represented >80% of solubilized peptides, and the 1.2M-NaCl precipitates contained collaginous polypeptides of identical electrophoretic mobilities to cartilage 1x2x3x collagen chains. However, significant differences were noted in the polypeptide compositions of the 2.0M-NaCl precipitates from the different cartilages. Two major bands of \( M, \) approx. 150000 and 420000 were present in the sternal fraction when samples were analysed under non-reducing conditions but on reduction these bands were lost and the sample yielded a number of low-\( M, \) peptides of approx. 100000, 55000, 48000, 36000, 31000 and <20000. Polypeptides of similar sizes and properties have been reported in other studies on peptic digests of chick sternal cartilage (Reese & Mayne, 1981; Reese et al., 1982; von der Mark et al., 1982). The 2.0M-NaCl fraction from tibiotarsal cartilage contained polypeptides of identical mobilities to those in the sternal fraction but also present was a major, non-reducible, highly soluble polypeptide (\( M, 45000 \)). This short chain collagenous component was further purified by CM-cellulose chromatography under native conditions, and shown to possess an electrophoretic mobility and amino acid composition identical to the pepsinized product of G collagen, previously detected only in chondrocyte cultures (Gibson et al., 1983). Both the cell culture- and tissue-derived polypeptides lack cysteine, but have relatively high methionine, aromatic amino acid and hydroxylysine contents when compared with the well-characterized interstitial collagens. Segment-long-spacing crystallites of the purified native species of \( M, 45000 \) showed a banding pattern distinct from chick type I and II collagens, and were approx. 130nm in length corresponding to about 43% of the normal \( \alpha \)-chain size.

Investigation of the synthesis of short-chain collagens in short-term organ culture was conducted with tissue from zones 1/2 and zone 3 of tibiotarsal cartilages and with sterna from 17-day-old chick embryos. Sliced tissue was incubated for 6h in Dulbecco's modification of Eagle's Medium containing L-ascorbate (25\( \mu \)g/ml), \( \beta \)-aminopropionitrile (64\( \mu \)g/ml) and 50\( \mu \)Ci of L-\( ^{3} \)H]proline. After the incubation, proteinase inhibitors were added to the samples and the \( ^{3} \)H-labelled collagens in the medium were collected by precipitation after the addition of \( (NH_{4})_{2}SO_{4} \) to 30% saturation. Labelled collagens in the tissues were extracted into 50mM-Tris/HCl buffer, pH7.4, containing 4M-guanidinium chloride, for 48h at 4°C. The newly synthesized polypeptides were analysed by SDS/polyacrylamide-slab-gel electrophoresis and fluorography as described by Gibson et al. (1983).

Remarkable differences were observed in the low-\( M, \) collagens synthesized by the sternal cartilage and the epiphyseal cartilage, especially from zone 3 of the tibiotarsal cartilage. In all the incubations it was possible to demonstrate the synthesis of type II collagen and 1x2x3x chains and these species were recovered from both tissue and medium. The sternal tissue also synthesized considerable amounts of the collagenous J and H chains. No evidence was obtained for the synthesis of G collagen by sternal tissue, an observation consistent with the failure to detect G-related peptides in the peptic digests of intact sterna. In contrast, the tibiotarsal explants from zones 1/2 synthesized detectable levels of G collagen as well as very significant levels of J and H chains. However, the tibiotarsal zone 3 synthesized large amounts of G collagen but no J or H chains could be detected in either tissue or medium.

The presence of the pepsinized product of G collagen in chick embryo tibiotarsal cartilage, and the synthesis of G collagen by tibiotarsal cartilage, provides corroborative evidence that this short-chain collagen is synthesized particularly by the hypertrophying chondrocytes in zone 3 of the epiphyseal growth region. Also noteworthy is the fact that preliminary studies of G collagen synthesis in pulse-chase experiments with tibiotarsal cartilage indicate that the G collagen is processed with time to a smaller species, slightly larger than the pepsinized G (\( M, 45000 \)), in a manner analogous to the processing of interstitial collagens (Heathcote & Grant, 1981).

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A new carboxypeptidase responsible for the modification in vivo of MM-creatinine kinase in human plasma

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Agarose-gel electrophoresis of human plasma has revealed that there are three bands of CK activity all corresponding in mobility to the MM isoenzyme, whereas the MM isoenzyme isolated from skeletal muscle is found as only a single electrophoretic form. In normal plasma MM-CK undergoes electrophoretic transformation as a result of the action of an agent known as 'CK conversion factor' (Falter et al., 1981). The object of this study was to determine the nature of the electrophoretic conversion.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of MM-CK before and after treatment by CK conversion factor showed that the activity of the CK conversion factor was not that of an endopeptidase. In addition, phenylmethanesulphonyl fluoride did not inhibit CK conversion factor (Edwards & Watts, 1983).

