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pH lability in serum during equilibrium dialysis

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Changes in pH were determined in previously frozen normal human serum during dialysis against sodium phosphate, Krebs Ringer phosphate or Krebs Ringer bicarbonate buffers of pH 7.4. Serum was either untreated (native) or adjusted to pH 7.4 before dialysis. pH in native serum was 7.7–7.9 before dialysis, showed a decrease after 1 h, and an increase after 3 h. pH-adjusted serum showed a continuous pH increase during dialysis. The increase in serum pH during dialysis was larger at 37° C than at 22° C, larger at low than at high buffer molarity, and larger in native than in pH-adjusted serum. The observed changes in serum pH during dialysis are associated with unacceptably large errors in unbound fraction in serum for a number of important drugs. Drug binding determination in serum by equilibrium dialysis should be performed with buffers providing appropriate and stable pH level.

Keywords equilibrium dialysis drug serum binding pH lability

Introduction

pH-dependent serum binding has been shown for a number of acidic and basic drugs, such as methyl orange (Klotz *et al.*, 1952), barbiturates (Goldbaum & Smith, 1954), theophylline (Vallner *et al.*, 1979), warfarin (Wilting *et al.*, 1980), fusidic acid (Henry *et al.*, 1981), propranolol and oxprenolol (Henry & Mitchell, 1981), imipramine (Kristensen & Gram, 1982), quinidine and prazosin (Brørs *et al.*, 1984), and pethidine (La Rosa *et al.*, 1984). For several of these drugs, 30% or more decrease in free fraction is observed if precautions to avoid a pH increase in serum are not taken (Henry & Mitchell, 1981, Brørs *et al.*, 1983). Frequently, pH during the experiments on drug serum binding is not accounted for, and procedures which in our experience lead to inappropriate pH levels are used.

The pH of serum left in air or frozen and thawed is usually elevated to 8 or higher. This is due to loss of CO_2 if precautions are not taken. In our experience, stable pH of about 7.4 in serum requires either equilibration with CO_2 and dialysis against a bicarbonate-containing buffer, or total removal of bicarbonate and then adjustment of pH before starting the experiment.

Equilibrium dialysis of serum is often performed against phosphate buffers neglecting the presence of bicarbonate in serum. Usually, pH of serum is not adjusted before the start of the experiment, but exceptionally, pH is adjusted to pH 7.4 by titrating with hydrochloric acid.

The aim of this study was to determine the pH changes in serum during dialysis against phosphate buffers of different molarity with and without initial pH adjustment of serum by adding hydrochloric acid.

Methods

Normal human serum was obtained from blood drawn in plain glass tubes. Serum was stored at −20° C until used.

Sodium phosphate buffers of different molarities up to 150 mM were prepared by dissolving the dibasic sodium salt and adjusting pH with 1 M HCl or NaOH. The composition of Krebs Ringer phosphate buffer and of Krebs Ringer bicarbonate buffer was as described previously (Brørs *et al.*, 1983), except that gassing with CO_2 was not performed in the present study.

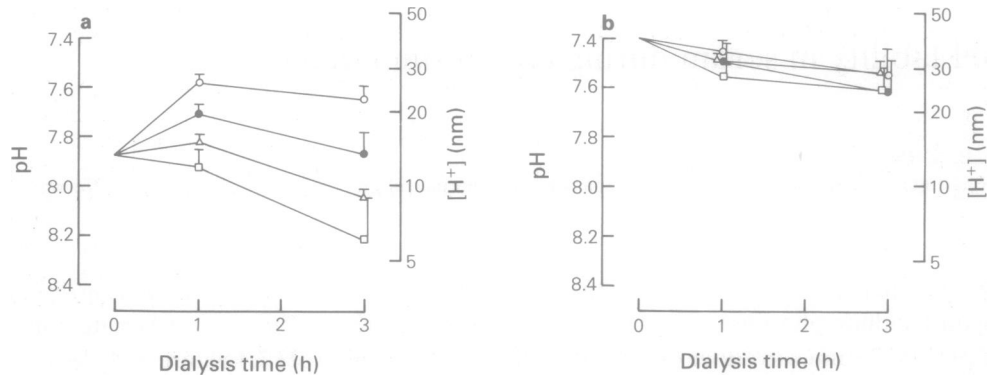

Figure 1 (a) pH and proton concentration in native serum during dialysis at 22 °C against different buffers. (b) pH and proton concentration in HCl-adjusted serum during dialysis at 22° C against different buffers. o—o 150 mM sodium phosphate, •—• 100 mM sodium phosphate, Δ—Δ 67 mM sodium phosphate, □—□ Krebs Ringer phosphate. Values are mean and s.d. from eight experiments.

Serum (800 μl) was dialysed against buffer (800 μl) under constant shaking in a thermostated incubator in normal atmosphere at 22 or 37° C, using dialysis membrane 20/32 (Union Carbide Corp., Chicago, Illinois, USA) between two Perspex® cells of total volume 1 ml each. The declared pore size of the dialysis membrane was 24 Å. The cells were sealed with tape, which was temporarily removed when measuring pH.

pH was determined with Micro Electrode Unit or Blood Micro System (Radiometer Copenhagen, Denmark) using Precision Buffer S1510 (Radiometer Copenhagen) for calibration.

