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Urinary Peptides derived from the Cross-linked Regions of Connective Tissue Proteins

WE have used 'Sephadex *G*-15' to examine the molecular weight distribution of urinary peptides obtained from normal males, aged between 22 and 24 yr, by a method designed to protect labile groups within peptides. We obtained a peptide fraction, of approximate molecular weight 600, which gave several slowly developing yellow Ehrlich positive zones after electrophoresis in pyridine acetate buffer at *p*H 5·5. This staining property was lost after chromatography in systems containing acetic acid, presumably because of oxidative decomposition on the paper surface in acetic acid. High voltage electrophoresis of this peptide fraction in pyridine acetate buffer at *p*H 3·7 showed that at least fifteen peptides were present.

Total hydrolysis of the whole peptide fraction in sealed tubes containing 6 N hydrochloric acid under nitrogen at 100° C for 24 h yielded a complex mixture of amino-acids. These amino-acids were separated with the Technicon autoanalyser system under nitrogen using the sodium buffer elution technique[1]. The hydrolysate contained the following. (*a*) Desmosine, isodesmosine and lysino-norleucine which are thought to be unique to insoluble elastin where they are known to be the acid stable cross-linkages[2,3]. (*b*) Hydroxyproline and hydroxylysine, the latter of which is known to be involved in glycosidic linkage with galactose and glucose in collagen[4] and has only been isolated from connective tissue proteins. (*c*) Two unidentified amino-acids, described as V* and Hi*, which have recently been observed for the first time in a protein fraction of the human intervertebral disk (unpublished results of F. S. Steven, D. S. Jackson and K. Broady). This protein is very firmly bound to collagen in the disk. The quantity of V* in the urinary peptide fraction is equivalent to 0·3 mg/l. of urine. (*d*) Several unidentified ninhydrin positive peaks which seem from their positions on the chromatographic elution pattern to be derived from basic amino-acids.

Table 1. R_F VALUES OF COMPOUNDS GIVING A YELLOW EHRLICH REACTION

Solvent	Iso-propanol : water 160 : 40	*n*-Butanol : ethanol : water 120 : 30 : 50	Colour development
Urea	62	41	Fast
Homocitrulline	27	17	Fast
Unknown	39	31	Slow

Total hydrolysis of the disk protein fractions referred to in (*c*) also yielded a yellow Ehrlich positive spot after two dimensional chromatography in systems which did not contain acetic acid. This yellow spot again failed to appear in hydrolysates which had been subjected to chromatography or electrophoresis in systems containing acetic acid. Table 1 shows the R_F values of urea, homocitrulline and the unknown Ehrlich positive spot.

Hydroxyproline peptides are commonly found in urine, but hydroxylysine has only once been observed[5] in normal adult urine. Desmosine, isodesmosine, lysino-norleucine and the unidentified amino-acids have not previously been reported in urine. The modified amino-acids have probably escaped detection in previous studies of urine, because little work has been carried out on these low molecular weight peptide fractions.

The presence of this peptide fraction suggests that the catabolism of connective tissue proteins proceeds normally by proteolytic digestion until a cross-linked region is reached. At this stage a fragment is released which contains the cross-link plus perhaps two amino-acid residues adjacent to the cross-link on each peptide chain. This fragment might be an enzyme-resistant H-peptide. The urinary peptides containing desmosine, isodesmosine or lysino-norleucine as cross-linkages would be derived from catabolism of elastin. Those peptides containing hydroxylysine would probably be derived from collagen catabolism and those peptides containing V* and Hi* might be expected to be derived from the disk type protein complex with collagen.

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Sedimentation Behaviour of Rapidly Labelled RNA from *Escherichia coli*

RAPIDITY of labelling after exposure to radioactive precursor is one of the distinctive characteristics of bacterial messenger RNA (*m*RNA)[1]. In early studies, rapidly labelled RNA prepared from bacterial extracts obtained by alumina grinding was of small size and sedimented as a broad peak at about 8–10*S* (ref. 1). When steps were taken to reduce enzyme degradation, rapidly labelled RNA showed a very heterogeneous distribution with fractions sedimenting at 6–8*S*, 14–16*S* and heavy chains at 16–30*S* (refs. 2 and 3). The heavy chains of rapidly labelled RNA could be dissociated to 18*S* by centrifugation in gradients of sulpholane, a compound known to decrease interactions between ribosomal components[4]. There is also evidence that *m*RNA may become fragmented by sonication. Thus *m*RNA labelled with ^{3}H-uridine from *E. coli* which sedimented at 10–12*S* became fragmented by sonication to chains sedimenting at 4*S* (ref. 5).

Chromatography of RNA on columns of methylated albumin (MAK)[6] resolved rapidly labelled RNA from *E. coli* into several fractions which eluted around the