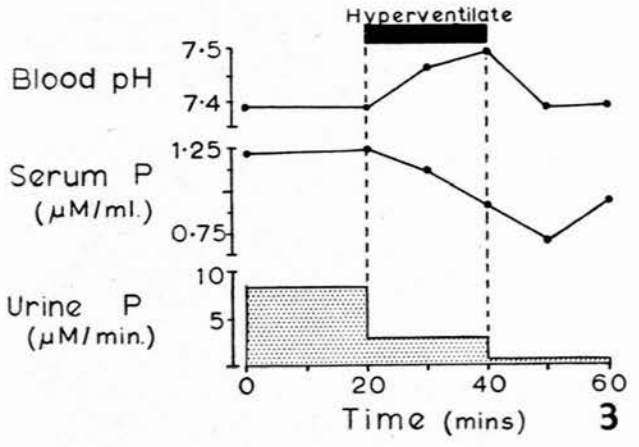


Fig. 1. Effect of hyperventilation on blood pH and serum inorganic phosphate concentration of a healthy adult.
Fig. 2. Effect of intravenous sodium bicarbonate infusion (200 mEq. in 7½ minutes) on blood pH and serum inorganic phosphate concentration of a healthy adult.
Fig. 3. Effect of hyperventilation on blood pH, serum inorganic phosphate concentration and rate of excretion of inorganic phosphate in the urine.

fall in pCO_2 that is responsible for the fall in serum-phosphate concentration. In this experiment the rapid intravenous infusion of sodium bicarbonate (200 mEq. in 7½ minutes) resulted in a rise in blood pH from 7·41 to 7·51, with a fall in serum inorganic phosphate concentration from 1·25 to 0·94 μM/ml.

As can be seen from the experiment summarized in Fig. 3, the fall in the serum-phosphate concentration is not due to a loss of inorganic phosphate in the urine. In this experiment timed urine samples were taken before, during and after hyperventilation, and it can be seen that the rate of excretion of phosphate in the urine fell in concert with the serum inorganic phosphate concentration in response to alkalosis.

In Vitro Experiments

Since the fall in serum inorganic phosphate concentration with alkalosis could not be explained on the basis of loss of phosphate in the urine, it seemed likely that it was due either to a movement of phosphate into the cells or to the conversion of inorganic phosphate to organic phosphate esters in the serum.

The possibility of conversion to organic phosphate esters was easily excluded as an explanation by determining both total serum phosphorus and serum inorganic phosphate before and after hyperventilation. The total serum phosphorus was determined by hydrolyzing an aliquot of the serum with concentrated sulphuric acid, clearing the carbon with hydrogen peroxide, and developing the blue colour as for the inorganic phosphate determinations. As

TABLE I. EFFECT OF HYPERVENTILATION ON THE BLOOD PH, SERUM INORGANIC PHOSPHATE CONCENTRATION AND SERUM TOTAL PHOSPHORUS CONCENTRATION

	Resting	*After hyper-ventilation*
Blood pH	7·41	7·58
Serum inorganic phosphate concentration (μM/ml.)	1·20	0·84
Serum total phosphorus concentration (μM/ml.)	1·35	1·02

can be seen from Table I, the concentrations of both serum inorganic phosphate and serum total phosphorus fell to the same extent, indicating that the fall in serum inorganic phosphate concentration was not the result of an increase in the concentration of extracellular organic phosphate.

It seemed likely, therefore, that with increasing plasma pH, inorganic phosphate left the plasma and entered the cells. To investigate this possibility experiments were done in which washed human red cells were incubated in an isotonic buffered medium at varying pH levels and the concentration of inorganic phosphate was measured in the medium before and after incubation for 90 minutes. As can be seen from Fig. 4, there was a linear relationship between the pH of the medium and the rate at which phosphate was taken up by the red cells. In some instances phosphate actually leaked out of the cells at the lower pH values, but there was invariably an uptake of phosphate at the higher pH values. It is also apparent from the experiment summarized in Fig. 5, that the effect of

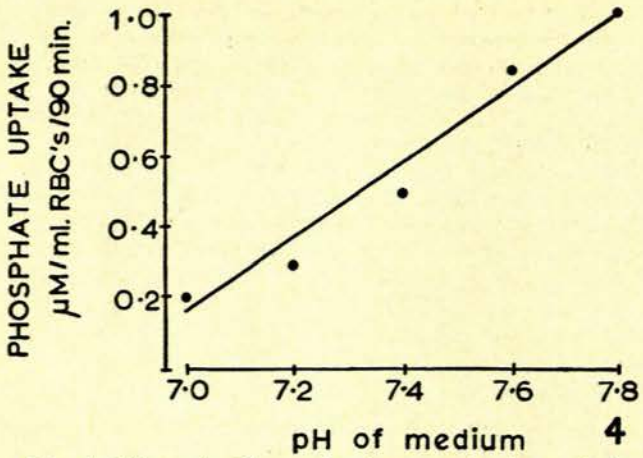


Fig. 4. Effect of pH on phosphate uptake by washed human red cells incubated *in vitro*. Washed human red cells (1·6 ml. of RBCs + 2·4 ml. of medium) were incubated in media of varying pH for 90 minutes at 37°C. under 93% O_2 - 7% CO_2. The adjustment of the pH of the medium, calculation of phosphate uptake and composition of the medium are described under Methods.

