JAN DYCK

STRUCTURE AND SPECTRAL REFLECTANCE OF GREEN AND BLUE FEATHERS OF THE ROSE-FACED LOVEBIRD (*AGAPORNIS ROSEICOLLIS*)

Det Kongelige Danske Videnskabernes Selskab
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Synopsis

The morphology of green and blue feathers of the Rose-faced Lovebird (*Agapornis roseicollis*) is described from light-, fluorescence-, and electron microscopical findings and discussed in relation to earlier works. The description is intended to provide a basis for future comparative studies.

Special attention is given to the colour-producing elements (pigments and the short-wave reflecting spongy structure ('Blaustruktur', 'cloudy medium') of specialized medullary barb cells (spongy cells, box cells)), and the findings are correlated with macro- and microspectrophotometric measurements.

Green barbs differ from those of blue barbs in having their cortex yellow pigmented, but are further distinguished by their spongy structure which is denser (wider keratin rods and correspondingly narrower air-filled channels) than that of blue barbs. This difference corresponds to the wave-length of maximum reflectance being shifted c. 30 nm towards longer wave-lengths compared to that of blue barbs. Thus green barbs are not the same as blue barbs only with a yellow pigmented instead of an unpigmented cortex, as usually stated.

Dark green back feathers reflect approximately half as much light throughout the visible spectrum as do green belly feathers. This difference is due to variations in yellow and black pigmentation of the barbules. These variations are described quantitatively and the importance of barbules for the resulting feather colour is stressed.

Variation in size and shape of barbs and barbules are discussed, principally in relation to their optical effects and the presumed functions of the colours.

The colour produced by the spongy structure cannot be explained by Tyndall (Rayleigh) scattering as is usually done. This follows from the shapes of the barb reflectance spectra which are not in agreement with the Rayleigh equation (scattering inversely proportional to λ^4).

A new model for colour production is forwarded. It is based on a model of the spongy structure in which this is considered to consist of short hollow keratin cylinders (diameter 0.3–0.35 μ) with air-filled cores. Backscattering from these cylinders is considered responsible for colour production and good agreement is obtained between values of λ_{max} calculated from the model and those measured spectrophotometrically.

The backscattering from the individual cylinders can be regarded as an interference phenomenon. The colour of the spongy structure thus is an interference colour. That it appears diffuse and not iridescent, as is generally the case for interference colours in feathers, is due to the presence of many hollow cylinders oriented in all directions in the spongy structure.

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Introduction

Non-iridescent green feather colours of birds can be produced in a number of ways (Haecker 1890, Auber 1957 a). Most often a vivid green colour is associated with the barbs where a structure reflecting blue and green light is found in the inner parts; the outer portions of the barb are yellowish pigmented and hence absorb most of the reflected blue light with the result that green light dominates in the light leaving the barb. This explanation was first given by Haecker (1890).

The morphology of green feathers, in particular that of the barbs, has since been described by various workers (Häcker & Meyer 1902, Strong 1902, Kniesche 1914, Kawamura 1935, Frank 1939 and Auber 1941, 1957 b, 1964). It is evident from these investigations that the barb from an optical point of view may be considered to consist of three parts: 1) a cortex containing yellow pigments (carotenoids, yellow melanins or pigments of unknown chemical constitution, specific to parrots (Völker 1937, 1942)), often restricted to the obverse part of the cortex, 2) a layer of blue-producing cells (box cells (Fox & Vevers 1960), cloudy, medullary cells (Auber 1957 b), here called spongy cells (p. 63); these cells may be arranged in many different ways, often they are situated immediately beneath the cortex and form a ring encircling the central parts of the barb or they are restricted to the obverse part of the barb, and 3) a dark-pigmented layer, nearly always containing melanin granules, mostly situated in the central or reverse parts of the barb. The melanin granules may be found in the central parts of the spongy medullary cells (so-called "two-media" cells (Auber & Mason 1955)) or in separate medullary cells; in other cases they are restricted to the cortex or the barbules.

Fine structure of spongy cells. The spongy cells have been subjected to much investigation and speculation since they were first localized in the barbs by Fatio (1866). Gadow (1882) considered the colour to be partly due to diffraction; this assumption he based on the presence of fine ridges on the surface of the spongy cells. Haecker (1890) found that the colour was dependent on the presence of small, air-filled pores in the keratin of the spongy cells and supposed that the colour was due to a difference in the reflection of blue and red light (he believed that the refractive index of keratin is greater for blue than for red light) from the many small air/keratin surfaces. Later Häcker & Meyer (1902) found that the dimensions of the air-filled pores were less than the wave-length of light, and consequently considered the colour

to be due to light scattering. They also showed that the intensity of the scattered light of a blue feather in the wave-length region 480–600 nm varied inversely proportional to the fourth power of the wave-length in accordance with Rayleigh's equation for the scattering of small, independent, isotropic particles (KERKER 1969). Later investigations by BANCROFT *et al.* (1923) confirmed this explanation.

RAMAN (1935), however, found that the individual medullary barb cells in reflected light appeared with different colours and from this considered it unlikely that the colours were produced by simple Rayleigh scattering. He inferred that the air-filled cavities were either elongated or flattened in which case the colours should rather be classified as interference colours.

KNIESCHE (1914) considered the pores to be in the form of canals arranged perpendicular to the surface of the barbs. GOWER (1936) gave an entirely different explanation, suggesting that the scattering particles were small solid particles imbedded in keratin. FRANK (1939) on the basis of light microscopical investigations supposed that the pores formed an irregular network, and he was able to confirm this by one of the first electron microscopical investigations on a biological material (FRANK 1939, FRANK & RUSKA 1939). Subsequent electron microscopical investigations (NISSEN 1958, SCHMIDT & RUSKA 1962) have confirmed this. For a more detailed review and explanation, see FOX (1953), FOX & VEVERS (1960).

Olive-green feathers. The olive-green plumage areas in numerous birds are produced by the juxtaposition of yellow and black pigmented feather parts (HAECKER 1890, FRANK 1939, AUBER 1957 a, AUBER *in* MOREAU 1957). In most cases the barbs and basal parts of barbules are yellow pigmented with the terminal parts of the barbules being black or brown pigmented. It is still unknown whether spongy medullary cells are present in the barbs of olive-green feathers. KNIESCHE (1914) is of the opinion that this is generally the case, while FRANK (1939) says that cells producing an imperfect blue colour are often found (e.g. in *Chloris*, *Phylloscopus*, *Oriolus* and *Picus*). AUBER (1957 a) stated this as incorrect in the case of *Chloris*. A study of the reflectance spectra (percentage of light reflected as function of wave-length) of olive-green plumage areas (DYCK 1966) has shown that these differ much from those where the green colour is based on the presence of spongy medullary cells, and from the form of the spectra it seems unlikely that a spongy structure is present in the olive-green feathers. I have later found (unpublished results) that some green plumage areas have reflectance spectra, which suggested an imperfect spongy structure. These observations induced me to perform electron microscopical investigations of green and olive-green feathers.

Programme. It was found advantageous to start with a detailed examination of the feathers of a single species to obtain a basis for comparison since no thorough electron microscopical investigations of *green* feathers with well-developed spongy medullary cells seem to have been made. A parrot, the Rose-faced Lovebird (*Agapornis roseicollis*), was chosen. This species has three plumage areas of different colours (dark green, light green and blue), based on well-developed spongy medullary cells.

In the paper is given a description of the morphology of the feathers based on light-, fluorescence- and electron microscopy. The morphological data are compared with spectral reflectance measurements in order to obtain a semi-quantitative expression for the factors responsible for the colour differences between blue and green on the one hand, and dark and light green plumage areas on the other hand. Finally, the physical explanation of the colour production of the spongy medullary cells is considered.

Material and Methods

Feathers were plucked from an adult female of *Agapornis roseicollis* obtained from a dealer. The plumage appeared to be fresh-moulted.

The air content of the barbs represents a difficulty in the embedding of barbs for electron microscopy. In order to obtain good ultra-thin sections it is necessary to have all air replaced by the embedding medium (epon 812). Due to the small dimensions of the air-filled pores this is difficult to achieve. If the barb is simply immersed in epon 812 no appreciable penetration takes place, not either if the barb is left in the medium for weeks, or at elevated temperatures or at vacuum. (The penetration of liquid into the spongy cells is easy to observe as it is followed by a change to a more or less brownish colour, the exact hue depending on the refractive index of the liquid. These observations confirm the old immersion experiments by HAECKER (1890) and BANCROFT *et al.* (1923) by which the nature of the colour-producing structure was demonstrated).

