J, Physiol. (1971), **212**, *pp.* 393–409
With 1 *plate and* 7 *text-figures*
Printed in Great Britain

SOME REFLECTIVE PROPERTIES OF THE TAPETUM LUCIDUM OF THE CAT'S EYE

BY J. A. COLES
From the Departments of Physiology and Biophysics, University College London, WC1E 6BT

(*Received 23 June 1970*)

SUMMARY

1. Observation of the fresh, isolated tapetum of the cat with a low-power microscope showed it to be composed of multilayer reflectors (domains) each being less than 6 μm in diameter. It is concluded that these are subdivisions of the intracellular bundles of rodlets seen in electron micrographs.

2. Apparatus was devised for illuminating a single domain with a pencil of white light and observing the colour and direction of the reflected light.

3. Those domains which lie near the vitreal surface of the tapetum tend to reflect blue light and deeper ones reflect longer wave-lengths; this is attributed to differences in the layer spacings of the domains.

4. Each domain has a set of reflecting planes lying within 15° of the general plane of the tapetal surface. In addition, many domains have surfaces inclined to these at angles greater than 20° which also can reflect visible light.

5. The mechanism of reflexion is discussed in relation to the lattice-like structure of the domain.

6. It is suggested that under some conditions the nature of the tapetum may lead to significant local variations in the spectral sensitivity of the visual system.

INTRODUCTION

Some doubt has been expressed concerning the mechanism by which light is reflected by the tapetum cellulosum of the cat's eye. Brücke (1845) ascribed the iridescent appearance to interference phenomena, and Schultze (1872) described how each cell contained a number of bundles of small rodlets and stated that each bundle reflected a particular colour by light interference. However, Gunter, Harding & Stiles (1951) and Weale (1953), having measured the spectral reflectivity, concluded that pigments were primarily responsible for the appearance. Since then, electron

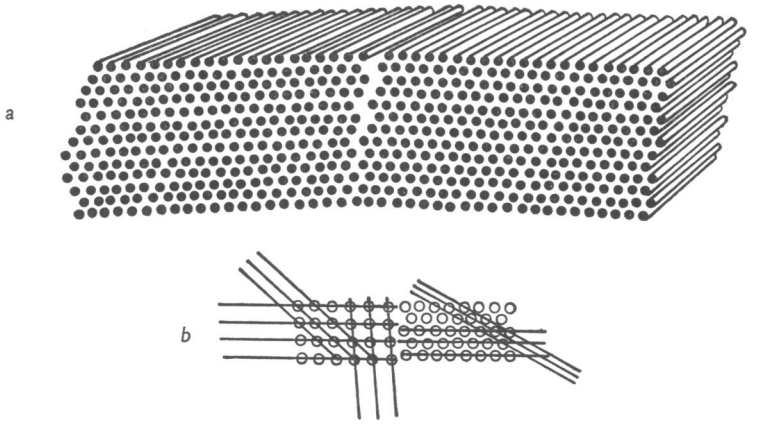
microscope studies have been made by Bernstein & Pease (1959) and Pedler (1963) which show that within each bundle the rodlets are arrayed with great regularity and the spacing between centres of adjacent rodlets is comparable to a wave-length of visible light. Because of this, Pedler suggests that reflexion according to Bragg's Law may occur from the layers of rodlets, rather as X-rays are diffracted by atoms in a crystal. This paper describes optical studies which indicate that the tapetum does indeed contain small domains each of which can reflect light in this way in several directions simultaneously. Although a layered structure is the basis of many biological reflectors (the principle of their operation is elaborated by Huxley (1968)) the optically effective periodicity usually occurs in one dimension only. This may still give rise to complex optical properties such as those described by Wilkins, Stokes, Seeds & Oster (1950) for crystals of the tobacco mosaic virus. The two-dimensional periodicity in the cat tapetum is to be expected to impose different constraints on the directions in which light can be reflected.

Anatomy. The tapetum of the cat covers about half the area of the fundus and has a shape roughly that of a spherical equilateral triangle with its base passing horizontally just below the optic disk and its apex upwards. Its predominant colour varies from cat to cat, from yellow to green, with a border which reflects mainly shorter wave-lengths. It lies immediately in front of the vascular layer of the choroid and is separated from the layer of rods and cones by Bruch's membrane and a transparent pigment epithelium. Near its centre, the tapetum has fifteen to twenty layers of cells, while towards the periphery it tapers until it is one layer thick. Each cell is 3·5–6 μm thick and has a polygonal outline with a largest diameter of 26–60 μm (Pl. 1, fig. 1). Only a small part of it is taken up by the nucleus and other normal cell organelles and the rest is packed with rodlets which, according to Pedler (1963), have diameters 0·19–0·35 μm and lengths 2–6 μm. Their axes lie within a few degrees of the plane of the choroid and they are grouped into bundles which normally extend through the thickness of the cell and whose vitreal surfaces are approximate parallelograms with shorter sides the length of a rodlet and longer sides 5–20 μm (Text-fig. 1*a*). Within each bundle, the rodlets, whose centres are spaced 0·3–0·5 μm apart, are arranged mutually parallel with remarkable regularity so that a section through a bundle cutting their axes shows an array similar to a crystal lattice in that there are sets of lattice planes as shown in Text-fig. 1*b*. In published electron micrographs (Bernstein & Pease, 1959; Pedler, 1963) one set lies within about 15° of the plane of the choroidal surface; the others are inclined to these at angles which vary from bundle to bundle so that although the lattices in some bundles can be described as square and in others as hexagonal, intermediate cases occur. The extent to which this represents a modification of the *in vivo* structure will be discussed.