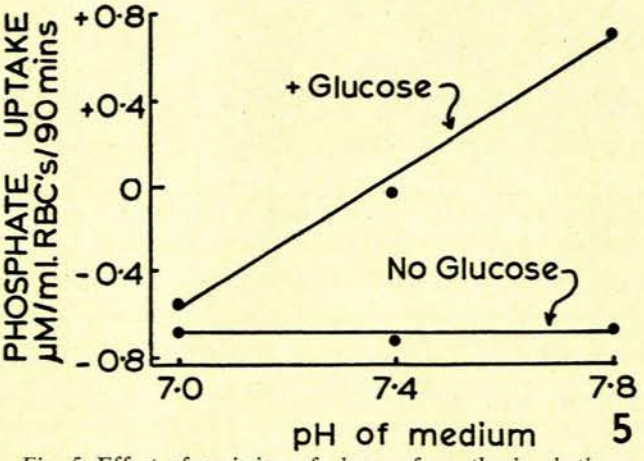


Fig. 5. Effect of omission of glucose from the incubating medium. Six flasks were incubated as described under Methods. In 3 of the flasks (upper line) glucose was added to the medium in a concentration of 30 µM/ml., and in the remaining 3 (lower line) glucose was omitted from the medium. The negative values for phosphate uptake represent a leakage of phosphate from the red cells into the medium.

pH on uptake of inorganic phosphate by red cells *in vitro* is dependent upon the presence of glucose in the medium.

DISCUSSION

These results confirm the observation that a rise in blood pH is accompanied by a fall in the concentration of inorganic phosphate in the plasma. This fall is not due to a sudden loss of phosphate in the urine, nor can it be ascribed to an increase in the concentration of plasma organic phosphate at the expense of inorganic phosphate. The results of the experiments conducted *in vitro* indicate that red cells, when exposed to an alkaline environment, accumulate inorganic phosphate. We suggest that a similar shift of phosphate from plasma into the red cells occurs *in vivo* when the blood pH rises.

The extent to which phosphate moves into other body cells, or into bone, in response to alkalosis, is not known. We have not been able to demonstrate any effect of pH on the rate of accumulation of phosphate by slices of rat liver or kidney, or by fragments of calvarial bone, when these tissues were incubated *in vitro*. These findings do not, however, exclude the possibility of accumulation of phosphate by these or other tissues *in vivo*.

Bartlett[10] has shown that if metabolically active red cells are incubated in the presence of ^{32}P-labelled orthophosphate, there is a very rapid incorporation of the label into inorganic phosphate in the cells and into the organic phosphate esters of intermediary red-cell carbohydrate metabolism. These studies indicate that extra- and intracellular inorganic phosphate and cellular organic phosphate are in a state of rapid dynamic equilibrium. It seems reasonable to suppose, therefore, that alkalosis disturbs this equilibrium in such a manner that a new steady state is achieved in the presence of an elevated equilibrium concentration of organic phosphate esters. This suggestion is supported by the findings of Tulin *et al.*,[11] who showed that alkalinization of blood accelerated glycolysis and led to an accumulation of organic phosphate esters within the red cells *in vitro*.

If one assumes, then, that alkalosis leads to an increase in the amount of organic phosphate in the cell, the failure of alkalosis to stimulate phosphate uptake in the absence of glucose, has two explanations. Firstly, glucose catabolism is necessary to supply the energy for increased phosphorylation and, secondly, glucose and its metabolic derivatives are essential substrates to serve as phosphate acceptors in phosphorylation reactions.

The precise mechanism involved in this phenomenon is still obscure, however, and its further elucidation must await quantitative determinations of the various moieties of intracellular phosphorus.

SUMMARY

Alkalosis in humans, whether induced by hyperventilation or bicarbonate infusion, is accompanied by a fall in the serum inorganic phosphate concentration. This effect is not due to a loss of phosphate in the urine, nor is it due to the formation of organic phosphate esters in the plasma. *In vitro* studies indicate that alkalosis causes a shift of inorganic phosphate into the red cells where, we suggest, it is incorporated into organic phosphate esters.

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