The following method was found to be usable: Feathers were washed in [1]hot detergent, [2]distilled water, [3]acetone and [4]xylene and the tips of the barbs were cut off. They were then treated with a basic solution of thioglycolate in absolute alcohol at 60° C (TEIGER *et al.* 1957) until air had been completely replaced by liquid, whereupon the barbs were transferred through absolute alcohol to propylene oxide. The replacement of propylene oxide with epon could then be achieved in one of two ways: a) immersion in gradually increasing epon concentrations (20, 40 . . ., 100 %) at room temperature; at least 24 hours in each concentration. b) a quicker method was to place the feather at the bottom of a 10 cm layer of the propylene oxide-epon mixture, and immediately afterwards place the glass vessel under pressure (nitrogen at 26 atmospheres) for one hour. The steps in epon concentration were 25, 50, 75 and 100 %. By neither method did penetration of epon occur in the total length of the barb, but usually a short part where the barb had been cut could be used for sectioning on the ultramicrotome (Reichert "Om U 2"). Sections were contrasted with lead hydroxide and uranyl acetate and examined in a Siemens Elmiskop I operated at 40 KV.

For thicker sections (3,5 μ) used for light- and fluorescence microscopy it proved advantageous, but not necessary, to have air replaced by epon. The sections were studied unstained or after staining with toluidine blue. Transmission and reflection investigations were carried out with air-filled spongy cells.

Some embedding media with lower viscosity than epon (butyl methacrylate and hydroxypropyl methacrylate (LEDUC & HOLT 1965)) were tried without great success.

Reflection measurements were carried out with a recording spectrophotometer (Beckman DK-2A) equipped with a reflectance attachment. The bird was placed at the sample

exit port of an integrating (Ulbricht) sphere and illuminated with monochromatic light (specular plates in position: total). Plumage areas measured were 8×10 mm. As reference was used a plate with a coating of magnesium oxide, which reflects very nearly 100 % in the spectral region used (380–740 mm). From the reflection curves values of hue, purity and lightness were calculated. These values designate the colour as viewed by a "standard" human observer in daylight from a completely overcast sky (CIE illumination C). For application of these methods in ornithology, see LUBNOW & NIETHAMMER (1964) and DYCK (1966). For more general treatment, see e.g. JUDD & WYSZECKI (1963).

Transmission measurements on pigment solutions were carried out by means of the same spectrophotometer.

Microspectrophotometric measurements on individual barbs and barbules (reflection and transmission) were carried out with a Reichert research microscope ("Zetopan") equipped with a microspectrophotometer. By means of a chopper (250 Hz) the apparatus was modified so that an A.C. signal was obtained. This was fed to an HR-8 precision lock-in amplifier (Princeton Applied Research). As detector a photomultiplier with S-20 spectral response (EMI 9698 B) was used. For reflection measurements a coating of magnesium oxide was used as a reference.

For extraction of the yellow pigment of the green feathers a slight modification of the method used by VÖLKER (1936) for the yellow pigment of the budgerigar (*Melopsittacus undulatus*) was used: The feathers were treated for three days with a mixture of 10 ml pyridine and $^1/_2$–1 ml concentrated hydrochloric acid at 60° C.

I was not able to transfer the pigment to petroleum ether as described by VÖLKER (*op. cit.*), and therefore used the pyridine solution for spectrophotometric measurements, using the pyridine-hydrochloric acid mixture as a reference.

Nomenclature. In the description of feather parts the nomenclature of CHANDLER (1914) is used, except as regards the different parts of the barbs, where the nomenclature of AUBER (1957 b) is followed. Also, the term barb is used instead of ramus (CHANDLER, *op. cit.*) for the primary branches of the feathers minus their barbules (cp. e.g. BRUSH & SEIFRIED 1968). In numbering the barbs the feather is placed with the obverse (exposed) surface upwards and the calamus pointing towards the observer. Numbering is from the distal towards the proximal end of the feather, barb r1 thus being the distalmost barb on right side. Terms of orientation are shown in text fig. 1.

Results

Description of Plumage Areas

The plumage of *Agapornis roseicollis* is dominated by green. The upper parts from crown to lower back and wings are a uniform dark lime, with the exception of blackish grey inner webs of remiges. Rump and upper tail coverts are light turquoise-cobalt (colour plate, fig. 1). The forehead is scarlet, while sides of head, chin, throat and breast are a light scarlet orange. The remaining under parts are lime (colour terms according to PALMER 1962, for the sake of clarity, however, green and blue are used instead of the correct terms, lime and turquoise-cobalt, in the following).

Text fig. 2 shows reflectance spectra of the blue and green plumage areas.

All spectra show a single reflection peak, at 452, 482 and 530 nm for blue, extracted green and green plumage areas respectively. Reflectance varies comparatively little in the red region (600–740 nm), where the two green spectra show a slightly indicated minimum at 660–670 nm. The green plumage areas reflect very little in

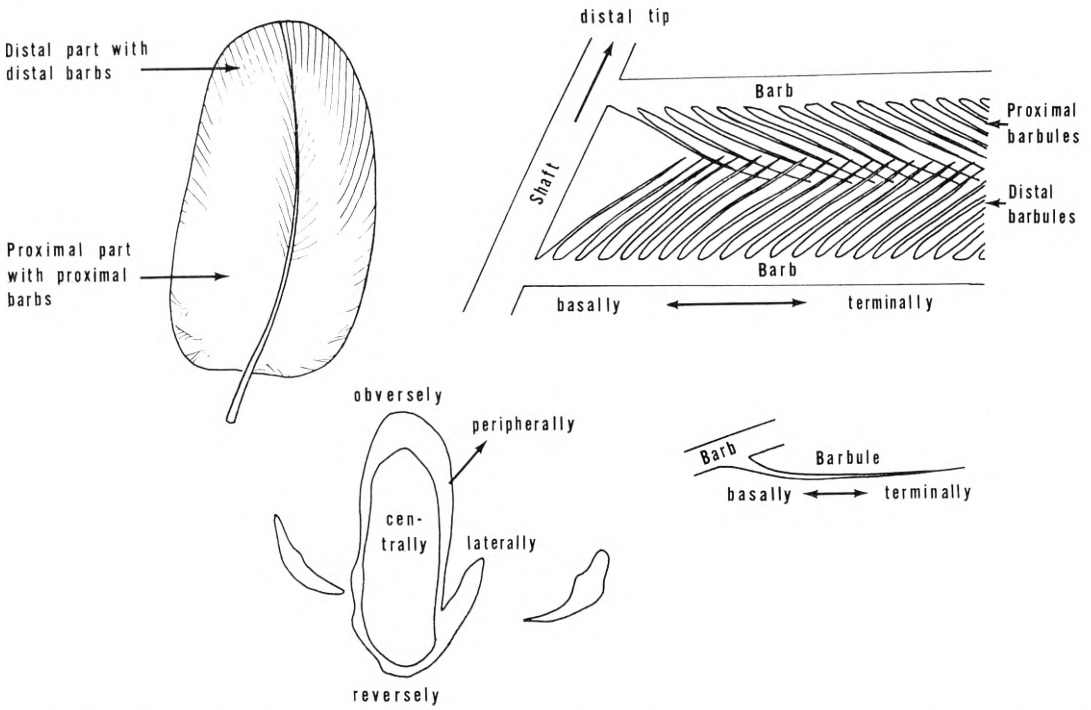

Fig. 1. Terms of orientation used. Lower part shows cross-section of barb and two barbules. The exposed (obvese) side of the feather is seen.

the blue and violet regions (380–480 nm) due to absorption by the yellow pigments. The absorption bands of the yellow pigments manifest themselves as reflectance minima (Dyck 1966), which in the two spectra occur at the same wave-lengths (c. 395, 419, 441 and 468 nm).

Reflectance spectra of the two green plumage areas are of almost identical form, the only difference being that the lighter green belly reflects almost twice the amount of what the darker green upper parts do.

Values of hue, purity and lightness, calculated from the curves in text fig. 2, are summarized in Table 1.

Table 1.
Hue, purity and lightness of plumage areas of *Agapornis roseicollis*.

Plumage area	Lightness (%)	Hue (nm)	Purity (%)
Green belly	28,83	564,5	53,37
Dark green back	13,64	561,4	52,19
Blue rump	16,54	479,0	39,65
Green belly, yellow pigment extracted	25,50	485,3	20,96

