Eleven patients had cholesterol gallstones and bile analysis revealed cholesterol crystals only in two and both types of crystals in six. Twelve patients had pigment stones; two had cholesterol crystals, three had both types of crystal and three had bilirubinate crystals only.

Bile was obtained in three of the five patients with a blocked cystic duct; one of these patients with empyema of the gallbladder and cholesterol stones had bile cholesterol crystals.

Inclusion of bilirubinate crystal analysis increased the sensitivity of diagnosing gallstones from 50% using cholesterol-crystal analysis alone to 66.7%. The use of an incubation period with re-examination of the supernatant also increased the diagnostic rate by 27%. The sensitivity of the test could be further improved by repeating the test on different days (Marks & Bonouros, 1984). The finding of bilirubinate crystals only in 25% of patients with pigment stones (compared to none in those with cholesterol stones) might be of practical value as these patients would not be suitable for gallstone dissolution by methyl-tert-butyl-ether (Neoptolemos et al., 1986).

In conclusion, bile-crystal analysis was found to be useful for identifying patients with gallstones. Although rather poor for gallstone-type identification, further refinements in technique are possible. Bile-crystal analysis should receive a wider application in clinical practice.


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The role of citrate in oral rehydration therapy

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Oral rehydration solutions represent the major advance in the treatment of dehydration from diarrhoeal disease (Donowitz et al., 1984). Bywater has shown that the inclusion of citrate increases the intestinal water absorption by these life-saving formulations (Bywater, 1977). Studies with the Ussing chamber have found that citrate significantly increases the transepithelial sodium flux, and that net absorption of citrate takes place from the intestinal lumen (Newsome et al., 1983). Citrate transport has also been demonstrated in kidney tissue using brush-border vesicles, which exhibit a sodium-dependent citrate uptake system (Wright et al., 1982). However, our understanding of intestinal citrate absorption, and the role of citrate in rehydration solutions is limited.

We have used isolated enterocytes (intestinal epithelial cells) as a model to study citrate absorption and the effects of citrate on sodium fluxes.

The enterocytes were isolated from chicken small intestine using the hyaluronidase method of Brown & Sepúlveda (1985), and were rendered metabolically inert by treatment with rotenone and ouabain (Montrose et al., 1985).

We have found that citrate influx is stimulated by a sodium gradient, with a $V_{max}$ in the physiologically attainable region, of 50 mM (Fig. 1a). In addition to the sodium-dependent flux, we have detected a saturatable sodium-independent citrate flux. This citrate flux is pH dependent and is increased fivefold by varying the transmembrane pH gradient (Fig. 1b). The reported presence of a one unit pH gradient across the entire chicken small intestine (Montrose et al., 1985), and the gradient across the upper regions of the mammalian small intestine, may attach physiological relevance to this finding. Whether this change in flux is due to an increase in concentration of transportable species, or the protonation of amino acid residues essential for activity is not known. Indeed, a further explanation could be the sodium and proton competition proposed by Hoshi et al. (1986) for the intestinal sodium-hexose symport.

The similarities in function between the intestine and kidney, present the possibility that the intestinal sodium

![Gastroenterology](https://example.com/gastroenterology.png)

Fig. 1. Effects of transmembrane sodium and pH gradients on citrate influx after 4 min

(a) Effect of transmembrane sodium gradients on citrate influx. Enterocytes were isolated in sodium-free media and introduced to buffers containing sodium. Error bars indicate duplicate samples. (b) Effect of a transmembrane pH gradient on citrate influx. Enterocytes were isolated at pH 7.2 and introduced to highly buffered solutions of different pH. The similarity between the intestine sodium gradient; * sodium-free media throughout. Error bars indicate duplicate samples.
Effects of synthetic incubation media on phosphate metabolism in human erythrocytes

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The relationship between cellular P, metabolism and the detailed composition of the extracellular medium has not been investigated systematically. For example, even though there is evidence that some mammalian cells can regulate their intracellular P, concentration in the face of changes in extracellular P, concentration (Bevington et al., 1986) the cell nutritional requirements for maintaining this regulation are unknown. For this reason, most of our early studies on P, metabolism in human erythrocytes have been performed in autologous plasma, to keep the experimental conditions as close as possible to those in vivo. Unfortunately, some experimental manoeuvres (for example removing P, from the extracellular medium) cannot readily be performed in plasma. We have therefore studied two synthetic substitutes: a balanced salt solution with glucose, buffered with Hapes (Hapes Ringer or HR) (Challa et al., 1985) and a more complex tissue culture medium (Eagle's minimum essential medium, MEM (Gibco 042–1430M) with 2 mm-glutamine and 0.1 mm-pyruvate). We show here that, even though these media have the same pH and P, concentration as normal human plasma, they produce marked differences in important parameters of erythrocyte phosphate metabolism.

Human erythrocytes were incubated in autologous plasma or in Eagle's minimum essential medium (MEM) at 2–5% packed cell volume under air/CO, 95:5 at 37°C for 24 or 48 h, under aseptic conditions. Extracellular pH and P, concentration were manipulated with HCl, NaOH or Na,HPO,.

In other experiments, cells were incubated under air at 37°C in HR (Challa et al., 1985) containing P, at a range of concentrations. Cells were separated without washing by centrifugation through di-n-butylphthalate (Kepner & Tosteson, 1972). Cell water was measured using H,O with [14C]inulin as an extracellular marker (Challa et al., 1986). P, was measured by a selective colorimetric assay (Challa et al., 1985). The measured cellular P, concentration was compared with that calculated from the transmembrane distribution ratio (r) for P, ions, on the assumption that P, (like P,) distributes passively across the cell membrane (the passive distribution model) (Bevington et al., 1986). All concentrations are expressed in mmol/l of cells or extracellular medium.

The transmembrane distribution ratio (r) for P, was 0.50 ± 0.03 (mean ± S.E.M., n = 21) for cells incubated for 24 h in plasma, and was similar in HR. However, it was higher in MEM (0.58 ± 0.04, n = 16, p < 0.05), possibly because the concentration of 2,3-bisphosphoglycerate (2,3-BPG) was much lower in these cells (see below), which will have affected the Donnan distribution of P, across the membrane (Hladky & Rink, 1977). After 24 h, (i.e. at steady state) the measured cellular P, concentrations were 0.55 ± 0.05 in plasma (n = 40), and 0.59 ± 0.04 in HR (n = 21) at 1 mm-extracellular P,. These values are 45 and 70%, respectively, higher than those calculated from the passive distribution model (Bevington et al., 1986) using the values of r above. A similar result is seen in MEM at 1 mm-extracellular P,. Similarly, after 24 h in P,-free HR, cellular P, concentration was maintained at 0.24 ± 0.02 mmol/l cells (n = 16), and was similar to this, 0.21 ± 0.02 mmol/l cells (n = 8), after 24 h in P,-free MEM. Therefore, the composition of the medium seemed to have no marked effect on the cellular P, concentration when the cells were allowed sufficient time to reach steady state.

However, it was apparent that, in the short term, MEM was able to move cellular P, metabolism away from steady state. When human erythrocytes were incubated in MEM at P, concentrations from 0 to 2.5 mmol/l, at pH 7.3, the cellular