

was tentatively supported by Blakely *et al.*⁵, who also observed an increase in the β -protein content of prolapsed disk tissue, although they assumed that this was due to the same protein as is prominent in the disks of elderly subjects. Lyons *et al.*⁶ reported the results of biochemical investigations during which they isolated an 'insoluble complex' from the disks of subjects over the age of 60 years taken as normal controls, and a 'similar type of complex' from eight 'herniated' specimens. Both complexes contained chondroitin sulphate B. These authors also supported the theory of pathologically accelerated ageing. The hexosamine/nitrogen ratios they reported for the insoluble complexes showed wide variations which could well be indicative either of a mixture or inconstant composition.

The changes in the intercellular matrix are, in the main, a reflexion of cell activity. In prolapse the polysaccharide content is reduced^{1,2} and the non-collagenous protein content increased, an alteration in the quality as well as the quantity of the matrix. This indicates disordered cell function, which may well stem from a disturbed nutritional state similar to that which has been proposed as a cause of osteoarthritis. No differences have been detected between the tissue from patients with or without a history of a significant spinal injury. In ageing there is also a change in the type of matrix, with the emergence of the dense β -protein as a major matrix component. Once this is present in appreciable quantities it seems reasonable to suggest that it affords a measure of protection against prolapse.

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BIOCHEMISTRY

Enzymatic Synthesis of the Skin-lightening Agent, Melatonin, in Amphibians

MELATONIN (*N*-acetyl-5-methoxytryptamine) has been shown to be the most effective skin-lightening agent in amphibians^{1,2}. This compound produces changes in pigmentation by causing the aggregation of melanin granules within the amphibian melanocyte. Although melatonin has been found to occur in mammals, no evidence for its formation has been obtained in amphibians where it exerts its most potent effects. High concentrations of radioactivity have been observed in the pineal area of the amphibian *Xenopus laevis* after the administration of the melatonin precursors ¹⁴C-5-hydroxytryptamine and ¹⁴C-methylmethionine³.

The formation of melatonin is catalysed by an enzyme, hydroxyindole-*O*-methyl transferase, which requires *N*-acetylserotonin as the substrate and *S*-adenosylmethionine as the methyl donor⁴. So far, this enzyme has been found only in the pineal gland of mammals⁵ and birds⁶. In mammals melatonin has been shown to inhibit the oestrous phase of the rat oestrous cycle and to produce decreases in ovary weight⁷. This communication describes the presence of hydroxyindole-*O*-methyl transferase in two amphibian species, *Xenopus laevis* larvae and adult frogs (*Rana pipiens*).

Table 1

Species	Hydroxyindole- <i>O</i> -methyl transferase activities
<i>Xenopus laevis</i> larvae	1.3
<i>Rana pipiens</i> adults:	
Brain	11.6
Pineal area	36.6
Optic tectum	34.5
Hypothalamus	36.0

Results expressed as μ moles/¹⁴C-melatonin formed per g tissue per h when incubated with *N*-acetylserotonin and ¹⁴C-methyl-*S*-adenosylmethionine.

Xenopus larvae, stages 48–50 (ref. 8), were homogenized in 0.5 ml. ice-cold water using an all-glass homogenizer. A 0.2-ml. aliquot was taken to measure the hydroxyindole-*O*-methyl transferase activity⁹. A control incubation was run concurrently without the addition of substrate to correct for a small amount of endogenous material present in the *Xenopus* larvae which forms a methylated metabolite. On incubation of *Xenopus* larvae with *N*-acetylserotonin and ¹⁴C-methyl-*S*-adenosylmethionine, a radioactive product was formed which was extractable into chloroform. This material had the same *R_F* value on three thin-layer chromatographic systems as authentic melatonin. The amount of melatonin formed enzymatically (Table 1) was more than enough to cause blanching in the *Xenopus*².

Hydroxyindole-*O*-methyl transferase was also examined in the adult frog brain using the procedure already described. Considerable enzyme activity was found in the frog brain (Table 1). Since the brain would also include the pineal, hydroxyindole-*O*-methyl transferase was measured in several brain areas. All areas examined contained enzyme activity. The melatonin-forming enzyme was found to be absent in the adult frog skin, liver, intestine and heart. These results demonstrate that the enzyme that forms the amphibian skin-lightening agent, melatonin, is present in the brain and pineal area of the frog, while it is present only in the pineal gland of all other mammalian and bird species so far reported. Later discoveries and more detailed analyses of hydroxy-*O*-methyl transferase activities in pineals, brain regions and eyes in various vertebrate species will be published elsewhere.

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Agar-gel Electrophoresis of Soluble Lens Proteins in Galactose-fed Rats

A NUMBER of reports have recently been published dealing with the electrophoretic properties of water-soluble lens proteins of albino rats and the presence of a 'fast' anodic fraction in electrophoretic patterns of lens proteins from galactose- and lactose-fed rats^{1,2}. It is well established that dense cataractous opacities develop in rat lenses within two to three weeks of galactose feeding³. Electrophoretic analysis of such lens extracts shows that the fraction with the highest anodic mobility is con-