p-Aminobenzoate (PAB) acts as a vitamin in rats, being mediated mechanism at low concentrations of the drug and gave identical absorption rates with duplicate experiments. The subject was a female Caucasian aged 21 years, who gave informed consent for the experiments. Isoniazid was assayed spectrophotometrically (Dymond & Russell, 1970) using a Cecil CE grating spectrophotometer. Buccal absorption of isoniazid was linear over the initial concentration range 1–8 μmol/ml. A mean ± S.E.M. of 72 ± 5.3 μmol/5min was absorbed at 8 μmol/ml. Replacement of the sodium salts in the buffer by equimolar amounts of potassium salts or choline salts at pH 6.0 gave identical absorption rates with duplicate experiments. Points represent mean absorption rates with standard errors shown as vertical bars. ○, Experiments using Na⁺-containing buffer; ▲, experiments using K⁺-containing buffer.

With an initial concentration of 29 μM the metabolic loss was 12%, 87 μM 5.5% and 146 μM 3.6%. Buccal mucosa was permeable to PAB. Absorption rates were linear with initial concentrations of PAB up to about 150 μM. Identical absorption rates were obtained using either Na⁺ or K⁺ buffers (Fig. 1). It was not possible to assess possible inhibition of isoniazid absorption by structural analogues tested with each analogue at a concentration fivefold that of the isoniazid at 2 μmol/ml. Nicotinamide, isonicotinic acid and acetylisoniazid (a major metabolite of the drug) were all without any effect upon the absorption of isoniazid.

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*p*-Aminobenzoate (PAB) acts as a vitamin in rats, being essential for normal growth (Ansbacher, 1941). Absorption of PAB from rat small intestine in vitro showed linear kinetics in relation to initial concentration in the range 0.5–50 mM. Non-saturable absorption with a lack of effect of added glucose, ouabain, PAB analogues or absence of Na⁺ all suggested passive diffusion (Arvanitakis et al., 1978). Transport was not against the concentration gradient with rat and hamster small intestine (Spencer et al., 1966).

Absorption of some nutrients from the human mouth mimics in many ways the characteristics of absorption from mammalian small intestine (reviewed by Evered & Vadgama, 1983). Our present aim was to investigate the absorption of PAB from the human mouth to compare with the results obtained with rat small intestine. Buccal mucosa was permeable to PAB. Absorption rates were linear with initial concentrations of PAB up to about 150 μM. Identical absorption rates were obtained using either Na⁺ or K⁺ buffers (Fig. 1). It was not possible to assess possible inhibition of isoniazid absorption by structural analogues tested with each analogue at a concentration fivefold that of the isoniazid at 2 μmol/ml. Nicotinamide, isonicotinic acid and acetylisoniazid (a major metabolite of the drug) were all without any effect upon the absorption of isoniazid.

We conclude that isoniazid is absorbed from the human mouth by Na⁺-independent passive diffusion with the concentration gradient as found for rat small intestine in vivo (Aznar-Ferreres, 1953) and in vitro (Barley et al., 1972). Isoniazid is poorly soluble in chloroform and insoluble in non-polar solvents. Lipid solubility encourages buccal mucosal absorption (Beckett & Hossie, 1971). Also isoniazid is poorly ionized at pH 6 and the un-ionized isoniazid must be the molecular species passing across mucosal membranes.

Abbreviation used: PAB, p-aminobenzoate.

Permeability of human buccal mucosa to p-aminobenzoate in vivo

Presence of ethacrynic acid (10 μmol/ml) did not alter absorption of isoniazid (2 μmol/ml). The diuretic ethacrynic acid is known to inhibit active extrusion of Na⁺ ions from cells.

Fig. 1. Absorption of PAB from the human buccal cavity from six experiments at pH 6
possible inhibition of absorption by folic acid as it interfered with the PAB assay.

We conclude that PAB passes across human buccal mucosa by a non-saturable, Na⁺-independent process presumably physical diffusion. These transport characteristics of PAB are similar to those found with small intestine of rats and hamsters in vitro (Spencer et al., 1966; Arvanitakis, 1978). PAB will be 92% ionized at pH 6 as in our experiments. Presumably the un-ionized molecule will be the permeant species.

Highly ionized at physiological pH values. Absorption may be limited by a binding or permeability phenomenon associated with the outer membrane we have used an m-chlorophenylhydrazone (CCCP), have the ability to increase the permeability of this membrane. In an attempt to elucidate more clearly this mechanism, we have used a photoaffinity label, TTFB, which is a highly polar compound and is generally lipophilic weak acid and from there may be able to penetrate into the cell. The results of these studies are consistent with the idea that the uncoupler acts as a protonophore, shunting the protons across the coupling membrane and hence enhancing the rate of respiration.

