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THE YELLOW COLOUR OF THE LENS OF MAN AND OTHER PRIMATES

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SUMMARY

1. Measurements have been made of the absorption spectra of the lenses of human, baboon (*Papio*), rhesus monkey (*Macaca*), squirrel monkey (*Saimiri sciureus*) and bush baby (*Galago crassicaudatus*).

2. In all these species an absorption maximum was found between 365 and 368 nm.

3. The pigment responsible for this absorption was water-soluble and aqueous extracts were examined. Protein-free aqueous extracts showed an additional maximum at 260 nm which could be only partially accounted for by the presence of ascorbic acid.

4. Chromatography of the protein-free solution from human lenses yielded a fast-moving yellow component with a blue fluorescence. Its absorption spectrum was very similar to that of the original protein-free solution. A fast-moving yellow component from the baboon lens had a yellow fluorescence.

5. The human lenses appeared to contain more of the yellow, water-soluble pigment at birth than in adult life. The concentration remains constant during adult life.

6. There is evidence for the appearance of another pigment in the human lens in later adult life. It is not water-soluble and has an absorption maximum at about 330 nm.

INTRODUCTION

Two groups of mammals, sciurids and primates, are known to have yellow lenses (Walls, 1942). However, although there has been considerable interest in the coloration of the human lens, only incomplete measurements of the absorption spectrum of human lenses are available (Wald, 1949; Weale, 1963). The data of Wald (1949) from a rhesus monkey is the only satisfactory information available on other primates. Little is known of the pigment responsible for the colour of the primate lens and no direct comparison of the coloration of primate and sciurid lenses has been made.

We have now examined the absorption spectra of lenses from several primate species including man and found them to be very similar to each other and to that of the squirrel lens (Cooper & Robson, 1967, 1969). Moreover, we have been able to extract a yellow, water-soluble, freely diffusible pigment from the primate lenses which is similar to but not identical with, the pigment found in the squirrel lens. The colour of the human lens is known to change with age (Weale, 1963) and so we have examined human lenses of different ages. The changes associated with senescence have been shown not to be due to changes in the extractable pigment.

METHODS

The methods adopted for the measurement of the absorption spectra of whole lenses, thin layers of lens material, solutions of lens material, dialysates and chromatographic eluates, were the same as those used for our investigation of the squirrel lenses (Cooper & Robson, 1969).

Lenses from baboons (*Papio*), rhesus (*Macaca*) and squirrel monkeys (*Saimiri sciureus*) were obtained immediately after death. Bush baby (*Galago crassicaudatus*) lenses came from a frozen specimen and human lenses were obtained at routine autopsy. Experiments with monkey eyes showed that no significant changes in lens colour occurred during the first 2–3 days of cool storage or during 2 months frozen storage.

RESULTS

Primate lenses

Figure 1 shows the axial optical density of the lenses of several primate species. Except for the bush baby these are extrapolations of measurements made on thin layers of lens material. All the lenses showed a pronounced absorption maximum at a wave-length between 365 and 368 nm with no sign of any specific absorption in the range 450–650 nm.

The maximum density in the near U.V. of the bush baby lens was considerably lower than that of the other species. That this low density was not a result of loss of pigment during storage is substantiated by Dartnall's measurements of the absorption spectrum of the fresh lens of this species (Dartnall, Arden, Ikeda, Luck, Rosenberg, Pedler & Tansley, 1965) which are also shown in Fig. 1. The apparent maximum of 0·9 in their results at 380 nm is probably an artifact due to the presence of stray light in the spectrophotometer (H. J. A. Dartnall, personal communication)

Humans lenses. The absorption spectra of human lenses from perinates were very similar to those of the other primates: they all showed an absorption maximum at 366 nm. However, by the age of 8 years the absorption spectrum of the human lens was significantly altered. Table 1 shows the results of all the measurements made on human lenses while Fig. 2 shows the absorption spectra of a selection of these to illustrate the effects of aging.

