

Ionised calcium in pathological human bile

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SUMMARY An automated ion-selective electrode system has been used to measure ionised calcium in bile from patients undergoing cholecystectomy for gallstones. Ionised calcium ranged from 0.63 to 1.73 mmol/l for a range of total calcium concentrations of 1.35 to 10.93 mmol/l. The correlation coefficient for ionised calcium regressed on total calcium was highly significant ($r = 0.97$). The slope of the line was 0.126 and the intercept 0.443.

Ultrafiltration of common duct and gallbladder bile has shown that much of the calcium present is bound to a substance or substances with a molecular weight greater than 10 000.¹ The remaining calcium consists of any bound to molecules with a lower molecular weight and ionised calcium. We have now investigated the ionised calcium concentration and its relationship to total calcium concentration in samples of pathological human bile.

Material and methods

Seven samples of gallbladder bile and three of common duct bile were collected anaerobically at 37°C from eight patients undergoing surgery for cholecystectomy.¹ The small number of common duct bile samples was due to technical difficulties in collection, and the corresponding gallbladder bile was not obtained for one of the common duct samples. All patients had functioning gallbladders, as determined by oral x-ray cholecystography. They also had gallstones, and analysis of these by the x-ray powder method² showed that in all cases cholesterol was the only crystalline constituent on the stone's surface.

Total calcium was determined in bile that had been millipore filtered anaerobically at 37°C.¹ As before, samples were taken after bile collection, but the actual measurements were made when several different bile samples had been collected.

Total sodium was determined on filtered bile using an AutoAnalyzer flame photometry procedure (Technicon method N-21a).

Ionised calcium measurements were made immediately after collection of the bile, which was millipore filtered anaerobically at 37°C into a 10-ml

plastic syringe³ so that the sample could be injected into the instrument for measuring ionised calcium. The instrument was an automated ion-selective electrode system, the Orion Model SS-20. The analyses are made anaerobically at 37°C, and the concentration is expressed as a digital readout on the display panel 3 minutes after injection of about 0.5 ml sample into the instrument. A chart recorder was attached to the analyser, and this gives a graphical recording of the change in potential between the calcium electrode and a flowing KCl reference electrode. The instrument was adjusted each day with two standard solutions of calcium salts (2.0 and 4.0 mmol/l, supplied by Orion). It was further adjusted immediately before use and after each bile measurement to avoid any error resulting from a change in the electrode potential during the sample measurement. Duplicate measurements were made on each bile sample.

The electrode is sensitive to variations in sodium ion concentration,⁴ but the error arising from this can be corrected to an apparent NaCl concentration of 140 mmol/l using the following formula derived in the above paper for use in serum measurements:

$$\text{Ca}^{2+}(\text{Na}) = \text{Ca}^{2+}(\text{M}) - \frac{\Delta\text{Na}}{374}$$

where $\text{Ca}^{2+}(\text{Na})$ is the sodium-corrected ionised calcium in the sample, $\text{Ca}^{2+}(\text{M})$ is the measured ionised calcium in the sample, and ΔNa is the measured NaCl concentration in the sample minus 140, all in mmol/l. The effect of a NaCl concentration of 140 mmol/l is eliminated by the incorporation of NaCl in the standard solutions used in the instrument to give this concentration. The total sodium concentration obtained for bile samples cannot be used

Received for publication 26 March 1979

in the equation because the sodium activity coefficient in bile salt solutions and in bile is lower than those for NaCl solutions at corresponding total sodium concentrations.⁵ The NaCl concentration equivalent to the activity of the sodium ion in bile must therefore be determined. From a plot of the data of Moore and Dietschy⁵ of total sodium concentration against sodium ion activity in bile, the sodium ion activity corresponding to the total sodium concentration in each bile sample was determined. The NaCl concentration corresponding to this estimated sodium ion activity was read off the graph of NaCl concentration against sodium ion activity plotted from the data again provided by these workers. This figure was incorporated in the above equation to give the corrected calcium ion concentration.