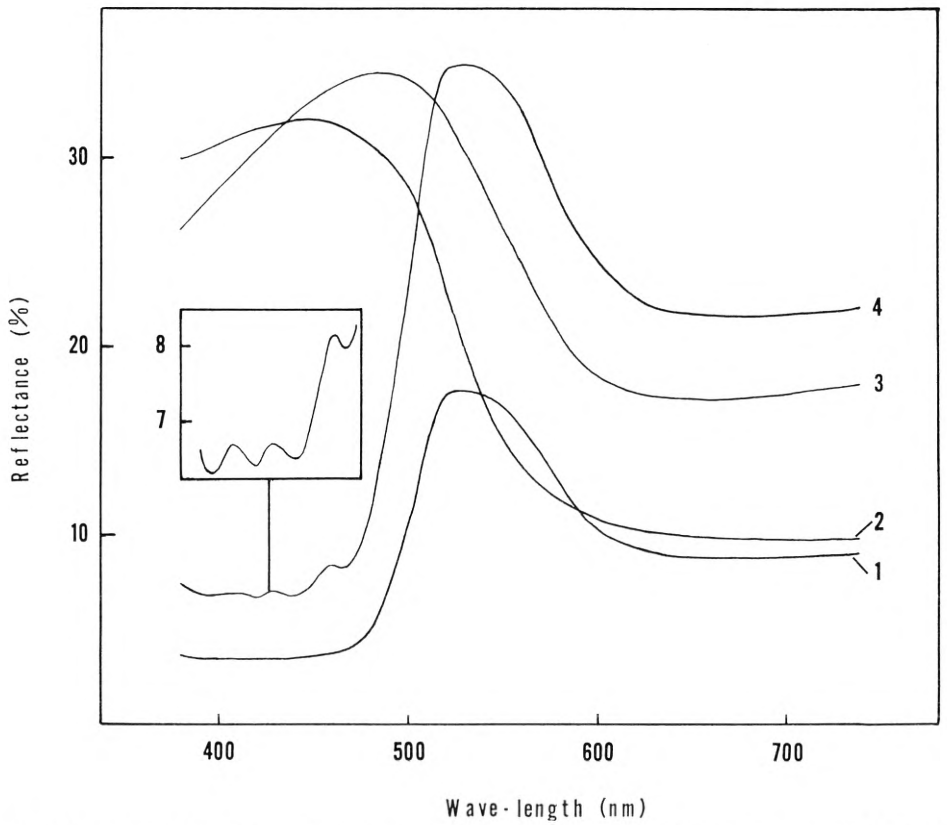

Fig. 2. Reflectance spectra of plumage areas of *Agapornis roseicollis*. 1: Dark green upper back, 2: Blue rump, 3: Green belly feathers with yellow pigment extracted, 4: Green belly. Inset: Part of curve 4 with ordinate enlarged to show reflectance minima. Area mesured: 8×10 mm.

It is seen from Table 1 that hue (or dominant wave-length) is 561 to 564 nm for the green, 479–485 nm for the blue feathers. The main difference between the two green areas is due to the lightness, which is twice as high for the green belly as for the dark green back. The colour of the blue rump is darker and more saturated (higher purity) than that of green belly feathers from which the yellow pigments have been extracted.

Light- and Fluorescence Microscopy

Description of individual feathers.

The coloured body feathers are typical contour feathers (15–30 mm long) with a proximal downy and a pennaceous distal part (colour plate, fig. 2). Generally speaking, only the pennaceous part is coloured, the downy part being silvery grey. The contour feathers possess a well-developed downy aftershaft, the length of which is c. 70 % that of the feather.

Two kinds of yellow pigment, both of unknown chemical constitution (VÖLKER 1937, 1940, 1942), are present in the feathers: a fluorescent and a non-fluorescent.

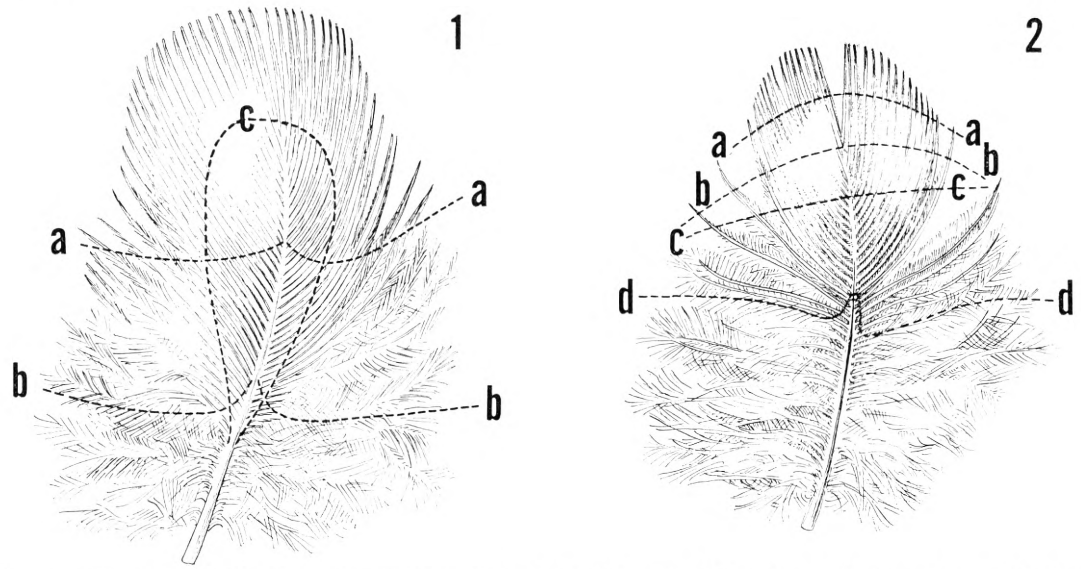

Fig. 3. Colour zones in feathers, obverse view. 1: Green belly feather. Distally to a–a barbs are green, between a–a and b–b barbs are light turquoise and proximally to b–b they are silvery grey. In the area encircled by c distal barbules are black pigmented. 2: Blue rump feather. Distally to a–a barbules are silvery grey, proximally to a–a they are yellow and black. Distally to b–b barbs are light turquoise-cobalt blue, between b–b and c–c barbs are greenish and between c–c and d–d they are light turquoise. Proximally to d–d barbs are silvery grey. Lines indicate transition zones, not abrupt changes.

According to the investigations by VÖLKER (*op. cit.*) the latter type is of primary importance in the colouration of the feathers, and is readily detected with the naked eye, whereas the fluorescent type is of a pale yellowish colour; the colour of this type is easily veiled by the presence of other pigments or structural colours and thus can be detected only with the aid of the fluorescence microscope. The distribution of the yellow pigment in the feathers given below is based mainly on the distribution of the yellowish fluorescence, as this is more easily detected in small quantities. In general, the two types of yellow pigment are distributed similarly in the barbules and barbs (but not in detail as shown below). It is considered likely therefore that the given distribution of the fluorescent yellow pigment is representative of both types of yellow pigment.

Green belly feather (colour plate, fig. 2). There is a gradual transition in the obverse colour of the barb, which changes from green in the distal portion of the feather to a light turquoise in the proximal portion (text fig 3). In the most proximal barbs the light turquoise colour is gradually replaced by the silvery grey colour of the downy barbs. The light tuquoise colour is also observed on the reverse surfaces of the barbs and, estimated visually, is matched by the colour of green feathers from which the yellow pigment has been extracted (reflectance spectrum 3, text fig. 2).

Light yellow barbules (colour plate, fig. 3) are present in the entire length of the barbs. In the distal part of the feather yellow pigments are found throughout the

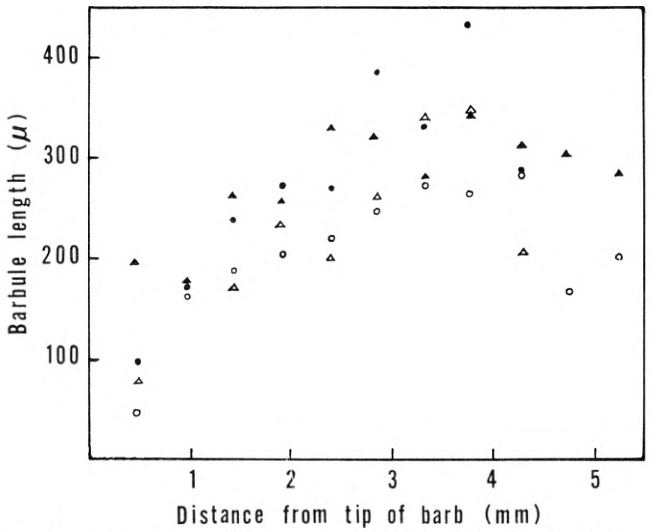

Fig. 4. Barbule length as a function of its distance (where it inserts on the barb) from the tip of the barb. All measurements carried out on distal barbules of the first barb on the left side. ●: dark green back feather (length of barb: 4.9 mm), ▲: green belly feather (length of barb: 6.3 mm), △: blue rump feather (length of barb: 5.6 mm), ○: blue upper tail covert (length of barb: 6.7 mm).

length of the barbules except the most terminal parts. Towards the proximal part of the feather the yellow pigmented parts of the barbules become gradually restricted to the basal parts, and most proximally less than half the length of the barbules is yellow pigmented. In the central part of the feather (text fig. 3) the distal barbules are black pigmented. Pigmentation is restricted to the portion of the barbules where the hooklets are found.

Dark green back feather (colour plate, fig. 2). The colours of the barbs appear visually identical with those of the green belly feathers (colour plate, figs. 3 & 4).

The barbules (colour plate, fig. 4) differ from those of the belly feathers in their much more intense melanization extending over more than half their length, the basal third being yellow pigmented and the tip unpigmented (or in some cases yellow pigmented also). The basal yellow pigmented parts of the barbules are shorter in the proximal portion of the feather.

Blue rump feather (colour plate, fig. 2). Distally the barbs are a light turquoise-cobalt blue. More proximally, the colour becomes distinctly greenish in a small zone and then becomes a light turquoise nearer the proximal end (text fig. 3). The light turquoise colour is very similar to that observed proximally in the green feathers.