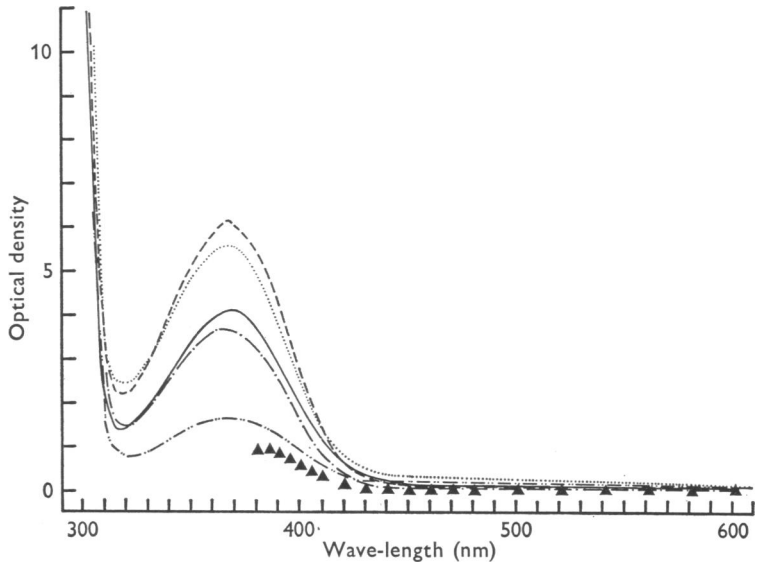

Fig. 1. Density spectra of various primate lenses. Human (perinatal) ——; baboon ——·——·; macaque – – –; squirrel monkey ·····; bush baby —·—··. Dartnall's measurements of the optical density of bush baby lens (from Dartnall *et al.* 1965) are shown as triangles (▲).

TABLE 1. Results of photometric measurements made on human lenses and aqueous dialysates of lens material. The optical density of the whole lens at the wave-length of the absorption maximum in the near U.V. was obtained from measurements made on thin layers of lens material. The concentration of extractable pigment present in the lens is given in terms of the optical density at 366 nm (for a 1 cm light path) of a solution containing in each litre the pigment that can be extracted from 1 g (wet weight) of lens material

	Whole lens		Aqueous dialysate of lens material		Concentration of extractable pigment in lens material
Age	λ max. (nm)	Optical density at λ max.	λ_1 max. (nm)	λ_2 max. (nm)	(D_{366}/cm/g/l.)
Stillborn	366	4·1	258	366	9·0
Stillborn	366	4·9	258	365	7·7
1 day	366	2·9	260	366	6·7
1 day	366	3·5	260	365	5·4
6 months	366	10·1	260	366	21
8 months	366	5·6	260	365	10·1
8 years	362	3·8	260	366	3·8
8 years	363	3·7	260	366	6·3
10 years	362	4·4	258	365	3·9
18 years	362	3·5	—	365	4·2
19 years	354	2·8	260	364	3·1
19 years	362	4·5	259	364	6·2
24 years	352	3·6	260	365	4·0
28 years	360	3·9	260	365	5·1
33 years	348	4·3	265	364	—
35 years	336	4·8	260	367	3·5
52 years	334	6·8	263	362	—
56 years	—	—	263	362	—
63 years	331	8·5	—	—	—
76 years	335	5·8	257	366	3·5
87 years	333	5·7	—	—	—

The most easily quantified change that occurred in the absorption spectrum was the wave-length of the absorption maximum in the near U.V. In Fig. 3 this is plotted against age. Although none of the lenses showed any specific absorption at wave-lengths greater than 450 nm, one of the signs of aging was a general increase of optical density in the blue and green regions of the spectrum. This could have indicated an increased scattering of light rather than a true absorption. However, the changes in the absorption spectrum at wave-lengths less than 400 nm were not consistent with