METHODS

Preparation. Eyes were taken from cats which had been used in acute experiments and further dissection was usually begun within 15 min of the arrest of the animal's heart. The details of the dissection were modified during the course of the work so as to treat the tapetum more gently, and the final method was as follows. The position

of the tapetum was located by pointing the eye at a lamp and noting the more opaque part of the fundus. The eye was placed with this part downwards in a nicely fitting cup whose depth was about half the radius of the eye and the wall was cut with a razor blade along the rim of the cup. The major segment was removed and the vitreous cleared from the remainder, which was slid onto a microscope slide. If necessary to permit the approach of the microscope objective, short radial cuts were made in the periphery of the tapetal area to allow it to lie flatter. Using fine forceps and taking care not to touch the tapetum itself, the retina was pulled away from



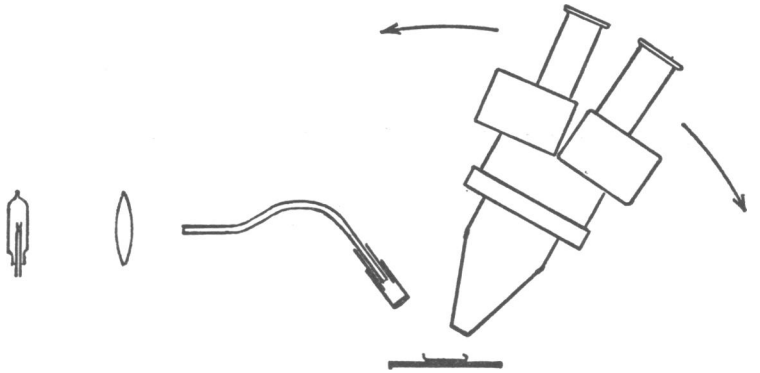
Text-fig. 1. The structure of a bundle. *a*. A perspective representation of a bundle. This particular one is divided into two domains whose corresponding lattice planes are not quite parallel. Usually there are twelve to fourteen layers of rodlets in a bundle. *b*. An idealized representation of a section perpendicular to the axes of the rodlets of parts of two adjacent domains with different lattice forms in one bundle. Some of the more widely spaced planes are indicated. The centres of the rodlets are 0·3–0·5 μm apart. The drawings are partly based on electron micrographs of Pedler (1963).

about 5 mm^2 of tapetum where there was least sign of wrinkling. In some experiments where the working distance of the objective was longer, the fundus was left in the cup and covered by saline solution (0·9 g/100 ml). Wrinkling of the tapetum, visible to the naked eye, and a loss of regularity in the structure of the upper cells, visible under the microscope, were usually apparent about an hour after dissection. The rate of this deterioration was unaffected when variants of Ringer solution were used instead of saline; e.g. (mM) NaCl 150, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 10, Na_2HPO_4 10 (cf. Baumann & Kurland, 1926).

Apparatus. Arrangements were devised to illuminate the tapetum from above in various ways, usually with white light, and for the observation of the reflected light. The light source was either a 6 V, 30 W tungsten filament lamp over-run at 7 V, or a 12 V, 50 W quartz-iodine projector lamp. For observations at low magnifications the approximate angle of incidence was conveniently controlled by passing the light down a flexible fibre light guide with a converging lens attached to its end as shown in Text-fig. 2. Observation at different angles was achieved by arranging a ×100 Watson binocular microscope body on its 'rackwork-long-arm' stand so that it could be rotated about a horizontal axis in the object plane (see Text-fig. 2). To illuminate the tapetum at angles of incidence close to normal and to permit obser-

vation at higher magnifications, a commercial top illuminator was installed in the barrel of an ordinary microscope and, when desired, the numerical aperture of both the incident light and reflected light was restricted by means of a stop in the back focal plane of the objective and the incident light was focused into the hole in this.

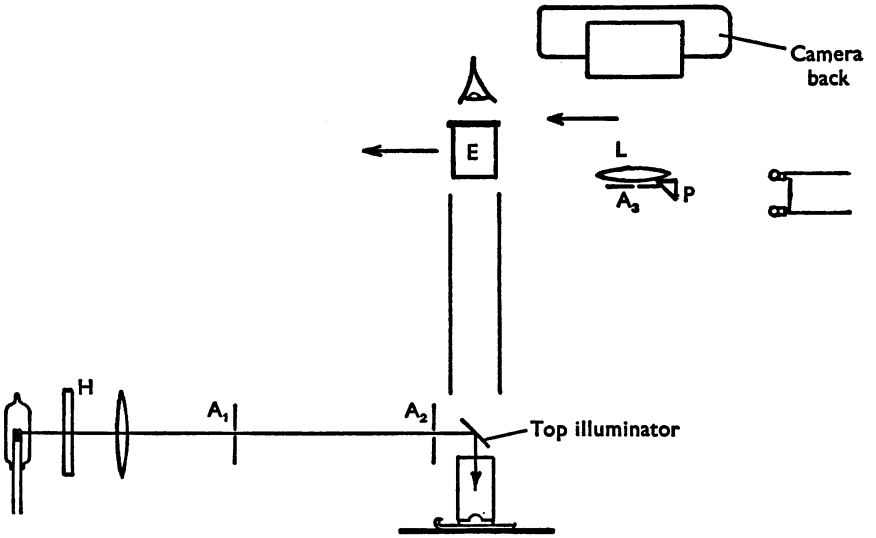
The study of reflexion from a single domain required the use of pencils of light less than 5 μm in diameter, and I am indebted to Professor A. F. Huxley for suggesting a way of using a microscope objective to achieve this. The top illuminator was modified by replacing the reflecting glass disk by one of better optical quality (a cover slip for a blood counting slide) which was coated with tungsten oxide on one face so as to reflect about half the light incident at 45°. As shown in Text-fig. 3, the aperture A_1 (from an electron microscope) was mounted in the image plane of the



Text-fig. 2. Arrangement for observing the tapetum at low magnification while changing the angle of observation or illumination. A Watson binocular microscope was mounted on its 'rackwork-long-arm' stand so that it could rotate about a horizontal axis passing through the object. Near-parallel illumination was provided using a fibre light guide and a 50 W quartz iodine projector lamp.