Barbules (colour plate, fig. 5) attaching basally to the barbs are well-developed, but those attaching terminally are somewhat reduced. The reduced barbules are a silvery grey. In the remainder of the pennaceous portion of the feather barbules are light yellow in their basal halves, black pigmented in their terminal halves. Proximally in the feather the basal yellow pigmented parts of the barbules become shorter.

TABLE 2.
Length of fully exposed parts of barbs in the intact skin compared to length of intensely coloured terminal parts of distal barbs.

Plumage area	Length of exposed parts of barbs (mm)		Length of intensely coloured parts of distal barbs (mm)	
	Arithmetic mean (mm)	No. of measurements	Range (mm)	No. of feathers
Green belly.....	5,8	40	6–10	2
Dark green upper back	4,3	99	5– 7	2

The regional difference in barb and barbule colouration makes the feather appear blue in the distal and green in the proximal part of the pennaceous portion (colour plate, fig. 2).

Barbule length. Barbules of distal blue barbs are somewhat shorter than those of distal green barbs (text fig. 4). The figure also shows that the barbule length often varies irregularly along the barb; this undoubtedly is due in some degree to the terminal parts of the barbules having broken of.

In the intact plumage the proximal portion of each feather is covered by the distal parts of other feathers, so that only the distal zone of each feather is fully exposed to view. This distal part is so narrow that only the intensely coloured terminal portions of the barbs are exposed, whereas the less intensely coloured proximal parts are partly concealed. This has been confirmed by measurements on photographs of the intact skin, summarized in Table 2.

Table 2 confirms that in general only the intensely coloured terminal parts of the barbs are fully exposed to view. This indicates, that the intensely coloured parts of the barbs are longer the more of the feather is exposed.

Description of individual barbs and barbules.

Blue barb (fig. 2). The barb is oval in transverse section. Outermost is found a cortex, 2–5 μ wide, often most narrow laterally. The outer layer of the cortex (surface cells, AUBER & APPLEYARD 1951) stains darker than the interior layer. Inside the cortex the medullary part is found. This consists outermost of a ring of blue-producing, spongy structure. In the central medulla numerous vacuoles are found with small areas of spongy structure interspersed. The central medulla is enclosed by a dense ring of black melanin granules. In addition, rings of melanin granules are found around some of the vacuoles. The barbules insert near the reverse end of the barb.

In ultra-violet light no greenish yellow fluorescence is observed.

Green barb (fig. 1). Green barbs are smaller and more triangular in shape than blue barbs in transverse sections. The cortex is thicker obversely, 10–15 μ wide, and consists of a heavily staining surface layer and a much less staining inner layer (cortex proper, AUBER 1957 b). Both layers narrow laterally towards the reverse part.

Comparison with unstained sections shows that the heavily staining layer corresponds to the yellow pigmented part of the cortex. The few vauoles lie on a straight line from the obverse to the reverse part. The barbules insert near the reverse part of the barb.

The distribution of the fluorescent yellow pigment in the barb is different from that of the more prominent non-fluorescent yellow pigment, referred to above. Fig. 3 shows that it is found throughout the width of the obverse cortex and in the lateral parts as well. The fluorescence is not distributed evenly in the obverse cortex; irregular fluorescing areas are divided by narrow non-fluorescing zones, probably cell boundaries, compare DRIESEN (1953) and SCHMIDT (1961). In the lateral cortex the fluorescing areas become long and narrow. The emitted light is of an intense greenish yellow colour.

In some cases (not illustrated) a few of the most obverse medullary cells also do fluoresce (this fluorescence is not a mere faint reflection of the fluorescing cortex, which follows from the observation that the fluorescing cells contrast sharply with non-fluorescing medullary cells). Areas along cell boundaries and areas around the vacuoles are parts of medullary cells fluorescing most intensely.

Variations in cross-sections of barbs. Shape and size of cross-sections of barbs vary along the length of the barbs and are different in barbs of different colours (text fig. 5). Both the two green barbs and the blue one showed the less intense, light turquoise blue colour in the basal 2–4 mm of their length, and the typical green, respectively blue, colour in their greater terminal parts.

The essential features of text fig. 5 are:

1. Yellow pigments are not present in the blue barbs.
2. In the basal part of green barbs, yellow pigments are restricted to a small part of the obverse cortex. Terminally the yellow pigment spreads from the obverse cortex laterally towards the reverse part so that terminally nearly the whole cortex is yellow pigmented.
3. Terminally the blue barbs are oval, the green ones more triangular in cross-section, as mentioned above.
4. The blue barb becomes more triangular with a thickened massive cortex basally, thereby becoming similar in shape to the green barbs. The change in shape corresponds to a change in colour from a light turquoise-cobalt blue terminally to a light turquoise basally (8–10 mm from tip). The shape of green barbs does not vary significantly throughout the length of the barbs. Thus blue and green barbs are similar in shape of cross-section and colour basally.
5. Barbs of green belly and dark green back feathers do not differ in shape, but the latter are somewhat smaller in cross-section than the former. The difference in colour between the two types of feathers depends on difference in barbule pigmentation.
6. The layer of spongy structure is mostly more narrow reversely than laterally and obversely.

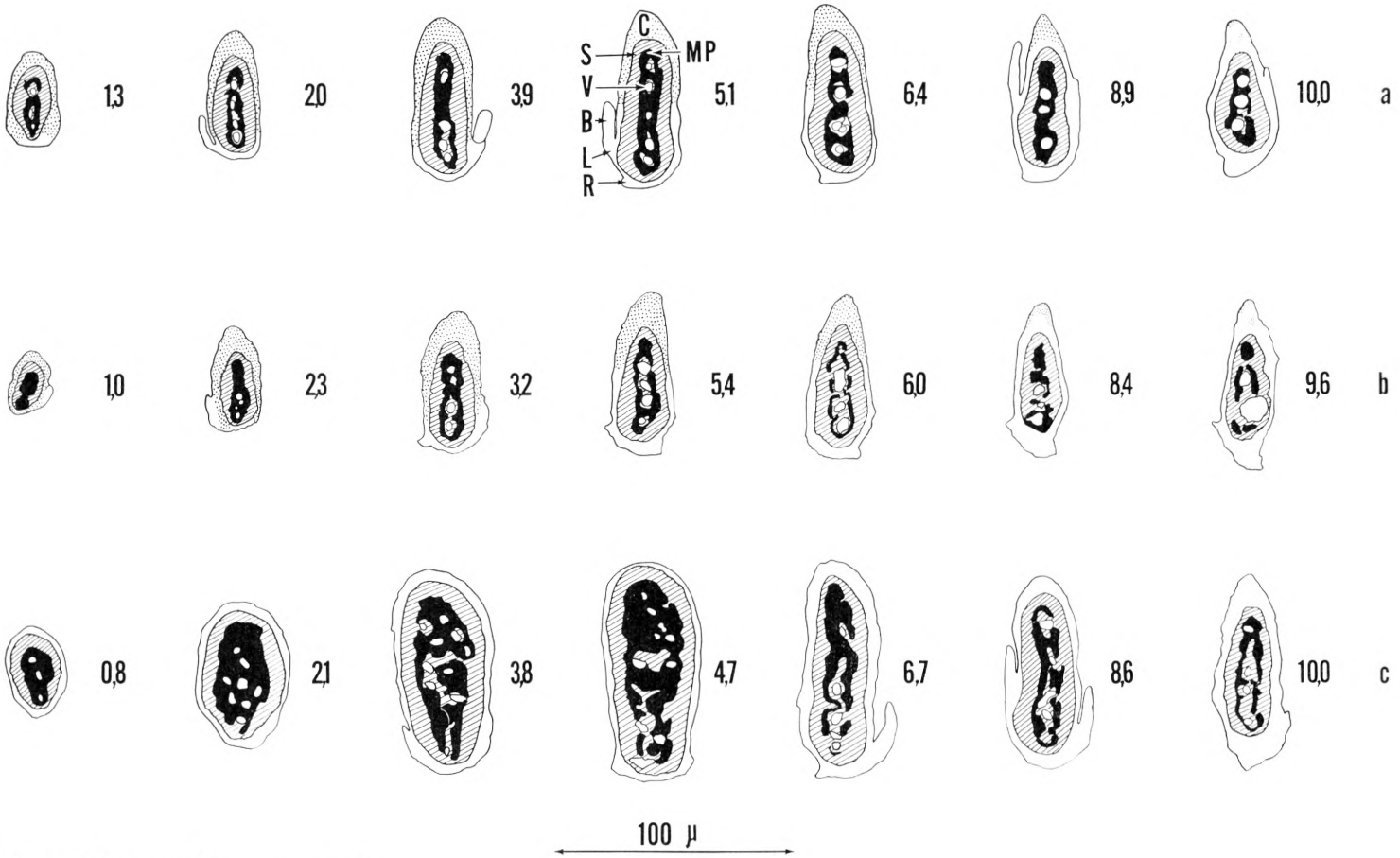

Fig. 5. Diagrams of transverse sections of barbs. a: barb 17 r (length: 13.2 mm) of a green belly feather, b: barb 10 l (length: 10.5 mm) of a dark green back feather, c: barb 10 r (length: 10.6 mm) of a blue feather. Figures at sections give their distances (in mm) from the terminal tips of the barbs. B: Barbule, C: Cortex, L: Ledge for barbules, MP: Melanized portion, R: Reverse ridge, S: Spongy structure, V: Nuclear vacuole. Dotted: Parts of cortex fluorescing greenish yellow.