Results

Dialysis at 22° C

pH of thawed native serum after storage at −20°C was 7.7–7.9. When such serum was dialysed at 22° C against sodium phosphate buffer with initial pH 7.4, serum pH initially decreased and then increased (Figure 1a). The magnitude of the pH changes depended on the molarity of the buffer. Even with 150 mM buffer serum pH failed to reach pH 7.4. Thus, after 3 h, mean pH was 7.6 with 150 mM phospate buffer and above 7.8 with 100 mM.

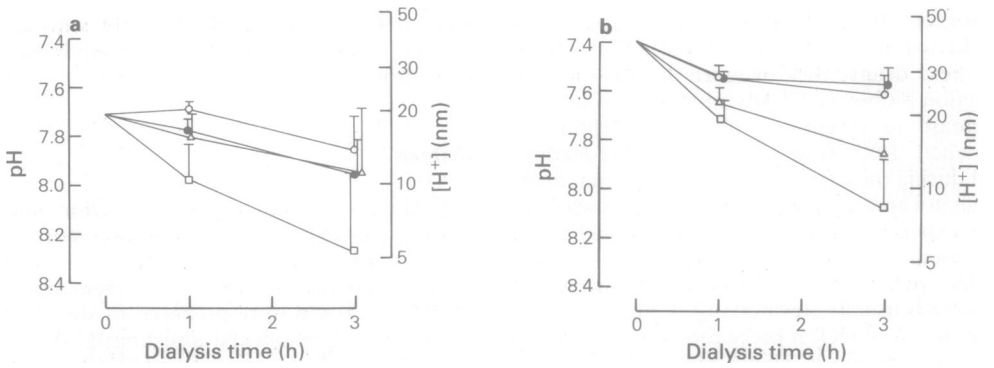

Figure 2 (a) pH and proton concentration in native serum during dialysis at 37 °C against different buffers. (b) pH and proton concentration in HCl-adjusted serum during dialysis at 37° C against different buffers. o—o 150 mM sodium phosphate, •—• 100 mM sodium phosphate, Δ—Δ 67 mM sodium phosphate, □—□ Krebs Ringer phosphate. Values are mean and s.d. from four experiments.

When serum was adjusted with HCl to pH 7.4 and then dialysed against sodium phosphate buffers at 22° C, pH increased slowly and mean pH was between 7.55 and 7.60 after 3 h (Figure 1b).

When HCl-adjusted serum was dialysed against HCl-adjusted, ungassed Krebs Ringer bicarbonate buffer, serum pH increased to 7.74 ± 0.03 (n = 4) after 1 h and to 8.37 ± 0.06 (n = 4) after 3 h.

Dialysis at 37° C

When thawed native serum was dialysed against sodium phosphate buffer at 37° C, serum pH increased markedly (Figure 2a) and more than at 22° C. Mean pH of serum was above 7.8 for all buffers after 3 h. When HCl-adjusted serum was dialysed against sodium phosphate buffers at 37° C (Figure 2b), pH changes of serum were larger than at 22° C. With 100 and 150 mM sodium phosphate buffer, mean serum pH of about 7.55–7.60 was observed after dialysis for 3 h.

When serum was adjusted to pH 7.4 with HCl and dialysed against HCl-adjusted, ungassed Krebs Ringer bicarbonate buffer of pH 7.4 at 37° C, serum pH increased to 7.85 ± 0.21 (n = 7) after 1 h and to 8.49 ± 0.19 (n = 7) after 3 h.

Discussion

The high pH (7.7–7.9) in frozen and thawed serum must be accounted for by reduced CO_2 content. The present results show that dialysis of the bicarbonate-containing serum in air against phosphate buffers is associated with unstable serum pH. The gradual increase in pH during dialysis is accounted for by continuous CO_2 loss, which causes a declining concentration of protons and bicarbonate in serum. Although dialysis of serum against a buffer with pH 7.4 initially may tend to bring native serum pH down toward 7.4, the pH after 1 and 3 h was markedly elevated. The gradual pH increase from 1 to 3 h was larger at low buffer molarity and at higher temperature.

According to published data, when pH of serum increased by 0.1 unit from 7.4, the free fraction of propranolol decreased by 9% (Henry & Mitchell, 1981), of oxprenolol by 8% (Henry & Mitchell, 1981), of imipramine by 6% (Kristensen & Gram, 1982), of pethidine by 7% (La Rosa *et al.*, 1984), and of theophylline by 8% (Vallner *et al.*, 1979) or 3–4% (Shaw *et al.*, 1982; Brørs *et al.*, 1983).

The relative error in determining free fraction of a large number of drugs by equilibrium dialysis under conditions described in the present paper may then exceed the acceptable limits for analytical error. We believe that a major part of published data on drug serum binding suffers from such errors. Determination of serum binding of drugs should be performed with buffers and experimental procedures providing stable and physiological pH.

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