microscope objective as reflected by the top illuminator. With a $\times 45$ objective a diameter of 100 μm was used, and with $\times 100$, 200 μm. The image of part of the lamp filament was focused on it and an image of the hole was formed in the object plane of the objective. The microscope stage was held by a Prior micro-manipulator with horizontal and fine vertical movements so that a chosen part of the object (the tapetum) could be brought simultaneously into focus and into coincidence with the illuminating spot. Some light was scattered back from the tapetum away from the focus of the illuminating cone so that the magnified image appeared as a bright spot surrounded by a diffuse patch. To study the directions in which light was reflected from this bright spot alone, the extraneous light was largely cut out by introducing aperture A_3, of the same diameter as A_1, in the image plane and concentric with the bright spot. This necessitated the use of an eyepiece of the Ramsden type. The diffraction pattern could then be observed by swinging the eyepiece out of line and using lens L, focal length 4·5 cm, to focus an image of the back focal plane of the objective into a reflex camera without its lens. The radial distance of a feature of this image is proportional to the sine of the angle at which the light enters the objective and the relating constant was found by shining parallel monochromatic light upwards through a diffraction grating on the microscope stage and photographing the pattern produced, the angles corresponding to this being calculable. A frame of

reference was provided by two dim 'lilliput' pilot bulbs mounted in such positions that after reflexion in the 45° prism P their images also were focused onto the camera film. To maintain a constant relationship between the three during an experiment the prism and the aperture A_3 were attached to the lens L. To restrict the incident light to a narrow pencil, aperture A_2, diameter 400 μm, was advanced into the illuminating cone close to the top illuminator. It was held by a micro-manipulator so that its position, which determined the direction of incidence on the tapetum,



Text-fig. 3. The apparatus used for observing reflexions from small areas of tapetum. Light from the quartz iodine lamp passes through heat filter H and an image of the filament is focused onto the plane of aperture A_1 (diameter 100 or 200 μm) lying in the image plane of the microscope objective as reflected in the top illuminator. An image of A_1 is formed in the object plane of the objective and the image of this, as reflected by the tapetum, can be observed with the Ramsden eyepiece E. The aperture A_3 is moved to coincide with the image of A_1 in the object plane of the eyepiece. This last is removed and the lens L and the reflex camera back can be swung into line so that an image of the back focal plane of the objective is visible in the viewfinder. The prism P reflects light from two 'lilliput' bulbs and their images on the camera film provide a frame of reference. Aperture A_2, diameter 400 μm, selects from the incident beam a narrow pencil of light whose direction of incidence on the tapetum is controlled by the position of A_2.

could be identified by the readings of the vernier scales on the horizontal and vertical movements. These were correlated with the directions of incidence by placing a plane, horizontal, front-silvered mirror under the objective and photographing reflexion patterns for a grid of positions. An alternative method of recording the direction of incidence was used in some early experiments in which a spot indicating this was superimposed on the tapetal diffraction pattern, but it was found that this sometimes obscured a feature in it.

To focus a wide cone of light onto a domain not at the surface of the tapetum it was necessary that the liquid in which the objective was immersed should have the same refractive index as the tapetum, estimated to be about 1·46 (see below). Liquid paraffin satisfies this requirement, and was found not to hasten appreciably the deterioration of the tapetum. A Cooke × 45 water immersion objective was available which had been modified by the makers so that the front lens surface was part of a sphere centred on the object and its functioning was independent of the refractive index of the immersion liquid. It permitted observation at angles up to about 31·5° to the normal; this was insufficient for some observations for which a × 100 oil immersion objective, N.A. 1·32, was used. The immersion oil, which was placed directly on the tapetum, did not appreciably hasten deterioration of the domains. In this case, the extreme rays, passing through the upper layers of the tapetum at about 60° to the normal, focused closer to the surface (i.e. higher) than the central rays.

RESULTS

When the tapetum was observed through a × 100 dissecting microscope its surface was seen to be almost entirely covered with small bright spots each reflecting what appeared to be a more or less saturated spectral colour. With the microscope mounted as shown in Text-fig. 2, individual spots could be observed continuously as the angle of observation was changed. When the tapetum was illuminated by a broad source it was seen that as the angle of reflexion increased up to about 30° the colours of individual spots changed progressively towards the shorter wavelengths. When the illuminating light was in a nearly parallel beam, spots appeared and disappeared as the angle of observation changed. These observations suggest that the spots arise from reflexion in small multilayer reflectors, of different spacings, whose inclinations deviate by only small angles from the general plane of the tapetum, a conclusion consistent with that of Schultze (1872). Multilayer reflexion has been found in a number of biological reflectors such as fish scales (Denton & Nicol, 1965) and the scallop eye (Land, 1966), and has been treated mathematically by Rayleigh (1917) and Huxley (1968). The wave-lengths λ_n reflected most intensely obey Bragg's Law:

$$\lambda_n = (2n_t d/p)\cos\theta, \tag{1}$$

where $n_t d$ is the optically effective distance of repeat of the structure, p is an integer and θ is the angle from the normal within the structure.

