intermolecular cross-linking were established by noting their absence when the fibres were reduced after rupture of the bonds with β -aminothiols, semicarbazide, or thermal denaturation (Bailey & Lister, 1968). These two peaks were again absent from the chromatograms when tropocollagen solutions were reduced, confirming that they were not involved in the intramolecular bond.

One of these labile components has now been isolated from rat tail tendons and its structure determined. When isolated from rats previously injected with [^{14}C]lysine the component was radioactive, indicating that it was derived from lysine. Electrophoretic mobility indicated it to be slightly less basic than lysinonorleucine isolated from elastin (Franzblau, Sinex, Faris & Lampidis, 1965). The mass spectrum of the *N*-trifluoroacetyl methyl ester derivative identified the component as hydroxylysinonorleucine [N^{ϵ} -(5-amino-2-hydroxy-5-carboxypentanyl)lysine] from the fragmentation pattern and identification of the mass ion 703.44 (peak matched against perfluorokerosene).

The labile intermolecular bond exists in the fibre in the unreduced form, $\Delta^{6,7}$ -dehydrohydroxy-lysinonorleucine. The probable route of biosynthesis is through the condensation of the 'lysine aldehyde' formed in the telopeptide region of the molecule (Bornstein & Piez, 1966) with an ϵ -amino group of hydroxylysine. Amino acid analysis demonstrated the absence of hydroxylysine from the telopeptide region, indicating that this new amino acid must exist as an intermolecular bond from the telopeptide region of one molecule to the body of the second molecule.

The 'lysine aldehyde', α -aminoadipic δ -semi-aldehyde, was isolated in its reduced form as hydroxynorleucine and identified by the mass spectrum of the bis-*NO*-trifluoroacetate and confirmed by comparison with authentic material synthesized from dihydropyran (Gaudry, 1948).

A second minor radioactive peak in the acidic region was identified as chloronorleucine (*N*-trifluoroacetyl methyl ester mass ion +1,276.01). This compound was formed from hydroxynorleucine during hydrochloric acid hydrolysis, the conversion reaching a maximum of 50% after 24 hr. at 105°.

The second labile bond appears to be more complex and probably cross-links several molecules. Its structure is currently being investigated.

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The Isolation of Human Calcitonin

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Human thyroid is a poor source of calcitonin (Galante *et al.* 1968), but medullary carcinoma contains a high concentration of the hormone (Cunliffe *et al.* 1968).

Human calcitonin was isolated in pure form from a mediastinal mass of medullary carcinoma. The dry defatted tumour was extracted three times with butan-1-ol-acetic acid-water (150:15:42, by vol.), and the pooled extracts were concentrated and freeze-dried.

The resultant extract (potency 10 M.R.C. units/mg. in the rat bioassay of Kumar *et al.* 1965) was distributed in a 32-tube steady-state apparatus. Fifty upper-phase transfers were made in a system of butan-1-ol-0.1 *N*-acetic acid (1:1, v/v) containing 250 mg. of ammonium acetate/l. of mixed phases. One major ($K=0.5$) and one minor ($K=7.5$) region of activity were found.

The major activity was dissolved in 0.1 *M*-formic acid containing 1 *M*-2-mercaptoethanol and chromatographed by gel-filtration on Bio-Gel P-6 (<400 mesh) in 0.1 *M*-formic acid. The emergent fractions were monitored by thin-layer chromatography, bioassay and measurement of protein by the Folin-Lowry method.

The peak of activity coincided with a protein peak homogeneous by electrophoresis and thin-layer chromatography in several systems. The specific activity of this material was 135 M.R.C. units/mg. The amino acid composition was (residues/mol.) (values for porcine α -calcitonin in brackets): Asp 1 (0), Asn 2 (4), Thr 5 (2), Ser 1 (4), Glu 0 (1), Gln 2 (0), Pro 2 (2), Gly 4 (3), Ala 2 (1), Val 1 (1), ($\frac{1}{2}$ Cys)₂ 2 (2), Met 1 (1), Ile 1 (0), Leu 2 (3), Tyr 1 (1), Phe 3 (3), Lys 1 (0), His 1 (1), Arg 0 (2), Trp 0 (1). This gives a molecular weight of 3419.

