occur in much less than 24 h, as the apparent first-order rate constant for net P influx was 1.7 ± 0.2 h⁻¹, and the value for efflux was similar; this corresponded to a halftime of only 25 min and was independent of extracellular P₄ concentration up to 13 mm. The measured cellular P₄ concentrations at 24 and 48 h therefore suggest that erythrocyte P₄ does not follow the passive distribution model. However, ouabain (up to 10⁻⁴ mol/l) had no effect on cell P₄, implying that erythrocyte P₄ is not maintained by an active sodium-linked P₄ pump (Bevington et al., 1987).

At 24 h, the concentration of 2,3-biphosphoglycerate (2,3-BPG), the principal OP of erythrocyte cytosol, increased slightly with the P₄ concentration in both plasma and HR; the overall mean slope was 0.3 ± 0.1 mmol per 1 cells/mmol per 1 extracellular medium. However, in P₄-free HR, 2,3-BPG concentration was still 2.5 ± 0.1 mmol/l, compared with 3.7 ± 0.7 mmol/l in plasma at 1 mm-extracellular P₄. This is consistent with the apparent regulation of 2,3-BPG concentrations observed in patients with a wide range of plasma P₄ concentrations (Bevington et al., 1985).

In incubations over the extracellular pH range 7.0-8.0, the Cl⁻ distribution ratio r at 24 h decreased with increasing pH, in agreement with earlier reports (Hladky & Rink, 1977) and therefore did the cell P₄ concentration predicted from the passive distribution model (slope = -0.5 ± 0.1 mmol/l cells per pH unit). However, the dependence of measured cellular P₄ on pH was significantly steeper (-1.1 ± 0.1, p < 0.001). There was no significant change in cell P₄, from 24 to 48 h at any pH, but unfortunately it was not possible to conclude that the discrepancy between observed values and those predicted from passive distribution was maintained when steady state was reached, as r became high (mean value 0.7), variable and independent of pH at 48 h, possibly indicating a decline in cell viability.

Cellular 2,3-BPG concentration at 24 h increased steeply with pH (slope = 14 ± 1 mmol/l per pH unit) and this was not significantly different at 48 h. In agreement with our earlier suggestions (Kemp et al., 1986) this implies that, at least within the first 24 h of pH perturbation, the unexpectedly steep dependence of cell P₄ on pH arises from a non-steady-state flux of phosphorus from P₄ into 2,3-BPG with increasing pH.

The general conclusion from these experiments is therefore that, even after allowing 24 h for cellular P₄ metabolism to attain steady state, cell P₄ concentrations are still not close to those predicted by the passive distribution model. This discrepancy could arise from the non-ideality of the solutions in cytosol and plasma, but the presence of a large P₄ pool in a non-exchanging thermodynamically inactive form in the cell seems unlikely, in view of the fact that measured cellular P₄ can approach zero on prolonged incubation in medium at pH 8.0.

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Effect of a fraction of goat milk serum proteins on milk accumulation and enzyme activities in rabbit mammary gland

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Milk synthesis in the mammary gland is regulated by local chemical feed-back inhibition, a mechanism which is manipulated by changes in milking frequency (Henderson & Peaker, 1984). Synthesis of milk constituents by rabbit mammary explants in organ culture has recently been described previously (Wilde et al., 1987a). In this study, the active protein fraction was introduced into the mammary gland of lactating rabbits by injection via the teat ducts, and its effects on milk secretion and mammary enzyme activities were measured.

The 10-30 kDa milk serum fraction was prepared as described previously (Wilde et al., 1987a). Before intraductal injection, lactating Dutch rabbits were accustomed to 1 h suckling and 23 h separation from their litter. On day 10-12 of lactation, after sucking the rabbit was lightly anaesthetized and the teat ducts were cannulated. The animals were injected unilaterally in four glands, each gland receiving 1.0-1.25 ml (0.25 ml/duct) of a solution of the milk serum proteins concentrated 20-fold relative to milk. Other rabbits received the carrier solution (10 mM-Hepes pH 6.7 containing 0.3M-sucrose). After 24 h, the mammary glands were carefully removed, weighed and frozen in liquid N₂, and the milk yields of treated and untreated glands were calculated from a pre-determined relationship between rabbit mammary weight gain and milk accumulation (Calvert et al., 1985).

