A or crude pancreatin, indicating that the brush-border peptidase activity was not due to adsorbed gastric or pancreatic enzymes.

Analysis of the products of hydrolysis indicated that the oligopeptides were hydrolysed sequentially. For example, when pentaglycine was used as a substrate, stoichiometric amounts of tetraglycine and free glycine were released. It was also found that the pH optimum for the hydrolysis of tetraglycine was the same as that for the release of smaller peptides and of free glycine. Studies of the relative rates of hydrolysis of the glycine peptides indicated that the longer peptides were hydrolysed more rapidly than the shorter.

Studies were performed to determine whether the glycine residues were cleaved from the *N*- or the *C*-terminal of the peptide. A series of *N*- and *C*-terminal-protected glycine derivatives were incubated with brush borders to select suitable blocking groups. Free glycine was liberated from the methyl, ethyl, *tert*-butyl and benzyl esters of glycine, but not from glycine amide, benzyl-oxy-carbonylglycine and benzoylglycine. Incubation of peptide derivatives showed that free glycine was liberated from triglycine amide, but not from benzyloxycarbonyltriglycine, benzoyltriglycine or benzyloxycarbonyltetraglycine, suggesting that the glycine residues were cleaved sequentially from the *N*-terminal of the peptide.

This conclusion was confirmed by synthesizing two tetraglycine peptides, labelled at either the *N*- or the *C*-terminal glycine residue (Merrifield, 1964; Gutte & Merrifield, 1969). When the [$1-\alpha\text{-}^3\text{H}_2\text{-glycine}$]tetraglycine was incubated with brush borders labelled glycine was released but the liberated diglycine and triglycine were unlabelled. When the [$4-\alpha\text{-}^3\text{H}_2\text{-glycine}$]tetraglycine was hydrolysed no labelled glycine was released but the diglycine and triglycine were radioactive. These experiments indicate that the brush-border hydrolase is an amino-oligopeptidase.

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Partial Purification and Characterization of an Iodide Peroxidase from *Enteromorpha linza*

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The occurrence of iodinated compounds in marine algae is well established (Roche & Yagi, 1952; Scott, 1954), but the mechanism by which they are formed is not fully understood (Tong & Chaikoff, 1955; Roche, Thoai & Lafon, 1949). A peroxidase is responsible for the iodination of tyrosyl groups of thyroglobulin (Coval & Taurog, 1967; Hosoya & Morrison, 1967), but similar activity could not be detected with red-algal peroxidases (Murphy & Ó hEocha, 1969). However, iodination is associated with a peroxidase that we have partially purified from the green alga *Enteromorpha linza*.

The alga, which was collected on the shores of Galway Bay, was ground in the presence of liquid N_2 and extracted overnight in 10 mM- NaHCO_3 , pH 8.3. The extract catalysed the iodination of tyrosine in the presence of iodide and hydrogen peroxide. In the absence of tyrosine, iodide was peroxidized to iodine, a process that can be conveniently followed spectrophotometrically in the presence of excess of iodide (Hosoya, Kondo & Ui, 1962; Alexander, 1962), thus providing a sensitive and rapid assay procedure for monitoring the activity. The extract was centrifuged at 35000g for 90 min. The supernatant, which contained most of the activity, was decanted and fractionated with ammonium sulphate. The fraction precipitated by between 0.5 and 0.7 saturation with ammonium sulphate was redissolved in 0.1 M-glycine-NaOH buffer, pH 9.0, dialysed against the same buffer and further fractionated on a column of Sephadex G-200.

Starch-gel electrophoresis of the peroxidase-containing fraction of the purified preparation in tris-citrate buffer, pH 7.0 (*I* 0.018 in gel and 0.27 in electrode), resolved two components, detected with a staining system that contained iodide as electron donor (Murphy, 1970). Isoelectric focusing of this fraction in a sucrose gradient also revealed two components; the major component had isoelectric point 4.45, and the minor one isoelectric point 4.7.

The peroxidase activity, as determined with *o*-dianisidine as electron donor (Worthington Biochemical Corp. Descriptive Manual), ran parallel with the iodide peroxidation activity during the above purification steps including starch-gel electrophoresis and isoelectric focusing, suggesting that one enzyme is responsible for both reactions.

Elution of the peroxidase activity from a calibrated column of Sephadex G-200, equilibrated with 0.1M-NaCl in 0.02M-phosphate buffer, pH 7.0, indicated that the molecular weight of the enzyme is about 220 000.

The purified enzyme did not display a Soret band, but was strongly inhibited by cyanide and azide, as are the activities of most haemoproteins. The inhibition with cyanide was competitive with hydrogen peroxide, as is the case with conventional haem-containing peroxidases.

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Metabolism of Sodium Oestrone [³⁵S]Sulphate in the Rat

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Earlier studies on the metabolic fate of orally administered and injected sodium oestrone [³⁵S]-sulphate in the rat (Hanahan & Everett, 1950; Davis *et al.* 1950; Lewison, Levi, Jones, Jones & Silberstein, 1951) have shown that the ester undergoes appreciable desulphation with the rapid excretion of inorganic sulphate via the kidneys. Subsequently several workers have reported that mammalian liver, placental and kidney preparations are able to desulphate oestrone sulphate, the responsible enzyme being localized mainly in the microsomal fraction of the tissue cell (Pulkkinen & Paunio, 1963; French & Warren, 1967; Zuckerman & Hagerman, 1969). However, the site of desulphation of injected oestrone sulphate in the mammalian system has never been established with certainty, and a reinvestigation of the metabolism of oestrone sulphate by using new and improved techniques was therefore undertaken.

Free-ranging male and female rats receiving oestrone [³⁵S]sulphate either orally or by intravenous or intraperitoneal injection excreted approx.

70% of the radioactivity in the urine as inorganic sulphate within 48 h. The remainder of the dose was recovered mainly as ester-bound sulphate in the faeces (10–20%) and the urine (5%), although this material was not identified. In antibiotic-treated animals a similar excretion pattern was observed when the ester was administered intraperitoneally or intravenously. However, when given orally to treated animals, only 25% of the dose appeared in the urine as inorganic sulphate, the major portion being recovered in the faeces as unidentified ester sulphate (40%).

Administration of the ester to normal animals with bile-duct and ureter cannulae resulted in excretion of most of the radioactivity in the urine within 12 h. Relatively small amounts (4%) of the dose appeared in the bile, although this was shown by chromatography to consist of at least six components including inorganic sulphate oestrone sulphate and oestradiol 3-sulphate.

The organ distribution of administered oestrone [³⁵S]sulphate was examined by whole-body radioautography by the method of Curtis, Powell & Dodgson (1967). It was observed that in young male and female rats receiving the labelled ester intraperitoneally ³⁵S readily accumulated in the liver within 30 min. Subsequently ³⁵S was detected in kidney bladder and gut.

These collective findings suggest that desulphation of the ester *in vivo* is an intracellular process and that the liver is the principal organ where this occurs. This was supported by the observed rapid liberation of ³⁵SO₄²⁻ that occurred when isolated rat liver was perfused with blood containing oestrone [³⁵S]sulphate.

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