To estimate the sizes of the domains which produce these spots a moist tapetum was placed between a microscope slide and cover slip and observed through a microscope with top illumination and a dry 4 mm objective, N.A. 0·85. It appeared blotchy yellow and green and the polygonal cells containing bundles of rodlets could be seen (Pl. 1, fig. 1). When the effective N.A. of the objective was reduced, so that the tapetum

was illuminated by a narrow cone of light whose half angle was typically 7° in air and only light reflected within the same cone observed, the coloured spots were revealed again (Pl. 1, fig. 2). Each had a largest diameter less than about 6 μm which is considerably less than that of the surface area of any but the smallest bundles. This might arise because the reflecting planes in the bundles are curved and only parts of them are able to reflect light. However, this is not a major factor because the sizes of the spots were not noticeably affected by alteration in the width of the illuminating cone, and the diffraction patterns to be described were not those to be expected from curved reflectors. Study of electron micrographs shows that often a bundle is divided into two or more parts distinguished by their having different spacings between the layers of rodlets, or deviating from the mean plane by up to 10°. Hence, such a subdivision is the largest area which would reflect a homogeneous spot, and it is suggested that it is this which corresponds to a domain (Text-fig. 1*a*). Presumably the low N.A. objective is necessary to make spots visible for two reasons. Firstly it increases the saturation of the colour reflected by any one domain and secondly it reduces overlapping of spots because reflectors inclined at more than a small angle to the plane of the tapetum will not reflect the light back through the aperture. For more than half the light reflected to reach the microscope eyepiece it can be shown, using simple geometry and assuming the refractive index of the tapetum to be about 1·45, that the reflectors must lie at inclinations less than about 2°. If the spots nearly fill the field, then, on average, one domain in about twenty must fulfil this requirement, in approximate agreement with the e.m. plates.

The possibility that this misalignment was an artifact of the preparation was considered. Weale (1953) noted that the tapetum *in situ* with its blood supply intact but with the vitreous and retina removed, crinkled after about 2 hr. The change which had occurred which might be expected to affect alignment was the removal of the intra-ocular pressure. An attempt to test whether this affected the appearance of the tapetum was made by clamping a piece of the sclera with the tapetum attached between two rings and applying pressure to the Ringer solution on the upper, tapetal, side, while observing the tapetum through a low power microscope. Various pressures up to about 30 mm Hg for periods up to half an hour were applied, but no changes were seen. The fundus of the eye, intact and *in situ*, was observed using an ophthalmoscope and again by using Daniel's application of Mèry's principle (Daniel, 1963), but when the N.A. of the system was reduced the resolution became too poor to permit observation of domains, probably because of the poor optical quality of the ocular media. However, reflexion from the fundus was shown to be quite diffuse as follows. The reflecting disk from the microscope top illuminator was used to direct a pencil of light about 1·5 mm in diameter into the pupil of a lightly anaesthetized cat. The reflected light was sampled by a pin hole which was moved over all parts of the cornea other than those very close to the incident pencil, where the card surrounding the pin hole cut off the incident light. No variation was observed (by eye) in the brightness of the illuminated part of the fundus as seen through the

pin hole. Alternatively, the pin hole was held close to the cornea in the path of the incident pencil, and the illuminated part of the fundus again observed through it. No variation in brightness was seen when the pencil entered the pupil at different points so that it fell on the retina at various angles to the normal (provided that it illuminated a part overlying the tapetum). It is concluded that unless the retina acts as a strong diffuser the domains are inclined at a range of angles to the general plane of the tapetum.

The distribution of domains of different layer spacings within the tapetum. Using apertures A_1 and A_2 in the apparatus shown in Text-fig. 3, a pencil of light was passed obliquely into the tapetum. Observation of the image plane with the microscope eyepiece showed a blurred spectum corresponding to reflexion of different colours at different depths within the tapetum, red being reflected from the deeper domains (Pl. 1, fig. 3). A similar, although less pronounced, effect would be expected if reflexion were caused by Rayleigh scattering from small particles. However, it is unlikely that this plays a major part as individual red domains can be seen with quite sharply defined borders when an image of A_1 is focused on the deeper layers of the tapetum (in the absence of A_2). An objective of N.A. about 0·75 is suitable for this as it permits sufficiently high resolution while each domain has a definite, although unsaturated, colour. This variation in colour through the thickness of the tapetum is believed not to be an artifact because the overall colour of the tapetum remained unchanged, provided it was kept moist. If all domains in the fresh tapetum reflected yellow or green and if there was preferential shrinking of the superficial layers, then to prevent a change in the over-all colour there would have to be a compensatory swelling of the deeper layers, which seems unlikely. When the illuminating light included only wave-lengths greater than 640 nm no reflexions of red light were seen from the upper domains and conversely, when the illuminating beam was filtered through a solution of copper sulphate, no blue light was seen to be reflected from the deeper domains.

Reflexions from other lattice planes

As described earlier, although electron micrographs of transverse sections of the tapetum show the domains as having lattice planes inclined at angles less than 15° to the mean plane, they usually have well defined planes at other inclinations and, from the symmetry of the structure, it is to be expected that these planes will also reflect light.

To estimate the angle at which light passes obliquely into the tapetum it is necessary to know its refractive index, and to this end the refractive index of the rodlets was measured. They were separated by gently homogenizing pieces of tapetum in distilled water, then centrifuging to remove most of the water. Samples of the sediment were immersed in comparatively large volumes of potassium mercuric iodide solution (see e.g. Holmes, 1921) and samples of this mixture were observed with a phase contrast microscope. The rodlets were invisible when the solution had a

refractive index of 1·57–1·59 and if it can be assumed that the rodlets are impermeable to the solution, then their refractive index is in this range. If the ground substance has a refractive index similar to that of the ground substance in the cornea, about 1·35, and the rodlets occupy about one half of the volume then, according to the law of Gladstone and Dale, the effective refractive index, n_t, of the tapetum will be the mean of these values, i.e. about 1·46 ± say 0·03. On the basis of these confidence limits the maximum error $\Delta\theta$ introduced by this source in calculating the angle of incidence in the tapetum, which is given by