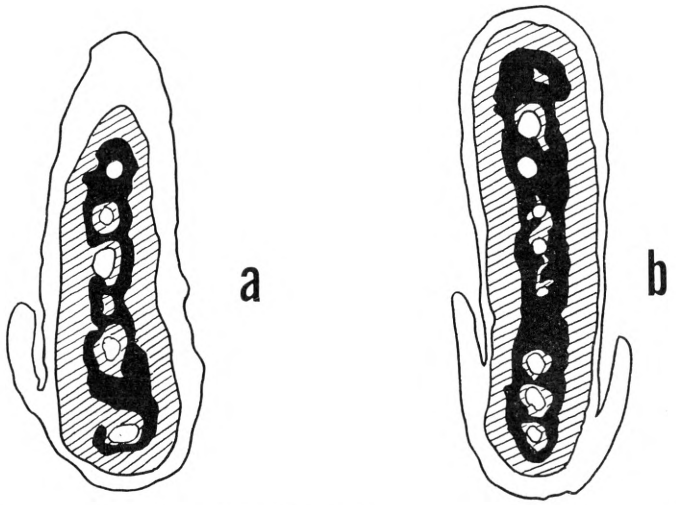

Fig. 6. Diagrams of transverse sections of distal barbs basally. a: barb 3 l of green belly feather, cut 7.3 mm from terminal tip (total length: 8.0 mm), b: barb 2 r of blue feather, cut 6.8 mm from terminal tip (total length: 7.3 mm).

7. The cortex becomes proportionately wider basally, especially in the reverse part, where a ridge is found in the basal halves of the barbs. The ridge is asymmetrical and points toward the shaft.
8. Terminally on the barbs the barbules insert at the reverse part of the barb, but towards the basal end of the barb they gradually insert nearer the obverse part, especially the distal barbules.
9. One would expect cross-sections of barbs to be largest at the basal ends of the barbs, but this is not the case. Instead, the barbs are highest midway out, where the blue barb attains a height of 90 μ, the green barbs of green belly and back feathers 74–75, and 67–68 μ respectively. From here the height diminishes regularly towards the tip, where the height is 10–20 μ. Towards the basal end the height also diminishes to increase slightly again shortly before the barb attaches to the shaft.

In the distal barbs, where the typical green or blue colours are observed throughout the entire length of the barbs, the change in cross-section towards the basal end, just described, does not occur. Instead of a thickening of the cortex with the developement of a reverse ridge, an increase of the height of the barbs is observed (text fig. 6). The height is greater than observed in any part of the more proximal barbs (91 and 98 μ for a green, respectively blue, barb). Apart from the increased height cross-sections of the barbs are similar to those found terminally in the more proximal barbs.

Distal barbule of dark green back feather (text fig. 7,1). This kind of barbule consists of a relatively broad base and a tapering pennulum, approximately equal in length. The pennulum bears hooklets and short cilia on the reverse side.

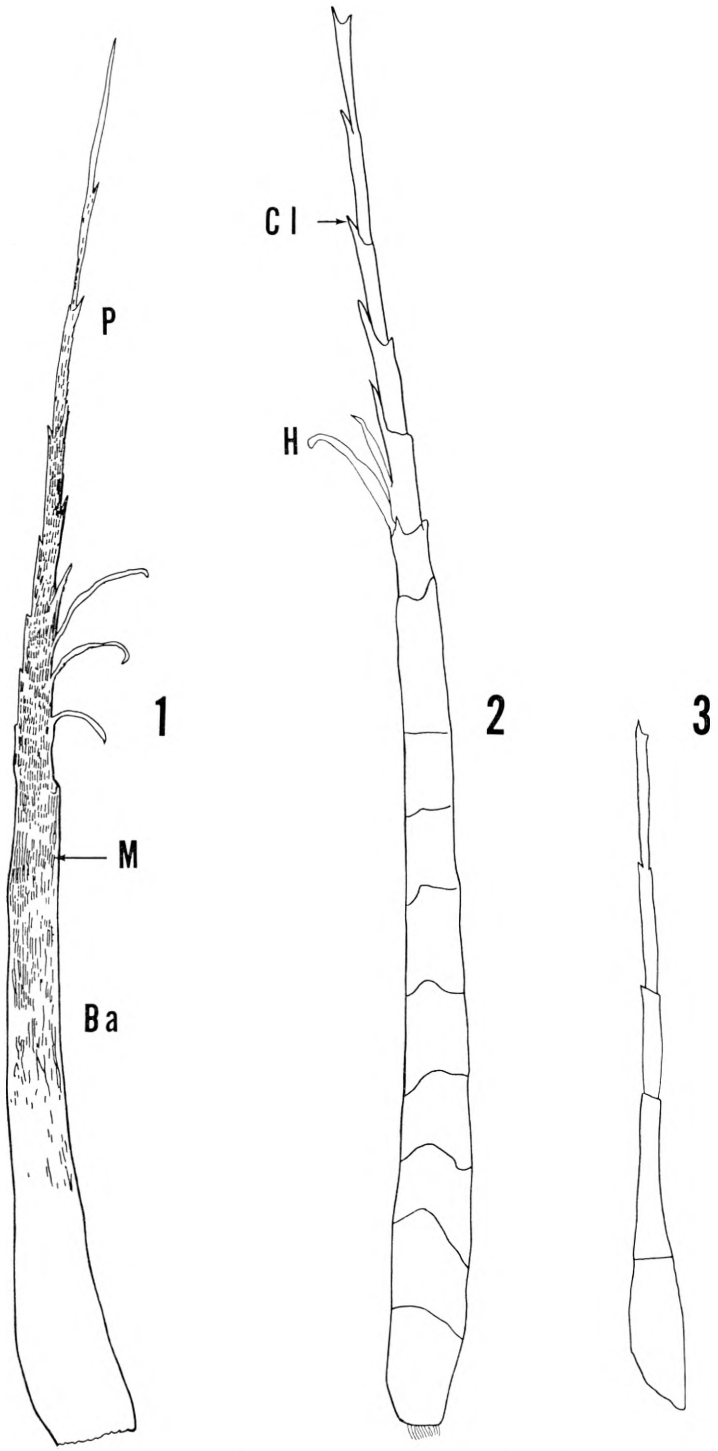

Fig. 7. Distal barbules from terminal parts of distal barbs. 1: 3.5–4.8 mm from terminal tip of barb 4 l of green back feather, 2: c. 5 mm from terminal tip of barb 4 l of green belly feather, 3: 2.5–3.5 mm from terminal tip of barb 2 l of blue feather. Ba: Base, Cl: Cilium, H: Hooklet, M: Melanin granules, P: Pennulum.

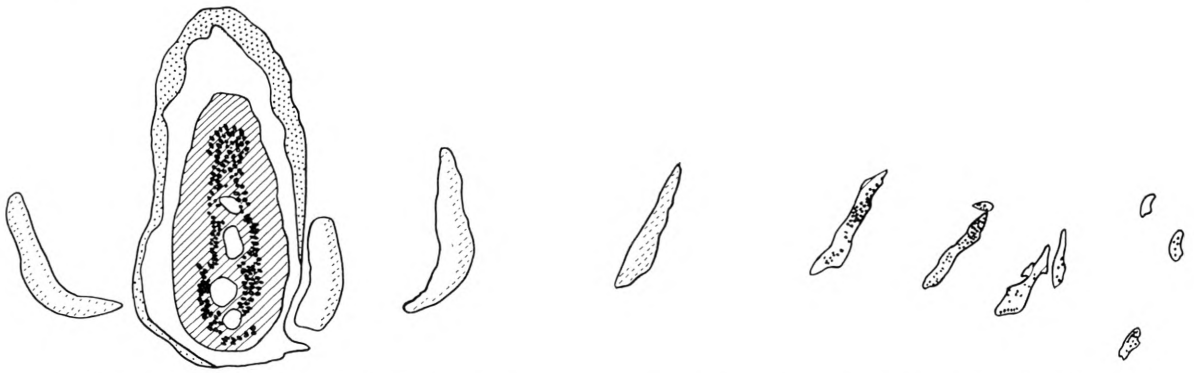

Fig. 8. Transverse section of barb and proximal barbules of dark green back feather. Barb 10 l (length: 10.5 mm) cut 3.2 mm from terminal tip. Barb signatures as in text fig. 5, except that dotted area here corresponds to intensely yellow pigmented surface layer. Melanin granules appear as black dots in barb and barbules. Fluorescing parts of barbules hatched. Inverted.

Part of base and most of the pennulum are filled with rod-shaped melanin granules, arranged with their axes parallel to the main axis of the barbule.