Activities of total acetyl-CoA carboxylase, fatty acid synthetase, glucose-6-phosphate dehydrogenase and galactosyltransferase were assayed in particle-free supernatants prepared by high-speed centrifugation (Shipman et al., 1987). DNA was assayed by a fluorimetric method (Labarca & Paigen, 1980).

Intraductal injection of the 10-30 kDa milk serum protein fraction resulted in a weight difference between treated and untreated glands (70.5 ± 6.3 and 92.1 ± 7.9 g/kg body weight, respectively for eight animals; p < 0.05) which was much greater than that observed in untreated rabbits (results not shown). This indicated that the goat milk serum fraction which reversibly inhibited lactose and casein synthesis by rabbit mammary explants in vitro also inhibited
Table 1. Enzyme activities in rabbit mammary glands treated with goat milk serum fraction

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Test group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>Acetyl-CoA carboxylase (nmol/min per mg DNA)</td>
<td>140.7 ± 14.1(5)</td>
<td>139.1 ± 15.8</td>
</tr>
<tr>
<td>Fatty acid synthetase (nmol/min per mg DNA)</td>
<td>0.81 ± 0.15(5)</td>
<td>0.77 ± 0.12</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase (nmol/min per mg DNA)</td>
<td>1.63 ± 0.25(5)</td>
<td>1.69 ± 0.27</td>
</tr>
<tr>
<td>Galactosyltransferase (nmol/min per mg DNA)</td>
<td>198.9 ± 9.0(5)</td>
<td>213.6 ± 20.2</td>
</tr>
</tbody>
</table>

Values are the mean ± s.e.m. with the number of paired samples in parentheses. Statistical significance was compared by the paired t-test: *p < 0.05, **p < 0.01.

Enzymic biosynthesis of peptide toxins by plant-specific fungi

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Several fungi are pathogenic by virtue of low molecular mass toxins which they produce (Scheffer & Livingston, 1984). Hc toxin, produced by Helminthosporium carbonum race 1, induces host-selective pathogenicity against certain crops of maize (Scheffer & Ullstrup, 1965) while tentoxin, produced by Alternaria alternata, is less specific, causing chlorosis in the seedlings of many plants (Fulton et al., 1965). Extensive studies in bacteria, particularly Bacillus sp. and to a lesser extent in fungi, have shown that these antibiotic and phytotoxic peptides are biosynthesized not by ribosomes, but by multifunctional enzyme synthetases (Kazir & Demain, 1977; Kleinkauf & Von Dohren, 1985). ATP-dependent amino acid activation, amino acid racemization and SAM-dependent N-methylation, transpeptidation, and cyclization activities are exhibited by these synthetase enzymes. We present evidence that the cyclic tetrapeptides Hc toxin and tentoxin are biosynthesized independently of protein synthesis by synthetase enzymes which we have partially purified and characterized.

Still cultures of H. carbonum race 1 were grown in 10 ml modified Fries medium (Ciuffetti et al., 1983). The mycelium (approx. 2.0 g) was separated from the medium and washed with distilled water before being suspended in 3 ml of 20 mM-sodium phosphate buffer, pH 6.8. Cycloheximide was added and the mycelium incubated for 1 h with gentle shaking. One microcurie of 3H-labelled protein hydrolysate (56 mCi/matom, Amersham U.K.) was then added and the incubation continued a further 2 h. The mycelium was frozen in liquid nitrogen and lyophilized, while the buffer was repeatedly extracted with ethyl acetate. The combined extracts were dried under a stream of nitrogen and redissolved in 100 μl of water and resolved by h.p.l.c. on a Du Pont Zorbax ODS (4.6 mm x 25 cm) column using a gradient of acetonitrile in water. Fractions (1 ml) were collected and assayed by scintillation counting. Labelled Hc toxin-containing fractions were confirmed by f.a.b. mass spectroscopy.

Cell-free extracts were made by powdering the lyophilized mycelium with a mortar and pestle, resuspending in 100 mm-