$$\Delta\theta = (\Delta n_t/n_t)\tan\theta \text{ radians}$$

is

$$\Delta\theta = 1{\cdot}2\tan\theta \text{ degrees}$$

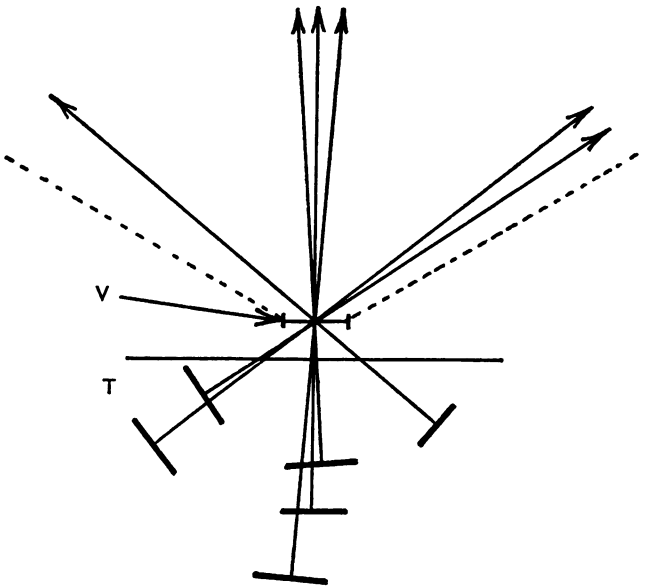
e.g. when $\theta = 50°$, $\Delta\theta = 1{\cdot}4°$. Unless otherwise stated, angles of incidence and reflexion described will be those calculated to occur within the tapetum using this value for the refractive index.

Observations at low magnifications. The fact that reflexion occurs from planes inclined steeply to the tapetal surface could be demonstrated crudely, without apparatus. The shape of the posterior half of a fresh eye was preserved by supporting it in a hemisphere and the whole area of the tapetum exposed. It was illuminated by a table lamp and, as expected for a multilayer reflector, the colour changed markedly when the observer's head was moved to change the angle between the incident and reflected light. However, the colour was much less strongly dependent on the actual value of the angle of incidence of the illumination. This was manifest in two ways; apart from the periphery, all parts of the tapetum were much the same colour, despite the curvature, and tilting the tapetum had little effect on the colour. The effect was seen more strikingly in green tapeta than yellow ones. This behaviour is roughly analogous to the formation of an X-ray diffraction pattern by a powdered crystal, with the difference that the tapetum includes a multitude of different lattice spacings.

A fibre light guide, with one end clamped close to the objectives of the rotating dissecting microscope described in connexion with Text-fig. 2 so as to illuminate the object, was used to observe the tapetum at ×100 magnification while the angle of incidence was increased to 60° in air. The gross appearance of the tapetum hardly changed; i.e. coloured spots were still seen, indicating that there were reflectors in the tapetum effectively inclined to the surface at $\sin^{-1}(1/n_t \sin 60°) = 36{\cdot}4°$. Rough measurements with a light meter confirmed that the reflexion was far more diffuse than that which would be produced by plane mirrors lying approximately parallel to the surface, but not completely diffuse. When the angle of observation was kept at zero, i.e. normal to the tapetum, while the angle of incidence was increased beyond about 35° in air, the appearance was somewhat different. Alternating light and dark bands with a period of about 100 μm and usually running tangentially round the tapetum were usually

seen. Illumination from alternate sides showed that these were produced by wrinkles. Where the illumination was at grazing incidence violet spots, and no others, were seen in some cases.

Diffraction by single domains. The reflectors steeply inclined at up to 36° to the tapetal surface described above might arise because of the wrinkling of the tapetum, and to eliminate this possibility similar reflexions were sought from areas of tapetum known to be flat. The surface

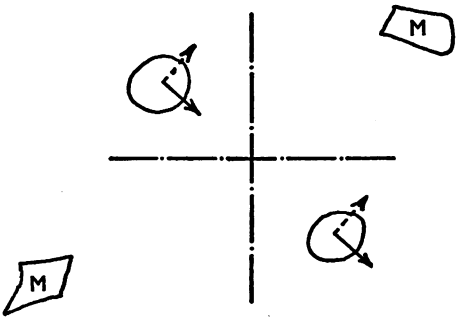


Text-fig. 4. Interpretation of Pl. 1, fig. 4. In the apparatus shown in Text-fig. 3, the microscope objective directs the incident light through the image V, diameter 2 μm, of the source aperture A_1. Because of aperture A_3 in the image plane of the objective, only light which is reflected back through V can reach the camera, so the image on the film represents reflexions at approximately normal incidence from reflecting planes orientated as shown within the tapetum T. The effect of the difference between the mean refractive index of the tapetum and that of the lens immersion oil is not shown directly; the dashed lines represent the estimated maximum angle of incidence within the tapetum, about 61°.

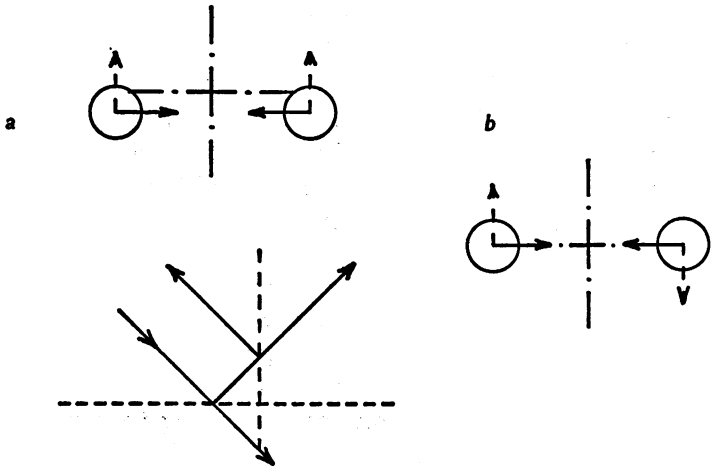
of the tapetum within the field of a $\times 100$ objective often appeared flat, and, over selected areas, the microscope stage could be moved horizontally through several diameters before the tapetum went out of focus. When a spot of light was focused just above the surface, using the arrangement shown in Text-fig. 3, the diffraction pattern for a tapetum recently and carefully dissected was of the type shown in Pl. 1, fig. 4. An interpretation of this is illustrated in Text-fig. 4. Aperture A_3 lets pass only that light which is reflected back through the image of aperture A_1. This occurs for