Distal barbule of green belly feather (text fig. 7,2). The general outline is the same as described above, but no melanin granules are present. The boundaries between the linearly arranged cells constituting the barbule are easily seen. A tapering terminal tip, as shown in text fig. 7,1, is present on a few barbules in the intact feather, but is mostly absent (broken of?).

Distal barbule of blue feather (text fig. 7,3). This barbule was placed more terminally on the barb than the two others, hence no hooklets are found. Individual cells are longer, and no clear distinction between the base and the pennulum is seen. No melanin granules are present.

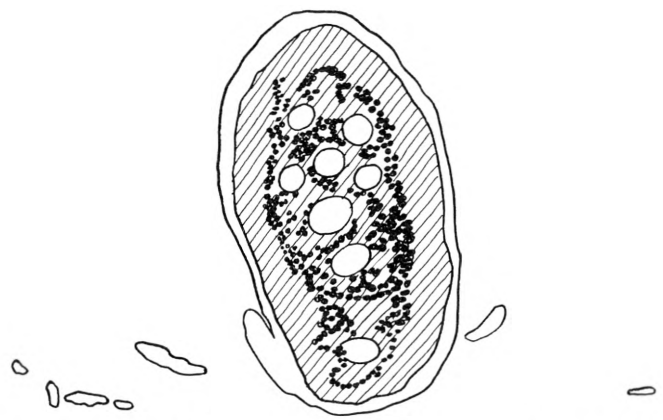

Fig. 9. Transverse section of barb and barbules of blue feather. Barb 10 r (length: 10. 6 mm) cut 3.0 mm from terminal tip. Signatures as in text fig. 5. Inverted.

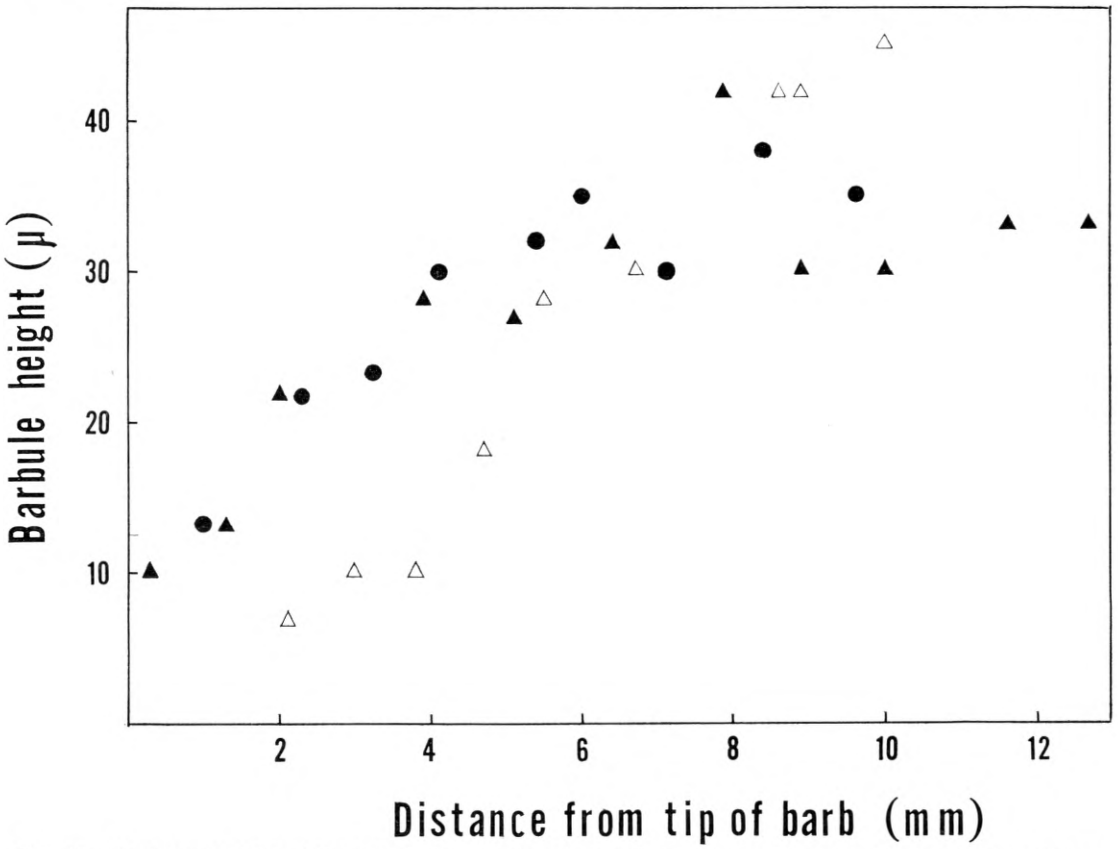

Fig. 10. Maximum height of distal barbules as a function of their distance from tip of barb. Height is taken as shortest distance between obverse and reverse tip. ●: barb 10 l (length: 10.5 mm) of a dark green back feather, ▲: barb 17 r (length: 13.2 mm) of a green belly feather, △: barb 10 r (length 10.6 mm) of a blue feather. No distal barbules were present on sections of the blue barb 0.65 and 0.8 mm from tip.

Transverse sections of barbules. The plane of section is perpendicular to the barb; as the barbules insert on the barb at an angle of 25–30° (colour plate, figs. 3–5) the barbules are sectioned at an angle of 60–65°.

The barbules attaching basally on the barbs are of the typical interlocking type, and no difference exists between those of green and blue feathers. In contrast to this, the barbules attaching terminally on the barbs, have quite different cross-sections according to whether they are from blue or green feathers (text figs. 8 & 9). These are described in some detail in the electron microscopical section (p. 29).

Only melanin-free sections of barbules of green feathers fluoresce. Barbules attaching terminally on blue barbs do not fluoresce.

Text fig. 10 shows maximum height of distal barbules as a function of their distance from tip of barb. The height was measured as the shortest distance from obverse to reverse edge, also in those instances where the reverse portion of the bar-

bules is bent towards the barb (text fig. 8). Text fig. 10 shows that height of barbules of a blue barb is much less than that of green barbs in the terminal 5 mm of the barbs, whereas there is no striking difference between the barbules of the basal portions of the barbs. Neither are barbule heights of dark green back and green belly feathers appreciably different. Text fig. 10 gives height of distal barbules only, since measurements showed that there is no consistent difference in heights of distal and proximal barbules from the same barb.

Electron Microscopy

Barbs.

a. Cortex.

Blue barbs (figs. 4–6). The cortex is made up of cells, which in transverse section appear flattened (figs. 4, 5). They are 4 to 20 (or more) μ wide and 0.3–1.2 μ thick. 3–7 cell layers may be present, mostly 5–6. In longitudinal sections the cells are found to be spindle-shaped and much elongated (fig. 6). The cells are too long to be present on the photographs in their entire length, but they are nearly always longer than 60–65 μ.

In the cell boundaries (fig. 5) a narrow gap (200–250 A wide) separates the two cell membranes. In this gap a central dense band (80–120 A wide) is generally present; it may be absent locally, however. On some photographs (not shown) a subdivision of this central band in two (or three) lamels (c. 30 A wide each) separated by a narrow gap (c. 30 A wide) can be observed. Total width of the cell membrane complex varies between 250 and 400 A. The structure closely resembles that described for other feathers by Filshie and Rogers (1962) and Schmidt and Ruska (1963) except that these authors do not mention a fine structure of the central band.

In the inner cell layers cell boundaries are comparatively smooth, whereas the boundaries between the cells of the outermost layer are more irregular (figs. 4 and 8). Cell boundaries become exceedingly irregular and complex laterally where the barbules insert (fig. 5) and circular cell profiles are often observed in this region indicating that finger-like projections extend from the cells.

Cell boundaries between the cortex and the medullary cells are smooth except that the cortex cells project a little in between the medullary cells (fig. 5, transverse section, the projections are not observed on longitudinal sections).

Between the keratin constituting most of the cell content a denser substance is found. In transverse sections it appears as an irregular network (fig. 4) or bands parallel to the perifery of the barbs (fig. 5). In longitudinal sections it appears as irregular bands (fig. 6) which diverge where a barbule inserts so that part of them becomes oriented in the longitudinal direction of the barbule. On the outer surface cells a dense, 0.04–0.07 μ thick, epicuticle (Philip *et al.* 1951) is found (fig. 4). The surface cells are further distinguished by their greater content of the dense material compared to the inner cortex cells (figs. 4 & 6). Filshie & Rogers (1962) and Schmidt

& Ruska (1963 a) referred to this dense material as non-keratinized cytoplasm. It seems not very likely to me, however, that the outermost barb cells and also the barbule cells (p. 29) which are mostly subjected to wear, rain etc. should be the cells which showed the most incomplete keratinization. The dense material should rather be considered indicative of a specialized keratinization of these cells.

Occasionally, small clusters of melanin granules as those found in the medullary cells occur in the inner parts of the cortex.

Green barbs (figs. 8–9). The thickened obverse cortex of green barbs is dominated by the presence of an extensively distributed, electron-dense material (fig. 8). When the yellow pigments of green barbs are extracted with pyridine and hydrochloric acid, the electron-dense material of the obverse cortex disappears (fig. 9). This, and the fact that the material is present in a much higher concentration in the surface layer than in the cortex proper (fig. 8), shows, that the material is the non-fluorescent yellow pigment (compare p. 14).