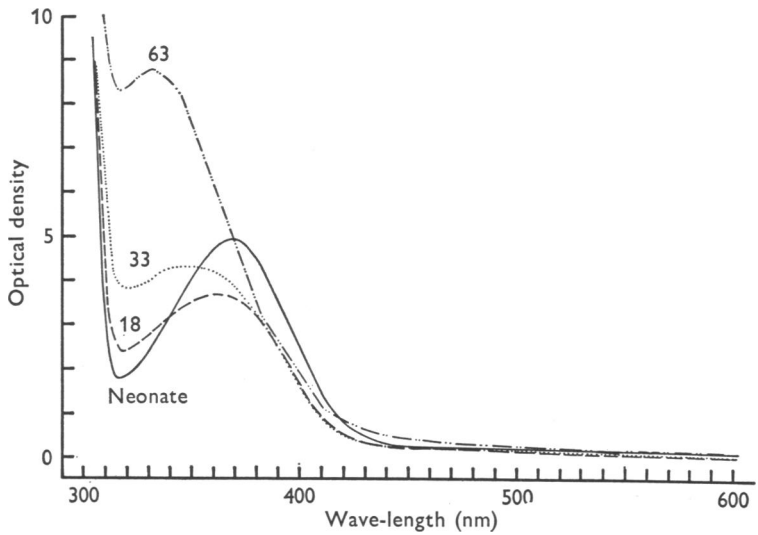

Fig. 2. Density spectra of intact human lenses of various ages. The age in years is shown against the curves.

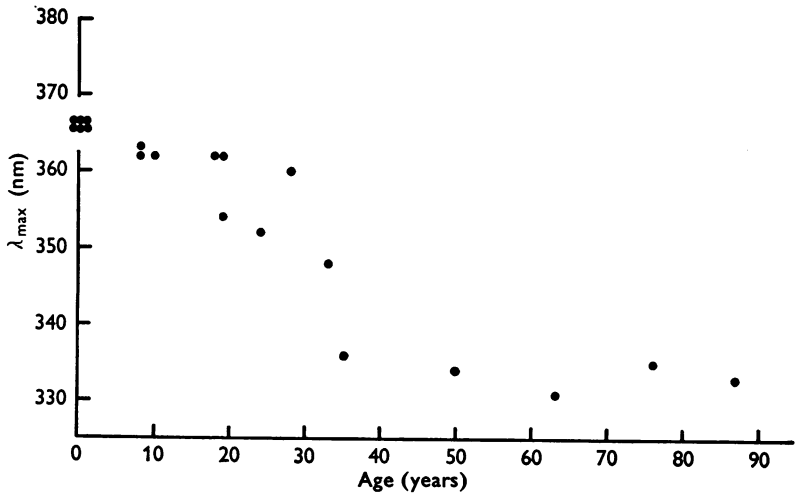

Fig. 3. Wave-length of absorption maximum of human lenses of various ages.

the hypothesis that the effect of aging was simply one of increased Rayleigh scattering.

Dialysates of lens material. Figure 4 shows the absorption spectrum of an aqueous extract of human lens material (age 6 months) together with that of a dialysate of this extract. The dialysate has absorption maxima at 365 and 260 nm, while only a maximum at 366 nm can be seen in the absorption spectrum of the crude extract as the protein absorption is so great at short wave-lengths. The absorption spectra of all dialysates prepared from

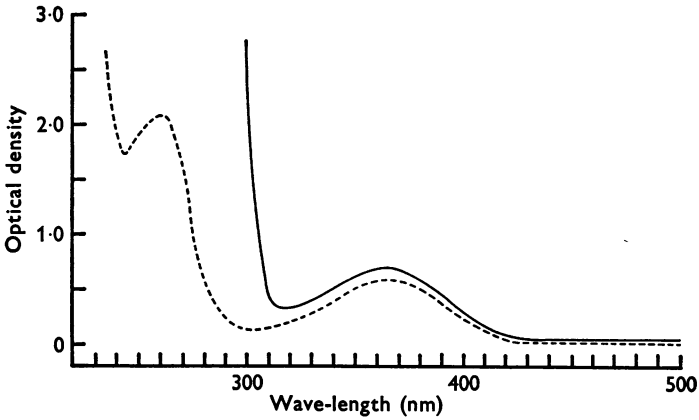

Fig. 4. Absorption spectrum of a crude aqueous extract (——) and dialysate (– –) of human lens material.

primate and human lenses were extremely similar. In the case of the human lenses the shape of the absorption spectrum of the dialysate did not appear to change significantly with the age of the lens from which it was prepared (see Table 1). The amount of pigment that was recovered in the dialysates of the youngest human lenses was sufficient to account for about 80% of the density of the lens material at 366 nm. The concentration of pigment present in the young lenses was rather greater than in the older lenses, but there was no obvious sign of further change in the concentration after the age of 8 years.