The pH of the bile was recorded.⁶

Results

Duplicate measurements of ionised calcium deviated from the mean by less than 1%, except in one case where the deviation was 5%.

During the automatic time cycle of the instrument, the value for ionised calcium on the display panel had become constant and usually had been so for 10-20 seconds. In some cases, though, the trace on the chart recorder representing the potential between the two electrodes appeared not to have quite reached a constant level, suggesting that equilibrium had not been reached. From the shape of these traces,

it was estimated that any error in the measurement due to this fact was unlikely to exceed 5%.

The correction for the electrode's sensitivity to variations in the sodium concentration ranged from -0.08 to +0.04 mmol/l but was usually of the order of ±0.02 mmol/l.

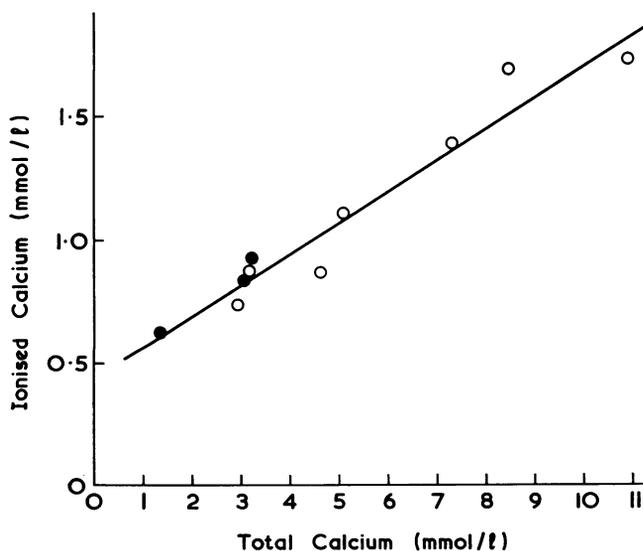
The corrected calcium ion concentrations ranged from 0.63 to 1.73 mmol/l and total calcium concentrations from 1.35 to 10.93 mmol/l (Figure). Ionised calcium was regressed on total calcium and the correlation was highly significant ($r = 0.97$, $n = 10$, $p < 0.001$). The slope of the linear regression line is 0.126 (SE ± 0.011) and the intercept is 0.443 (SE ± 0.063).

There was no correlation between pH and ionised calcium.

Conclusions

Ionised calcium, like ultrafiltrable calcium,¹ is related to total calcium. The regression lines are almost parallel, the slope being 0.126 for ionised calcium regressed on total calcium and 0.112 for ultrafiltrable calcium regressed on total calcium. The corresponding intercepts are 0.443 and 0.976 respectively; thus the amount of calcium bound to molecules with a molecular weight of less than 10 000 is on average 0.53 mmol/l.

Ionised calcium in urine is also markedly dependent on the total calcium and accounts for about 50% of it.⁷ Although total calcium concentrations cover a



Ionised calcium plotted against total calcium in bile from patients with cholesterol on the gallstone surface. Shaded circles represent measurements on bile from the common duct; all other measurements are for gallbladder bile. The equation of the line is $y = 0.126x + 0.443$ where y = ionised calcium and x = total calcium. The correlation coefficient is highly significant ($r = 0.97$).

similar range in the two fluids, ionised calcium is much lower in bile than in urine for corresponding values of total calcium. This suggests that the chief calcium-binding agent in bile, thought to be the mixed micelle,¹ has a much greater affinity for calcium than do citric acid and the other calcium-binding compounds in urine.

The absence of correlation between pH and ionised calcium which was also found for pH and ultrafiltrable calcium again shows that the chief binding agents are completely ionised over the biliary pH range which is the case for the principal conjugated bile salts (pK values less than 5).

We thank Professor C G Clark and the surgeons at University College Hospital for providing the bile, the Medical Research Council for financial assistance, and Professor R H T Edwards for permission to use the SS-20.

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