In the surface cells of the thickened obverse cortex the pigment occurs as a dense network. In the next cell layers the network is much more open, and in the innermost cells the pigment appears as irregular bands in the central parts of the cells, parallel to the periphery of the barbs. Laterally and reversely, where the cortex cells flatten, the network-like distribution of the pigment in the surface cells is retained, whereas only the bandlike distribution of the pigment is observed in the inner cell layers.

No granular structure can be observed in the pigment, neither is the pigment sharply delimited from the surrounding keratin.

The distribution of the pigment in the cells (network-like in surface cells, bandlike in inner cells) resembles the distribution of the electron-dense material in unpigmented cortex cells of blue barbs (fig. 4). This indicates that the pigment is primarily present in these zones, thus being distributed between the keratinized portions of the cells (*interfascicular* and possibly also *intramoenial* distribution, Schmidt and Ruska 1963 b). A similar distribution of the pigment turacin was found by Schmidt and Ruska (*op. cit.*) in barbs of *Turacus leucotis*.

The number of cell layers (mostly 4) is similar to that found in blue barbs. Thus the thickening of the cortex of green barbs relative to the cortex of blue barbs is not caused by an increase in the number of cell layers, but by an increase in thickness of the cells. In particular, the outer cell layers are thicker ($\rightarrow 6\ \mu$, mostly c. $4\ \mu$) obversely and correspondingly narrower ($\rightarrow 0.8\ \mu$, mostly $2–3\ \mu$). The remaining cortex cells are intermediate or similar to the type described for blue barbs, only they are somewhat thicker ($0.7–2.1\ \mu$) on an average.

b. Medulla.

The medulla is built of rather large, regular cells. The cells "stand" on the cortex and stretch towards the central parts of the barb. In cross-section (parallel to the tangent plane of the surface of the barb) they appear regularly polygonal (fig. 16),

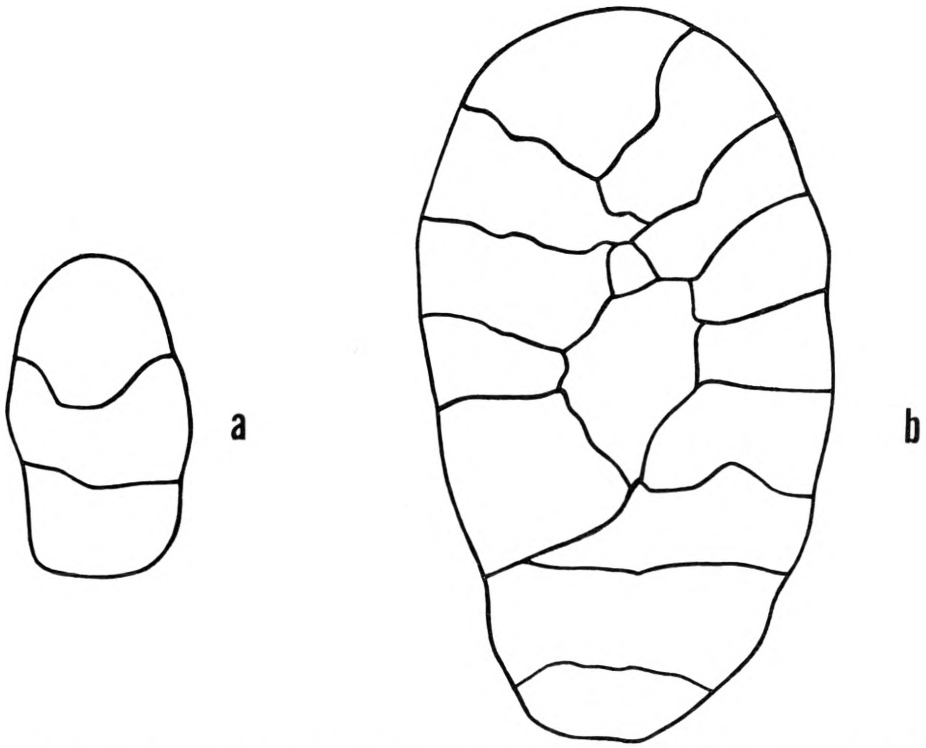

Fig. 11. Diagram showing arrangement of medullary cells in transverse sections of (a) green barb terminally of dark green back feather and (b) blue barb terminally. Drawn from photographs shown as figs. 7 and 10.

length (along main axis of barb) mostly varying between 8 μ and 14 μ. Their height (from the cortex to the central medulla) is mostly 12–17 μ.

The greater peripheral portion of each cell is entirely filled with a distinctive spongy structure (figs. 7, 10 & 16) which is responsible for the production of blue colour. More centrally in the cell a layer of melanin granules is found, and still more centrally the spongy structure becomes irregular, small air-filled spaces appear and a great vacuole is generally observed (fig. 10). No simple, air-filled medullary cells are present.

Terminally in blue barbs, where the cross-section is broad, elliptical, a few central cells (c. 10 μ wide), apparently without connection with the cortex, are often present (fig. 10). Where the medulla is smaller and narrower as in green barbs and basally in blue barbs all cells touch the cortex, and often a cell stretches from one lateral wall to the other (fig. 7). In the latter case vacuoles and melanin granules are found in the middle of the cells, so that the same picture of a peripheral layer of a spongy structure surrounding a central core of vacuoles as found in the broad elliptical barbs, is observed. Arrangements of medullary cells are shown diagrammatically in text fig. 11.

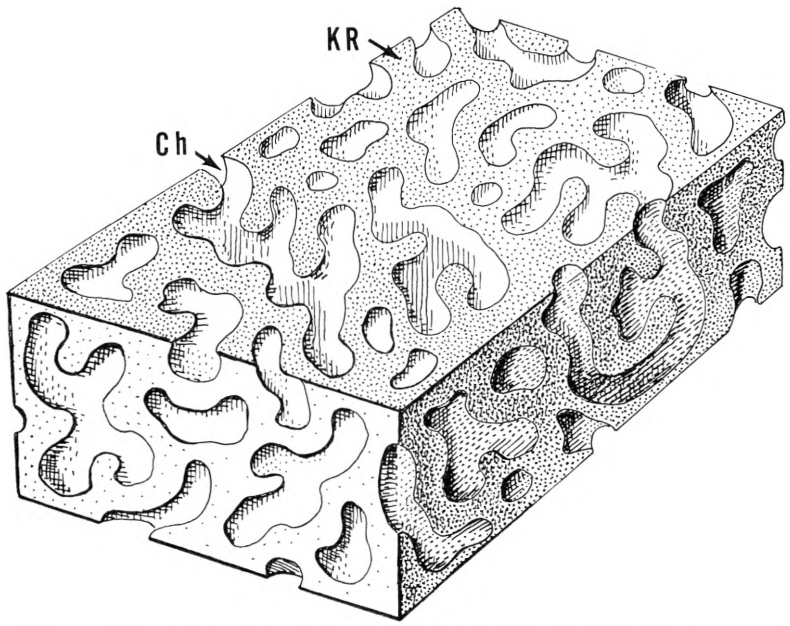

Fig. 12. Schematic drawing of spongy structure with air-filled channels (Ch) between keratin rods (KR). Note approximately constant widths of rods and channels and lack of preferred orientation.

Terminally, only a single medullary cell, approximately circular in transverse section (diameter c. 9 μ) is present (observed only in green barbs).

The homogeneous, peripheral layer of a spongy structure varies in thickness. In blue barbs it is mostly 6–8 μ thick, but reduces basally near the shaft to 3–5 μ. In green barbs it is mostly 3–5 μ thick, but decreases at the tip where only a single medullary cell is present, to 1–3 μ.

Spongy structure (figs. 11, 12 & 13). The structure consists of irregularly bent and oriented keratin rods separated by air-filled spaces of similar shape. The spongy structure shows no preferred orientation in relation to the main axis of the barb as can be seen by comparing transverse and longitudinal sections (figs. 10 & 16). On each section a number of approximately circular or somewhat elliptical keratin profiles are observed. The diameter of these correspond roughly to the width of longitudinally sectioned keratin rods. This shows that the keratin rods are nearly circular in cross-section. Similarly, circular cross-sections of air-filled channels surrounded by keratin are present on the sections indicating that the air-filled space consists of channels, also oriented in all directions. Thus, there is no difference in the shape of the keratin-filled and the air-filled spaces, air-filled spaces surrounding keratin rods as keratin tubes surround air-filled channels, the dominating feature being the fairly constant width of rods and channels (text fig. 12). Cross-wise sectioned channels appear to be somewhat more numerous than cross-wise sectioned isolated rods, compare Table 3. A typical feature is the many sharp bends of the rods and

TABLE 3.

Mean width of keratin rods and air-filled channels of spongy structures of blue and green barbs calculated from different photographs. Each set of values corresponds to a photograph. Values in brackets are from photographs of different parts of the same section.