reflectors normal to the incident light and hence the spots in the diffraction pattern indicate the existence of reflectors and their inclinations in a region of the tapetum some 200 μm in diameter. It is seen that there is a clear distinction between two classes of reflectors; those lying close to the horizontal whose many overlapping reflexions go to form the central spot of the pattern, and those steeply inclined at angles up to the maximum at which observation is permitted by the optics (60·7°). To observe reflexions from a single domain over a range of angles it was necessary to focus the image of A_1 within the domain; this required the use of the objective with the curved front face which limited the maximum angle of incidence to 31·5°. When the aperture of the incident cone was not restricted further, the diffraction pattern contained a radially symmetrical part (Pl. 1, fig. 5) in which the colours were progressively bluer as the radius (and hence the angle of incidence) increased and which was attributable to multilayer reflexion from a set of planes lying within about 15° of the horizontal. Superimposed on this there was a central, redder reflexion from deeper domains and faint spots which because of their small areas were presumed to come from other domains, as in the previous case. Some other vague features lacking radial symmetry were sometimes apparent, and were investigated by introducing aperture A_2 to restrict the incident light to a narrow pencil. This produced a single spot in the camera viewfinder which corresponded to reflexion from an approximately horizontal plane, and for about one domain in two there was, in addition, a second spot of similar intensity and not very different wave-length which appeared for a limited range of directions of incidence and corresponded to a ray reflected at an angle of at least 40° to the first (Text-fig. 5). This would arise if there were a reflector, coextensive with the first and inclined to it at half this angle, i.e. 20° or more; when the direction of incidence was changed the two spots moved in the direction predicted by this model. To work with larger angles of incidence the oil immersion objective was used. Despite the poor image of aperture A_1 in the tapetum, rays with approximately equal angles of reflexion must come from the same region, and pairs of such rays were observed with included angles from about 40–70°. In two cases with normal incidence three spots were seen, one corresponding to normal reflexion and the other two symmetrically disposed on each side at an angle of about 50°.

Other types of reflexions. In six cases two spots of similar appearance were seen simultaneously for a range of directions of incidence such that they always showed approximate mirror symmetry about a diameter which remained constant for a given domain. The spot not corresponding to reflexion only in a horizontal plane was attributed to further reflexion in an approximately vertical plane (Text-fig. 6*a*). Other pairs were seen



Text-fig. 5. A much enlarged drawing based on a photograph of a common type of diffraction pattern from a single domain. The two green spots were of roughly equal brightness. When the direction of incidence of the pencil of light falling on the domain was altered the two spots moved approximately together in the same direction for short distances, e.g. in the directions indicated by the solid arrows or the dotted arrows. The images of marker lights M provide a frame of reference. In this exposure the two spots were symmetrical about the centre and corresponded to two reflectors, one approximately horizontal and the other inclined to it at an angle calculated to be 22° 24′ ± 6′, where the error given is solely that estimated to arise in measuring the photograph.



Text-fig. 6. Two types of diffraction pattern. In *a* one spot corresponds to reflexion from an approximately horizontal plane and the other behaves as if it were reflected from vertical planes as indicated in the ray diagram. In *b*, the dotted arrows indicate a different type of response to change in direction of incidence. Often the spot not reflected from horizontal planes was red. No explanation for its formation is offered but it is believed to be distinct from the artifact due to back reflexion in the optics which had somewhat similar properties, but was generally whitish.

in which the spots were markedly different in colour and intensity and always lay on the same diameter (Text-fig. 6*b*). Back reflexion from optical surfaces in the apparatus accounted for the second spot in many of them, but in some cases both spots did arise in the tapetum, as they both disappeared when this was moved slightly, giving place subsequently to a pattern from a neighbouring domain.

Distribution of inclinations of reflecting planes. Repeated measurements on the patterns showed that for the angular co-ordinates associated with a spot, the average standard error of the mean introduced by random errors in the method could be reduced below about 4° for angles less than 40°. For the last thirty-eight pairs of planes studied whose included angles lay between 10° and 65°, the probability that these angles were distributed other than randomly among 5° intervals between these limits was calculated to be 0·2, and this small probability might be accounted for by the biased sampling inherent in this method. After the first moderate refinements of the method, diffraction patterns were observed from twenty-three tapeta.

DISCUSSION

The arrangement of the rodlets in the tapetal cells. It is concluded that the tapetum in the preparations studied has a structure similar to that shown in electron micrographs in that the rodlets are arranged approximately in lattices in which the angles between the sets of lattice planes vary from one domain to another, and reflexions can occur simultaneously from more than one set of lattice planes. It is possible that this is a consequence of dissection, that *in vivo* the structure is different and while studied it was slowly being distorted. In particular, the rodlets in each domain may originally have been packed in a hexagonal lattice. In this arrangement the domain would occupy the least volume and the rodlets could be held separate by radially symmetrical forces. However, the distance between adjacent rodlets is an order of magnitude greater than that over which conventional electrostatic 'long range' forces can act, e.g. less than 150 Å in liquid crystals of the tobacco mosaic virus (Bernal & Fankuchen, 1941), and even if each rodlet were surrounded by an extensive repulsive field it would be difficult to explain the observation of Schultze (1872) and Pedler (1963) that when a domain is broken open clumps of rodlets stick together. An alternative might be that each rodlet is surrounded by a shell having the same refractive index and the same appearance in electron micrographs as the matrix. However, this would mean that there would be a minimum separation between adjacent rodlets so that when a hexagonal lattice was deformed by relative movement of horizontal rows to become, say, square, the spacing between the horizontal lattice planes would

