glucose was not lost, suggesting that close association of the nucleotide with the tissue residue was involved. Replacing the ammonium acetate with acetate buffers of pH below 4 resulted in some retention of nucleotides by the tissue residue, presumably by ionic binding to protein. Similar but much smaller effects were produced in the absence of cellulose phosphate. We found that P11 cellulose phosphate washed and equilibrated with acetate buffer of pH 5.0 was a satisfactory alternative to 1,1,1-trifluoro-3-(2-thenoyl)acetone and gave good recoveries of the nucleotides, including UDP-glucose, without the risk of contamination that attended the use of the soluble metal-complex-forming agent.

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Identification and Determination of Hydroxylysine in Connective-Tissue Proteins

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A significant content of hydroxylysine has been tentatively reported in a non-collagenous fraction obtained from complete human intervertebral discs (Steven, Jackson & Broady, 1968) and protein-polysaccharide complexes derived from intercostal cartilage (Steven, Broady & Jackson, 1969). Robert and his colleagues have isolated a number of connective-tissue non-collagenous structural glycoproteins, which appear to contain considerable amounts of hydroxylysine (Robert & Comte, 1968). If hydroxylysine is present in these non-collagenous proteins, which are difficult to remove from the collagen, unreliable results for the hydroxylysine content of the associated collagen may be obtained.

Steven *et al.* (1968) isolated collagen from complete human intervertebral discs by using crude bacterial α -amylase. Most of the collagen was probably derived from the annulus fibrosus, because we have found this method ineffective when applied to the nucleus pulposus of the disc. However, a short treatment with porcine trypsin of the water-insoluble complexes from the nucleus pulposus released a non-diffusible non-collagenous glycoprotein (NT). Further digestion of the insoluble residue followed by dispersion in acetic acid and precipitation with sodium chloride gave a collagen apparently of 80–90% purity. The annulus fibrosus treated under similar conditions gave collagen of

purity comparable with that of insoluble tendon collagen (Steven & Jackson, 1967). Both disc collagens were higher in hydroxylysine content than most mammalian collagens.

Amino acid analysis, on the 130 cm. column of a Technicon apparatus, of the glycoprotein NT, which has been reported elsewhere (Pearson, Happey, Shentall, Naylor & Turner, 1969), showed one major peak (Xn) in the position expected for *n*-hydroxylysine. The following evidence proves that peak Xn is not 5-hydroxylysine. The ratio E_{440}/E_{570} for the ninhydrin reaction product was about 0.2 compared with about 0.5 for hydroxylysine. Elution with a single citrate buffer (pH 4.43, I 0.40) separated substance Xn from *allo*-hydroxylysine and *n*-hydroxylysine. The high-voltage electrophoretic mobilities of hydroxylysine and substance Xn at pH 1.85 were also considerably different. Under the same conditions that hydroxylysine was destroyed by periodate, substance Xn remained unchanged.

The compound reported as hydroxylysine by Steven and his co-workers in non-collagenous proteins from the disc and cartilage may be identical with the unknown component found by us in several similar preparations. The findings of Robert and his colleagues also require confirmation.

The identification of substance Xn is under investigation.

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Incorporation of Amino Acids into the Protein of Mitochondria Isolated from Soya-Bean Hypocotyls

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Early reports of amino acid incorporation *in vitro* by mitochondria isolated from higher plants were published (Webster, 1954; Das, Chatterjee & Roy, 1964) before the necessity of avoiding heavy bacterial contamination was realized (see Roodyn, 1968).

In view of the limited amount of information about protein synthesis by isolated higher-plant mitochondria (Lado & Schwendemann, 1967; Baxter & Hanson, 1968, Ellis & MacDonald, 1968)