sucrose and maintaining them in this medium overnight. Spontaneously fused tobacco protoplasts (Power & Cocking, 1971) were released by lowering a sterile cover slip on to a leaf piece floating in the medium and applying gentle pressure (Power, Cummins & Cocking, 1970). This gave a mixed culture of 30% fused protoplasts and 70% non-fused protoplasts, which were cultured aseptically in Petri dishes at 20°C and low light-intensity (4–60 ft-candles). Two culture media were employed: a medium similar to that used by Nagata & Takebe (1970) containing 2,4-D, 6-benzyladenine, glycine, *myo*-inositol and thiamin hydrochloride, but without mannitol; and a commercially prepared modified Whites medium (S-3; Biocult Laboratories Ltd.); both with added 23% (w/v) sucrose. For nuclear examination fused protoplasts were fixed in acetic acid–ethanol (1:3, v/v) for 3 h and stained with acetocarmine or acetoorcein.

After 24 h culture in the modified Nagata & Takebe (1970) medium systrophy occurred markedly within these spontaneously fused protoplasts, the nuclei coming together and remaining so during subsequent culture. Systrophy was less evident in Whites medium, but a general increase in size in both media preceded the first division of the fused protoplasts, which took place after about 4 days' culture. In fused protoplasts containing two or three nuclei, some nuclear division was synchronous and concomitant with the initiation of cell-wall regeneration. Fused protoplasts regenerated a wall and a few underwent repeated divisions, but there was no synchrony of regenerated cell division within the culture as a whole. Nuclear fusion could occur during any one of these divisions in these intraspecies fusion bodies. These results show that wall regeneration readily occurs; and that nuclear fusion probably, after common spindle formation, is possible in these spontaneously fused plant protoplasts, because in many instances the nuclei are closely associated with each other and enter mitosis synchronously.

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Fluorescent Derivatives of 3-Hydroxy-L-kynurenine in the Lens of Man, the Baboon and the Grey Squirrel

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It has been known for a long time that the lenses of certain species of animal, including the primates and the Scuridae, are coloured yellow (Walls, 1942).

The human lens has been found to contain a number of fluorescent compounds, of which the most concentrated is the *O*- β -D-glucoside of 3-hydroxy-L-kynurenine (van Heyningen, 1971). This shows a strong blue-white fluorescence on paper. It is split by hydrolysis with β -glucosidase, or 1M-hydrochloric acid, to give 3-hydroxy-L-kynurenine and D-glucose. There is also present in the human lens a closely related glucoside with the α -amino group of 3-hydroxy-L-kynurenine blocked (but not by acetylation).

I have now examined the lens of the baboon (*Papio* species) and the grey squirrel (*Sciurus carolinensis leucotis*). Cooper & Robson (1969*a,b*) found evidence that the pigments in the lens of man, the baboon and the squirrel were very similar to each other, but probably not the same. I have confirmed that the fluorescing material from the lens of man, the baboon and the squirrel is yellow and has an absorption peak at about 368 nm.

Examination by paper electrophoresis and chromatography showed that the major fluorescent compound in baboon lens was the same as that in the human lens. The presence of glucosides in these lenses is surprising, as it is generally held that phenolic glucosides are not formed in animal tissues.

The lens of the squirrel, on the other hand, contained a main fluorescent compound different from that in the primate lens. It fluoresced green-yellow on paper and had all the properties expected of *N*-acetyl-3-hydroxy-L-kynurenine (Dalglish, 1952). The presence of the acetyl group was confirmed by hydrazinolysis followed by dansylation (Schmer & Kreil, 1969). The acetyl dansyl hydrazide formed was identified by two-dimensional chromatography on polyamide layer sheets (Hartley, 1970).

Thus 3-hydroxy-L-kynurenine occurs in the lens of the primate (man and baboon) as the *O*-glucoside, and in the squirrel lens as the *N*-acetyl derivative.

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Nature and Properties of Retinol-Binding Proteins

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Retinol-binding protein was isolated from human serum by using two different procedures. The first, similar to that employed by Kanhai, Raz & Goodman (1968), involves the following stages: (i) fractional precipitation of retinol-binding protein from serum by ammonium sulphate to yield concentrate (preparation C₁), (ii) chromatography of preparation C₁ on (a) DEAE-cellulose to give preparation C₂ and of the latter on (b) a combination of Sephadex G-75 and G-100 gels to yield preparation C₃, followed by (iii) electrophoresis preparation of C₃ on polyacrylamide gel at 5°C, yielding preparation C₄. Retinol (0.5mg) in 0.5ml of ethanol was then added to maintain saturation of the carrier protein, which was finally chromatographed on Sephadex G-75 to remove excess of buffer salts and retinol (preparation C₅).

Preparation C₅ was resolved by disc-gel electrophoresis into three components with α_1 mobility, only two of which fluoresced. The major slower-moving protein was called fraction RBP_s and the other fraction RBP_r. The third component with greatest mobility and present in only small amounts was subsequently found to bind retinol and was called fraction apo-RBP. These correspond to the fractions H₂, H₁ and A respectively reported by Raz, Shiratori & Goodman (1970).

A second, shorter, isolation procedure was developed. This involves chromatography of serum on arginine-Sepharose (6B), prepared as described by (Porath & Fornstedt, 1970), by using a gradient buffer system and concentration of the various fractions by membrane ultrafiltration. The retinol-containing fractions are detected by fluorescence and two major pure retinol-binding protein fractions can be obtained after passage of concentrates through the column three more times with various conditions of pH and salt concentration to be described. The first to be eluted from the final

column contains mainly fraction RBP_s with a trace of fraction RBP_{r1} and the second mainly fraction RBP_{r1} with a trace of a third protein, fraction RBP_{r2}, as revealed by disc-gel electrophoresis and isotachophoresis. Analysis of the pure components shows them to have the same amino acid composition, which is very similar to that reported by Kanhai *et al.* (1968). The molecular weights as determined in the ultracentrifuge and by Sephadex-gel chromatography are also the same at 21900. Both fraction RBP_r and fraction RBP_s contain 1mol of retinol/mol of protein. Evidence from u.v. and fluorescence spectra indicate that fraction RBP_r is derived from fraction RBP_s by transition of the latter to a more stable conformation. Optical-rotatory-dispersion spectra of the latter at pH 8.0 indicate that the molecule is randomly coiled.

The electrophoretic mobilities of fractions RBP_s and RBP_r were α_1 in polyacrylamide gel, in agreement with Kanhai *et al.* (1968). However, on support systems such as paper and cellulose acetate free from molecular-sieving effects and with tris buffer at pH 8.9 the true mobility is α_2 and less than that of caeruloplasmin, as shown by Glover & Walker (1964).

Insufficient evidence has been obtained yet to determine whether fraction RBP_s is the sole component in fresh serum or whether fraction RBP_r is also present or has been produced as an artifact by the isolation procedures. In other experiments with pig serum similar phenomena have been observed.

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Studies on Olfactory Receptor Mechanisms

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The olfactory neurones of higher animals, in contrast with the pheromone-receptor cells of insects, exhibit a broad stimulus specificity. The cells respond to a wide range of chemicals, though no two cells appear to have an identical specificity profile (Gestland, Lettvin & Pitts, 1965). Although a large number of speculative mechanisms have been advanced for the olfactory transduction process, few experimental investigations have been