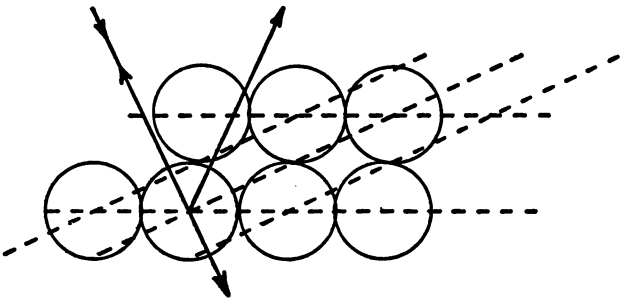
increase by sec 30° = 1·155. This should produce an easily visible change in the colour of the tapetum, and this was not seen. These considerations make the hypothesis unattractive. On the other hand, if the lattices with the various angles observed are stable (and the rodlets do not exert directional forces whose angles vary from one domain to the next) then they require some kind of framework. Since the vertical sides of many domains are irregular, these could not provide sufficient constraint and some further skeleton is postulated. This is in agreement with features in some electron micrographs in which rodlets in disrupted domains appear aligned in single file and, in some domains, there is only one set of clearly defined planes (the horizontal ones), which would account for the observation of domains whose diffraction patterns each contain only a single spot. Rutschke (1966), discussing the very similar morphology in the reflecting surfaces of duck specula, hypothesizes a structure of melanin rodlets and keratin which is similar, but not identical to this.

The spacing between the rodlets. The spacing between the centres of the rodlets can be estimated from the optical behaviour. By substituting numerical values for p in eqn. (1) it is seen that normal reflexion of wave-length λ_1 will occur only for optical layer spacings of $\frac{1}{2}\lambda_1$, λ_1, $\frac{3}{2}\lambda_1$, etc. The paucity of simultaneous reflexions in several directions suggests that the first two are more likely. An example of one common result was that simultaneous reflexion was observed from co-extensive reflectors mutually inclined at angles of about 25° (Text-fig. 7). In this case, if white light is incident at say 25° and there is first order reflexion of wave-length λ_1 from the inclined planes then there will be first-order reflexion of wave-length $2{\cdot}12\,\lambda_1$, and second order reflexion of wave-length $1{\cdot}06\,\lambda_1$ from the horizontal planes. Since two visible reflexions, both blue or green, were seen, it must be assumed that it is the second order reflexion from the horizontal planes which is visible, i.e. the spacing is λ_1. This is the spacing deduced by Pedler (1963) from his electron micrographs.

The differing optical spacings implied by the different colours reflected by different domains could arise either from differences in the geometrical distance of repeat or from differences in the mean refractive index which might be achieved by changing the diameters of the rodlets or the composition of the matrix (only a small range of refractive indices was found for the rodlets themselves). In the electron micrographs of Pedler (1963) different spacings are seen but these are not progressive through the thickness of the tapetum and may be due to uneven shrinkage during fixation. This change in optical spacing through the structure is similar to, but more regular than, that found in the scales of the herring, another case where a broad band reflector is achieved by the overlapping of narrow band components (Denton & Nicol, 1965). It might arise from a particularly simple

case of development controlled by a concentration gradient of a morphogen (Crick, 1970).

If eqn. (1) is the only condition necessary for reflexion to occur then more reflexions are predicted than were in fact seen. In particular, it is to be expected that there would be third order reflexion of blue light from the deeper, red reflecting domains, and some first order reflexion of red light incident obliquely on the upper, blue reflecting domains. In addition, when there were reflexions from more than one plane within a single domain and



Text-fig. 7. Interpretation of double reflexion from a single domain. If the separation of adjacent rodlets is constant as indicated by the circles surrounding each lattice point, then it can be calculated that when there is second order reflexion from the horizontal planes there can be simultaneous first order reflexion from inclined planes of a similar wave-length.

the normals to the planes were all on the same side of the incident ray (the common type of reflexion illustrated in Fig. 7) then the colours of the reflected pencils were not very different. It is suggested, therefore, that a rodlet has a structure factor strongly dependent on wave-length such that only those wave-lengths close to that appropriate for the lattice spacing are reflected. Schaefer & Grossman (1910) set up a formal solution of the equations for scattering of electromagnetic radiation by a dielectric cylinder. They evaluated this only after making the approximation that the wave-length of the radiation was large compared to the radius of the cylinder, but even in this case the intensity of back scattering is strongly dependent on wave-length, so it seems quite possible that a tapetal rodlet may have the properties predicted.

In the intact cat the overlapping of the different coloured domains may be sufficiently even to cause no appreciable variation in the spectral sensitivity of the retina over different parts of the tapetum; the calculations of Weale (1953) show that the spectral reflectivity curve of the tapetum must have quite a sharp peak to affect appreciably the shape of the spectral sensitivity curve. However, when recordings are made from

small regions of the opened eye it seems possible that the deteriorating tapetum may cause local variations in spectral sensitivity.

I wish to thank Professor A. F. Huxley for invaluable help throughout the course of the work, Dr C. M. H. Pedler for the loan of original electron micrographs, Mr A. Ness for criticizing the typescript, and Professor E. Neil and many others for arranging a supply of eyes. The processing of the colour photographs was carried out by the Central Photographic Unit of University College London. The work was undertaken during the period of an award for Further Education in Medical Sciences (Formal Courses), and a Scholarship for Training in Research Methods, both from the Medical Research Council.

REFERENCES

BAUMANN, E. J. & KURLAND, S. (1926). Changes in the inorganic constituents of blood in suprarenalectomized cats and rabbits. *J. biol. Chem.* **71**, 281–302.

BERNAL, J. D. & FANKUCHEN, I. (1941). X-ray and crystallographic studies of plant virus preparations. *J. gen. Physiol.* **25**, 111–146.

BERNSTEIN, M. H. & PEASE, D. C. (1959). Electron microscopy of the tapetum lucidum of the cat. *J. biophys. biochem. Cytol.* **5**, 35–40.