Chromatography. Chromatography of dialysates from both human and baboon lenses revealed a fast-moving yellow component (R_F about 0·88). However, although this component in the baboon chromatogram had a yellow fluorescence, the fast-moving yellow component in the human chromatogram fluoresced blue. A less intensely coloured slower-moving yellow component (R_F about 0·36, yellow fluorescence) was evident in the baboon chromatogram.

The absorption spectrum of the fast-moving yellow component of the human lens dialysate is shown in Fig. 5. The form of the absorption

spectrum is similar to that of the original dialysate, and it seems likely that this chromatographic component is responsible for most of the yellow colour of the infant human lens and makes a substantial contribution to the absorption of the adult lens in the far blue and near U.V.

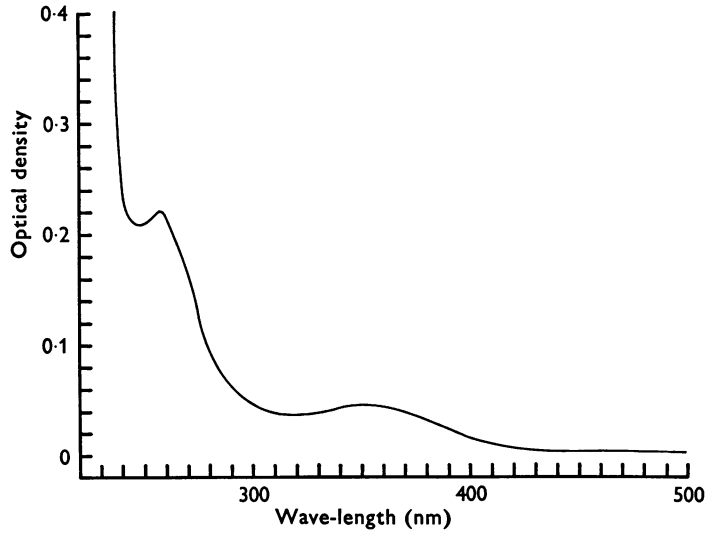

Fig. 5. Absorption spectrum of eluate of fast moving yellow component of chromatogram of human lens extract.

DISCUSSION

The yellow colour of primate lenses (including infant human lenses) appears to be mainly due to the presence of a freely diffusible, water-soluble pigment which has absorption maxima at about 365 and 260 nm. The pigment that is responsible for the colour of the human lens does not appear to be identical with that of the baboon lens (it has a quite different fluorescence) although its absorption spectrum is clearly very similar. Again it is not clear whether the pigment of the baboon lens is the same substance as the pigment of the squirrel lens (Cooper & Robson, 1969) although they are certainly very similar.

Although the amount of pigment in the human lens appears to be greater at birth than in adult life, the concentration of extractable pigment during adult life appears to stay more or less constant. Thus we must look to some other effect to account for the change in the absorption spectrum of the whole lens. An increase in Rayleigh scattering (optical density inversely proportional to the fourth power of the wave-length) does not by itself seem able to explain the change in the form of the absorption spectrum of the lens material in the U.V. and it seems likely that another pig-

ment substance with an absorption maximum in the region of 330 nm appears in later life. This could account for the more or less constant wave-length of the absorption maximum seen in human lens more than 50 years old. However, we have not been successful in extracting such a pigment with aqueous or organic solvents from senescent lenses.

Whilst we do not have any direct evidence indicating the function of these pigments in the primate lenses, it is possible that they serve optical or metabolic functions, as suggested by Cooper & Robson (1969) in the case of the squirrel lens pigment.

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