After feeding of rats with the raw soyabean diet, serum polyamine concentrations (primarily spermidine) were significantly increased at 6 and 12 h (Table 1). No similar increases were apparent in the control animals. After 24 h, the circulating levels of polyamines returned to control values and subsequently the total polyamine content in the blood was below normal. These later changes suggest that clearance of polyamines from the circulation occurred at higher rates in soyabean-fed rats than in controls.

In another experiment, feeding of rats (7 g/day per rat) for 7 days with a lactalbumin-based diet containing purified trypsin inhibitors (28 g/kg diet) resulted in a considerable increase in pancreatic dry weight (+ 82 ± 14 mg) and polyamine content (+ 1345 ± 350 nmol/pancreas) compared with that obtained for controls. The lectin (7 g/kg diet) had a similar effect upon pancreatic weight (+ 60 ± 14 mg) and polyamine content (+ 928 ± 179 nmol/pancreas). The control values for pancreatic dry weight were 158 ± 14 mg and for polyamine content 2209 ± 179 nmol/pancreas. Treatment of rats with 1200-1300 mg/day per kg rat body weight increased by feeding of rats with soyabean, trypsin inhibitors or lectin were not.

Pancreatic activity of spermidine/spermine N\textsuperscript{1}-acetyltransferase (SAT) [6] was high (25 ± 9 nmol of N\textsuperscript{1}-acetyl spermidine/h per pancreas). Thus, much of the required polyamines could have been generated in the pancreas via the interconversion pathway if they were available from the systemic extracellular fluid to bone extracellular fluid, where it may influence the cellular P\textsubscript{i} metabolism accompany stimulation of these cells with PTH [2].

In a recent detailed study [1], it was demonstrated that parathyroid hormone (PTH) stimulates active (Na\textsuperscript{+}-dependent) uptake of P\textsubscript{i} in the osteoblast-like cell line UMR 106. It was proposed that this effect might be of importance in vivo in the regulation of P\textsubscript{i} transport from the systemic extracellular fluid to bone extracellular fluid, where it may influence the rate of bone mineral deposition. As this might involve transcellular movement of P\textsubscript{i} across the bone-lining cells, and as we have already demonstrated effects of calcitonin [2] and 1.25-dihydroxyvitamin D\textsubscript{3}, the regulation of P\textsubscript{i} transport from the systemic extracellular fluid to bone extracellular fluid, where it may influence the rate of bone mineral deposition. As this might involve transcellular movement of P\textsubscript{i} across the bone-lining cells, and as we have already demonstrated effects of calcitonin [2] and 1.25-dihydroxyvitamin D\textsubscript{3} [3] on the P\textsubscript{i} concentration in UMR 106, we have now examined whether changes in intracellular P\textsubscript{i} metabolism accompany stimulation of these cells with PTH.

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mixture of 2-methylpropan-1-ol and petroleum spirit [7]. 32P uptake is expressed as the distribution ratio: (c.p.m./l of cell water)/(c.p.m./l of extracellular water). All results are expressed as means ± S.E.M.

In agreement with the observations of Selz et al. [1], PTH stimulated 32P uptake in UMR 106-06 cells in nine experiments, over a dose range of 10^{-11}−10^{-7} M (from a Friedman two-way analysis of variance, F_1 = 23, P < 0.0001). In a separate series of four experiments over the same dose range, this was accompanied by a comparable fractional increase in the cellular 32P concentration (F_1 = 15, P < 0.01). For example, at a dose of 10^{-7} M, the cellular 32P concentration was 7.5 ± 1.0 mmol/l of cell water, compared with 4.8 ± 0.6 in control cells; whereas, the total 32P distribution ratio was 9.6 ± 1.3 with PTH, compared with 6.5 ± 0.9 in control cells. This implied that PTH brings about a net movement of 32P from the extracellular medium into the cell.

However, on measuring the incorporation of extracellular 32P into cellular 32P, and organic phosphates, it was unexpectedly found that the fraction of cellular 32P in the form of P, did not increase in response to PTH (it. stimulation of P, transport at the plasma membrane). For example, at a dose of 10^{-7} M, the cellular 32P concentration was 7.5 ± 1.0 mmol/l of cell water, compared with 4.8 ± 0.6 in control cells; whereas, the total 32P distribution ratio was 9.6 ± 1.3 with PTH, compared with 6.5 ± 0.9 in control cells. This implied that PTH brings about a net movement of 32P from the extracellular medium into the cell.

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Overproduction and isolation of elongation factor Tu using Escherichia coli grown on protiated and deuterated succinate

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Elongation factor Tu (EF-Tu) is a protein with an important role in the elongation cycle during protein biosynthesis in prokaryotes. In Escherichia coli it has a molecular mass of 43 225 Da [1], its amino acid sequence is known [1] and the crystal structure of the 39 kDa tryptic digest fragment has been determined [2]. The protein has a limited stability in solution (a half-life in the order of a few hours at pH 7 and 4°C). Stability is increased by the presence of either GDP or the antibiotic kirromycin [1].

Kirromycin inhibits protein biosynthesis by binding to EF-Tu. As part of a programme aimed at elucidating, in detail, the molecular basis of kirromycin action using 1H n.m.r. techniques, we have attempted to produce large quantities of pure deuterated EF-Tu. Economic production of deuterated protein requires that the bacterial strain grows on a medium with succinate as the only carbon source.

E. coli MRE 600 (wild-type) produces about 1 × 10^{-7} mol of EF-Tu/l of culture medium (L broth), as measured using a GDP-exchange assay [3]. Two overproducers were available to us: LBE 1101 gp582 (‘LB clone’), a gift from Dr E. Vlijgenboom and Professor L. Bosch [4] and MRE600 pCP1857 (pCP40) (‘TDH clone’), the plasmids being gifts from Professor A. Parmeggiani [5]. EF-Tu production in both clones grown on L broth and succinate media was monitored by the GDP-exchange assay. On L broth the LB clone produced up to 3 × 10^{-7} mol of EF-Tu (see Fig. 1). As shown in the Figure, the concentration of EF-Tu falls sharply in early stationary phase, but quickly recovers achieving the maximum value 1−2 h later. When grown on succinate, however, this clone produces no more EF-Tu than wild-type MRE600. This strain is therefore not immediately suitable for production of deuterated EF-Tu.

Abbreviation used: EF-Tu, elongation factor Tu.