Crude pancreatic extracts were found to cause exactly the same electrophoretic changes as those found for CK conversion factor. Commercially obtained preparations of carboxypeptidase A and carboxypeptidase B were both found to be effective 'conversion factors'. Carboxypeptidase B was particularly active. Analysis of amino acids
The role of peptidyl dipeptidase (angiotensin converting enzyme) in the hydrolysis in vitro of gliadin by human intestinal brush-borders

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Peptidyl dipeptidase (EC 3.4.15.1) is widely distributed in endothelial tissues and plays a key role in the renin-angiotensin system, hence the name 'angiotensin converting enzyme'. The enzyme removes the C-terminal histidyl-leucine residue from angiotensin I to produce angiotensin II, a vasoconstrictor. The enzyme has been reported to be located in the microvilli of kidney tubules and small intestine (Takada et al., 1981; Ward & Sheridan, 1982). Peptidyl dipeptidase has been the focus for the development of a new generation of anti-hypertensive drugs which inhibit the enzyme at low concentrations (Cushman et al., 1977).

Human small intestine was obtained at surgery, and washed with 0.15M NaCl. The mucosa was separated from the underlying muscle tissue and frozen on dry ice. The material was stored at −20°C and used within 4 weeks. The mucosa was homogenized at 15% (w/v) in 50mm-mannitol/2mm-Tris/HCl pH 7.1, by using a blender for 1 min. The homogenate was made 10mm with respect to Ca2+ by the addition of solid calcium chloride, and stirred slowly in the cold for 30 min. The Ca2+-treated homogenate was fractionated by spinning at 2000gs for 20 min. The pellet (P1) was resuspended in the mannitol/Tris. The brush-border fraction was then obtained by spinning the supernatant at 25000gs for 30 min, washing the pellet twice with mannitol/Tris, and resuspending in the same buffer. The fractions were assayed for the brush-border marker Zn2+-resistant α-glucosidase by the fluorimetric method of Peters et al. (1972), and for peptidyl dipeptidase using hippurylhistidyl-leucine and measuring the released histidyl-leucine fluorimetrically after reaction with o-phthalaldehyde (Cheung & Cushman, 1973). Protein was measured by the method of Lowry et al. (1951).

The distribution of peptidyl dipeptidase in the subcellular fractions obtained is shown in Table 1. The brush-border fraction was enriched 15-fold as judged by the distribution of the brush-border marker enzyme α-glucosidase. The peptidyl dipeptidase is enriched just over 5-fold and 3 times as much is found in the cytosol. This indicates that the enzyme is located in more than one site in the cell. There is brush-border localization and also localization in the cytosol, or possibly the lysosomes which would be ruptured during the vigorous homogenization in dilute medium.

The breakdown of a PT digest of gliadin was measured by the sensitive fluorimetric method described by Bruce & Woodley (1981), which measures the release of free glutamine and glutamic acid residues (38% by weight of gliadin). Human intestinal brush-borders could completely hydrolyse PT-gliadin in an overnight incubation at a brush-border: substrate ratio greater than 1 (as measured by protein). The initial rate of breakdown was linear for 60 min.

The breakdown of the PT-gliadin almost certainly involves the concerted action of a number of brush-border peptidases. That peptidyl dipeptidase was one of them could be demonstrated by the use of the specific inhibitor captopril (donated by Mr. Lucania, Squibb Institute). The human brush-border peptidyl dipeptidase was very sensitive to captopril inhibition, having a KI of 7 x 10−5M and giving complete inhibition at 10−4M. In the presence of 10−6M-captopril the initial rate of PT-gliadin breakdown was inhibited by 34%. This indicated that the peptidyl dipeptidase was one of the enzymes involved in the digestion of the dietary protein gliadin.

The results presented suggest that in the small intestine, the brush-border peptidyl dipeptidase is highly unlikely to be functioning in the control of angiotensin levels, but is acting as a digestive peptidase involved in the terminal stages of protein digestion. The dipeptides produced will then be hydrolysed by aminopeptidases such as aminopeptidase N. Indeed, studies have also shown this enzyme to be involved in gliadin breakdown.