Surprisingly perhaps, buccal mucosa is also permeable to another vitamin-like compound, choline, which is also highly ionized at physiological pH values. Absorption was also Na⁺-independent (D. F. Evered & F. Mynah, unpublished work).

Uncoupler resistance in Escherichia coli

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The ‘classical’ or protonophoric uncouplers of oxidative phosphorylation, typified by 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole (TTFB) and carbonyl cyanide m-chlorophenylhydrazone (CCCP), have the ability to uncouple respiratory electron flow from ATP synthesis. As a result the latter ceases whilst the former proceeds maximally.

Several proponents of the chemiosmotic hypothesis have suggested that these uncouplers act as protonophores, shunting protons across the coupling membrane and hence dissipating the proton electrochemical gradient established by respiratory electron flow. Principal support for this mechanism comes from physicochemical considerations (uncouplers are generally lipophilic weak acids) and from positive correlations between protonophoric activity and uncoupling potency in mitochondria (Cunarro & Weiner, 1975) and liposomes (Bakker et al., 1973).

Over recent years, studies with photoaffinity labels (Katze & Wilson, 1978; Hanstein & Hatefi, 1974; Kurup & Sanadi, 1977; Partis et al., 1984) and the isolation of uncoupler-resistant mutants of Escherichia coli (Ito et al., 1983; Sedgewick et al., 1984) and Bacillus megaterium (Decker & Lang, 1977), and a line of Chinese Hamster ovary cells (Freeman et al., 1983), have led several groups to suggest more specific mechanisms for uncoupling. The results of these studies are difficult to rationalize with a purely protonophoric mechanism for uncoupling.

It has been recognized that the outer membrane in Gram-negative bacteria such as E. coli can pose problems when studying the bioenergetic properties of intact cells: it acts as a permeability barrier to high molecular weight compounds such as ionsophores. EDTA treatment has to be employed to increase the permeability of this membrane. In an attempt to ensure that any uncoupler resistance in our studies is not due to a binding or permeability phenomenon associated with the outer membrane we have used an E. coli mutant (designated Doc S) that has a defective outer membrane (Ahmed & Booth, 1983). The membrane in this strain does not act as a permeability barrier to penicillins, ionophores and bile salts. Our hypothesis is that these compounds can pass the outer membrane, so can negatively charged uncouplers such as TTFB and CCCP. The uncoupler-resistant mutants described in this communication have retained this leaky outer membrane, along with the other phenotypic characteristics of the Doc S strain (amino acid requirements and constitutive lac operon).

All mutant strains were isolated by streaking bacteria on minimal medium plates containing 44 mmm-sodium succinate as carbon source and supplemented with uncoupler. Plates were exposed to u. v. light for 3 h after inoculation. A TTFB-resistant mutant, designated TUV, was isolated by streaking Doc S on to plates supplemented with 100 µm-TTFB. This bacterium grew in liquid culture in the presence of 100 µm-TTFB, but only grew in liquid culture in the presence of 50 µm-CCCP after a lag phase of 45 h. In an attempt to enhance this CCCP cross-resistance, samples of TUV were streaked on to plates supplemented with 50 µm-CCCP. The bacterium isolated from these plates was designated CUV. It retained the growth characteristics of TUV with respect to TTFB but was markedly more resistant to CCCP.

The properties of TUV and CUV with respect to growth in the presence of uncoupler are mirrored in their relative abilities to accumulate proline in the presence of TTFB and CCCP. We have found that a titre of 34 nmol of TTFB/mg of total cell protein completely inhibits proline accumulation in Doc S. There is no inhibition of proline uptake at this titre with TUV or CUV. Similarly at 10 nmol of CCCP/mg of total protein, Doc S retains only 10% of its uninhibited capacity for accumulating proline whilst TUV retains 55% and CUV some 95% of this capacity. Thus uncoupler resistance is also manifest in the ability of these bacteria to accumulate proline.

The non-invasive technique of 32P n.m.r. has been used in recent weeks to study the transmembrane pH gradient in Doc S and TUV. Whilst this work is as yet incomplete, we can now say with some confidence that at 7 nmol/mg of total cell protein, TTFB collapses the ΔpH across the cytoplasmic membrane of both Doc S and TUV at an equivalent rate when the cells are respiring endogenously. We therefore conclude that the uncoupler resistance shown by TUV is not due to a permeability or binding effect.

REFERENCES


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Abbreviations used: TTFB, 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole; CCCP, carbonyl cyanide m-chlorophenylhydrazone.