BRÜCKE, E. (1845). Anatomische untersuchungen über die sogenannten leuchtenden Augen bei den Wirbelthieren. *Arch. Anat. Physiol.* 387–406.

CRICK, F. (1970). Diffusion in embryogenesis. *Nature, Lond.* **225**, 420–422.

DANIEL, P. M. (1963). A simple and inexpensive method for examining the fundus oculi. *J. Physiol.* **169**, 7–8*P*.

DENTON, E. J. & NICOL, J. A. C. (1965). Reflexion of light by external surfaces of the herring *Clupea harengus*. *J. mar. biol. Assoc. U.K.* **45**, 711–738.

GUNTER, R., HARDING, H. G. W. & STILES, W. S. (1951). Spectral reflexion factor of the cat's tapetum measured at different ages. *Nature, Lond.* **168**, 293–294.

HOLMES, A. (1921). *Petrographic Methods and Calculations*, p. 36. London: Thomas Murby and Co.

HUXLEY, A. F. (1968). A theoretical treatment of the reflexion of light by multilayer structures. *J. exp. Biol.* **48**, 227–245.

LAND, M. F. (1966). A multilayer interference reflector in the eye of the scallop *Pecten maximus*. *J. exp. Biol.* **45**, 433–447.

PEDLER, C. (1963). The fine structure of the tapetum cellulosum. *Expl Eye Res.* **2**, 189–195.

RAYLEIGH, 3RD BARON (1917). On the reflexion of light from a regularly stratified medium. *Proc. R. Soc.* A **93**, 565–577.

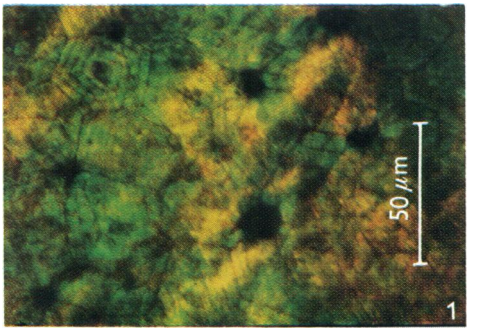
RUTSCHKE, E. (1966). Die submikroskopische Struktur schillernder Federn von Entenvögeln. *Z. Zellforsch. mikrosk. Anat.* **73**, 432–443.

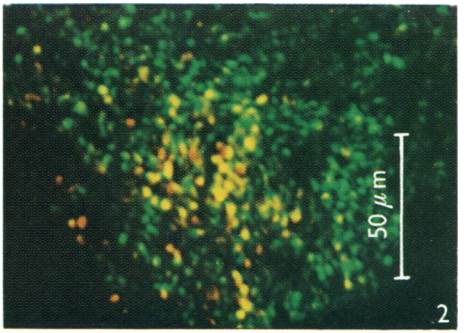
SCHAEFER, C. & GROSSMAN, F. (1910). Untersuchungen über die Beugung elektromagnetischer Wellen an dielektrischen Zylindern. *Annln Phys.* 4 Folge, Band 31, pp. 455–499.

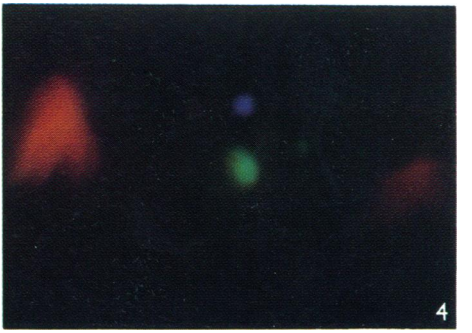
SCHULTZE, M. (1872). Uber das Tapetum in der Choriodes des Auges der Raubthiere. *Sber. niederrhein. Ges. Nat.-u. Heilk* **29**, 215–216.

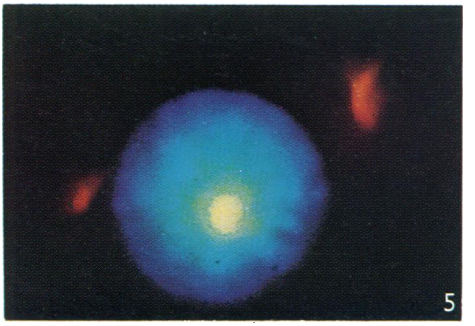
WEALE, R. A. (1953). The spectral reflectivity of the cat's tapetum measured *in situ*. *J. Physiol.* **119**, 30–42.

WILKINS, M. H. F., STOKES, A. R., SEEDS, W. E. & OSTER, G. (1950). Tobacco mosaic virus crystals and three dimensional microscopic vision. *Nature, Lond.* **166**, 127–129.









J. A. COLES

(*Facing p.* 409)

EXPLANATION OF PLATE

Fig. 1. The tapetum seen with top illumination and a 4 mm air objective, N.A.0·85. The polygonal cells divided into parallelogram-shaped bundles are visible. The dark spots are penetrating blood vessels. The shorter side of the figure corresponds to 119 μm.

Fig. 2. The tapetum observed with the same magnification but with the aperture of the objective restricted to pass a cone of light whose half angle is about 7°. The shorter side of the figure corresponds to 125 μm.

Fig. 3. Reflexions from a pencil of white light entering the tapetum with an angle of incidence of about 30°. The upper cells reflect blue and the deeper ones red.

Fig. 4. The back focal plane of the $\times 100$ microscope objective illuminated by reflexion from planes in an area of tapetum about 200 μm in diameter selected as indicated in Text-fig. 4. The field is estimated to cover angles of reflexion up to about 61° in the tapetum. The red flares are the marking lights.

Fig. 5. Diffraction pattern arising mainly from a single domain illuminated by a solid cone of light. The limits of the field represent light reflected at about 32° to the normal. The red spots are the marking lights.