In a separate set of experiments (Riniker *et al.* 1968) the initial tumour extract was first chromatographed on Bio-Gel P-6. Two active zones were found (D and M). The activities from both zones were purified by 360 transfers in a countercurrent-distribution apparatus in butan-1-ol-acetic acid-water (4:1:5, by vol.).

The products were homogeneous and calcitonin M was identical with the product from the first procedure. Calcitonin D had the same amino acid composition and specific activity as calcitonin M but differed in sedimentation coefficient *S* (0.51 $\times$ 10⁻¹³ sec. for calcitonin M and 0.81 $\times$ 10⁻¹³ sec. for

calcitonin D in 0.05 N-acetic acid). Calcitonin D was shown to be the dimer of calcitonin M, which is thought to be the natural hormone.

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Structure and Synthesis of Human Calcitonin M

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Enzymic degradation of human calcitonin M yielded several fragments, which were used for amino acid-sequence studies. Thus the complete structure of the hormone was found to be:

Pro-Ala-Gly-Val-Gly-Ile-Ala-Thr-Gln-Pro-Phe-Thr-His-Phe-Lys-Asn-Phe-Asp-Gln-Thr-Tyr-Thr-
Gly-Leu-Met-Cys-Thr-Ser-Leu-Asn-Gly-Cys

(Neher, Riniker, Rittel & Zuber, 1968). A comparison with porcine α -thyrocalcitonin (Neher, Riniker, Zuber, Rittel & Kahnt, 1968) shows that only 14 amino acids occupy identical positions, whereas the remaining 18 are different. Calcitonin thus represents the first known polypeptide hormone in which there occurs such a pronounced species difference.

The structure of calcitonin M was subsequently confirmed by total synthesis (Sieber, Brugger, Kamber, Riniker & Rittel, 1968) by a fragment-condensation approach which yielded a large number of crystalline intermediates. The α -amino group and all the functional side chains, except histidine, were protected by substituents, which could be split off by mild treatment with acid in the last step of the synthesis. Determination of amino acid composition, chromatography, electrophoresis, tryptic-degradation studies, and biological-activity tests proved the synthetic material to be identical with the natural hormone.

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Lactose Synthetase A Protein from Human Milk

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Lactose synthetase A protein catalyses the transfer of galactosyl units from UDP-galactose to *N*-acetylglucosamine, forming *N*-acetyl-lactosamine, but in the presence of α -lactalbumin this reaction is partially inhibited, and galactosyl transfer to glucose, forming lactose, is favoured (Brodbeck, Denton, Tanahashi & Ebner, 1967; Brew, Vanaman & Hill, 1968). The A protein occurs in cow's milk (Babad & Hassid, 1966) but in comparison the lactose synthetase activity/ml. of human milk is tenfold greater. Therefore human milk was chosen as a source of the A protein for its isolation and further study.

Galactosyltransferase activity was determined spectrophotometrically by the method of Brodbeck & Ebner (1966), with at least a 20-fold excess of the linking enzymes. Ammonium sulphate fractionation and chromatography on DEAE-cellulose and Sephadex G-150 gave the A protein from human milk in about 30% yield, with 100- to 200-fold purification and a galactosyltransferase activity of approximately 1 μ mole/min./mg. at 25°. Its gel-filtration behaviour indicated a molecular weight of 70000, similar to that of the cow's-milk enzyme (Ebner, Tanahashi, Brodbeck & Kiyosawa, 1968).

The partially purified A protein catalysed *N*-acetyl-lactosamine synthesis with K_m values of 0.9 mM and 40 μ M for *N*-acetylglucosamine and UDP-galactose respectively. *N*-Acetylglucosamine concentrations above 4 mM inhibited the reaction. Human α -lactalbumin did not stimulate the reaction at low *N*-acetylglucosamine concentrations, in contrast with the observations of Brew *et al.* (1968) on the bovine proteins, but behaved as an uncompetitive inhibitor at all *N*-acetylglucosamine concentrations tested (0.25–20 mM).

The human A protein preparation also possessed feeble lactose synthetase activity, with a K_m for glucose of 90 mM. Further gel filtration on Sephadex G-150 failed to eliminate this activity, suggesting that it may be an inherent property of the A protein and not due to contamination with α -lactalbumin.

Human and bovine α -lactalbumin were equally effective in stimulating the lactose synthetase reaction, each with a K_m of 3 μ M (43 μ g./ml.) in the presence of 80 mM-glucose. The K_m for glucose was