Blue spongy structure					Green spongy structure				
Keratin rods		Air-filled channels		Rod width: Channel width	Keratin rods		Air-filled channels		Rod width: Channel width
Mean (nm)	Number of measurements	Mean (nm)	Number of measurements		Mean (nm)	Number of measurements	Mean (nm)	Number of measurements	
99.6	43	97.2	44	1.02	116.0	40	95.0	43	1.22
98.8	32	110.0	54	0.90	128.0	40	105.8	32	1.21
91.2	34	118.8	60	0.77	125.0	45	111.6	35	1.12
112.8	30	100.0	37	1.13	126.2	45	112.6	32	1.12
91.4	14	116.2	32	0.79	118.0	27	100.8	56	1.17
105.0	25	129.6	40	0.81	107.8	29	100.0	38	1.08
101.6	38	98.4	36	1.03	107.2	31	101.0	39	1.06
102.4	25	121.4	16	0.84	108.8	21	107.2	44	1.01
102.4	23	120.0	54	0.86	110.0	8	110.0	16	1.00
107.0	26	124.8	26	0.86	90.0	6	100.0	17	0.90
88.8	37	122.0	42	0.73	123.0	20	97.0	38	1.27
103.6	50	117.0	33	0.89	127.2	25	102.4	51	1.24
101.0 ± 18.4		114.0 ± 24.6		0.89	119.4 ± 22.0		103.6 ± 19.5		1.15

All values combined: Arithmetic mean $\pm$ st. dev.

channels. The rods and channels are never long in the sections, but bend frequently and sharply, often at right angles. Not even along the cell boundaries do channels or rods of greater lengths occur. Characteristic are the cross-sections of the keratin tubes with air-filled cores (hollow cylinders) (fig. 12).

There is a difference in dimensions of the spongy structure of blue and green barbs (cp. figs. 11 & 12). In the green structure the keratin rods are wider, and the air-filled channels narrower than in the blue structure. Table 3 gives mean values.

Samples from which means have been calculated are with good approximation normal distributed.

For measuring widths copies enlarged to 50,000 times were made from plates taken at 20,000 times. Diameters of all circular and shortest axis of all elliptical sections of rods and channels were measured to the nearest whole mm. Widths were measured not only of rods or channels completely surrounded by the other medium, but also such that were in connection with their own medium, but protruded into the other medium (figs. 11 & 12). Several pictures taken of different sections of each kind were measured in order to eliminate intercellular variations and variations due to varying thickness of the sections. The thickness of the sections in many instances

make the boundaries between air and keratin indistinct in which case the decision as to whether a given width was, say 5 or 6 mm, might be rather arbitrary. I avoided bias due to this factor by arranging it so that I did not know to what type the picture belonged when I measured it. Sections of the two types of barbs were treated in exactly the same way as regards staining, and all exposures in the electron microscope were taken within a few hours.

Values given in Table 3 are not determined with the accuracy with which they are given (0.1 nm), since no calibration of the magnification of the electron microscope was carried out. But as relative values they probably are fairly exact.

Another source of error in determining widths could be alterations due to contraction or swelling of the keratin during the embedding procedure. Such alterations do not seem to have occurred to any significant degree, however, as the colour of the embedded barbs appears identical to the original one as far as can be judged when looking through the yellowish epon.

Table 3 shows that the keratin rods of the green spongy structure are 18.4 nm wider than those of the blue spongy structure and the channels of the green spongy structure 10.4 nm narrower than those of the blue spongy structure. The differences are statistically significant ($p < 0.002$ for the difference in rod widths, $p \simeq 0.02$ for the difference in channel widths ($n_1 = 12$, $n_2 = 12$)).

The variances are significantly different (F-test), so a simple t-test could not be used for testing the difference. Instead, a non-parametric test (MANN-WHITNEY U-test, SIEGEL 1956) was used.

In addition to average widths, fractions $\frac{\text{rod width}}{\text{channel width}}$ are given in Table 3. This fraction is generally less than 1 (mean: 0.89) in blue spongy structures and more than 1 (mean: 1.15) in green spongy structures. It is evident from Table 3, however, that this fraction varies considerably for both types of spongy structure, and there is overlapping as there is overlapping in mean widths of both rods and channels from the two types of spongy structure. Table 3 also shows that these variations occur between spongy structures from different portions of the same sections, which indicates intercellular variations rather than regional differences along the barb.

With reference to the discussion of the physical explanation of colour production by the spongy structure it is of interest to find out also whether the rods and channels may be considered to have a fairly constant length. Consider a sample of randomly tilted cylinders of diameter d and length l ($l > d$). It is easily perceived that in a given plane the sections of these cylinders will show a distribution of lengths with two peaks, one corresponding approximately to d and one to l, the latter peak being lower relative to the former, the higher the ratio $\frac{l}{d}$ is. Text fig. 13 shows the distribution of rod lengths as measured on a section of the spongy structure of a blue barb.

The histogram shows a very wide distribution of lengths with peaks at 4 and

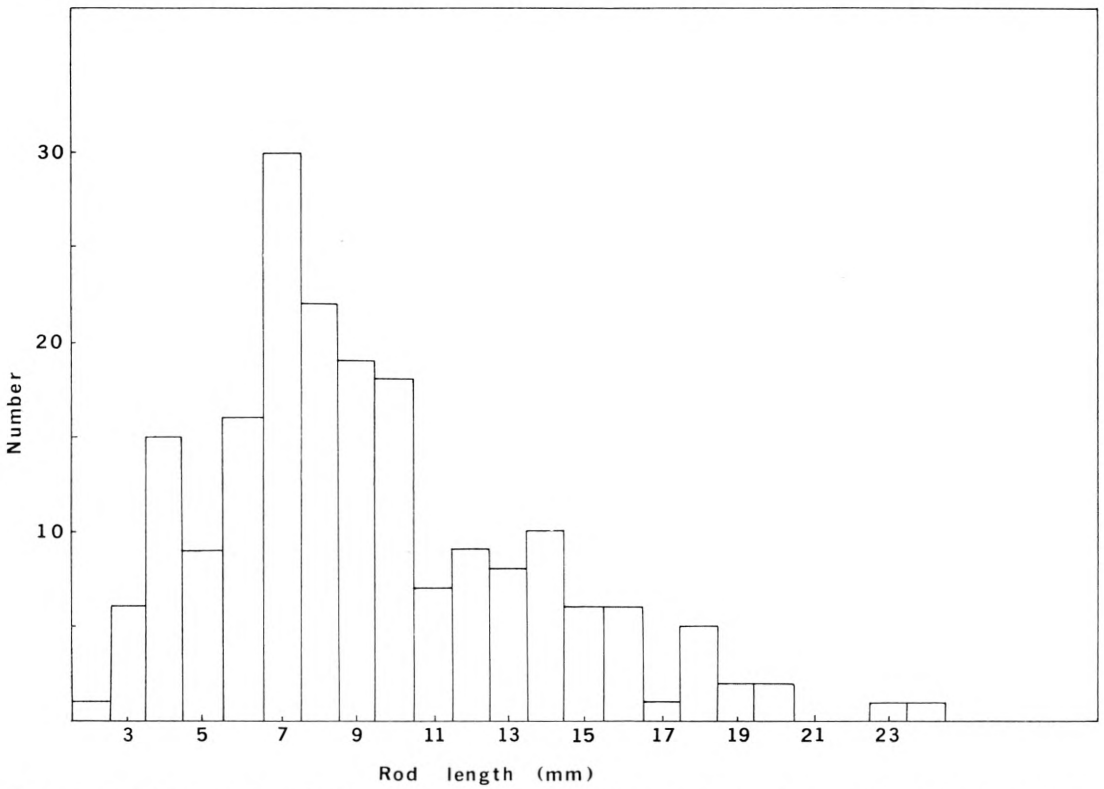

Fig. 13. Distribution of rod lengths as measured on a section of spongy structure from a blue barb. 3 mm $\simeq$ 100 nm.

7–8 mm and a shoulder at c. 13 mm. The peak at 7–8 mm should correspond to rod length. As a first approximation the rods may therefore be considered as cylinders with length c. 250 nm ($\simeq$ 7–8 mm), equal to $2^1/_2$ times diameter. It is evident from the histogram, however, that the rod length varies considerably and a glance at the photographs of the spongy structure makes it clear that it is a somewhat dubious affair to treat the irregularly bent keratin rods as straight cylinders of well-defined lengths.

Measurements show that the channels may similarly be considered as cylinders with length equal to $2^1/_2$ times diameter, but again the length varies considerably.

The relatively and absolutely greater width of the air-filled channels in the spongy structure of a blue barb makes this appear more open than the spongy structure of a green barb (compare figs. 11 & 12). Some quantitative estimates of volume percentages of keratin in the two types of spongy structure were made: Air and keratin areas in the photographs were determined by cutting and weighing. From the known areas keratin volume percentages were calculated according to $\frac{(\text{keratin area})^{\frac{3}{2}}}{(\text{keratin area})^{\frac{3}{2}} + (\text{air area})^{\frac{3}{2}}} \cdot 100$. Keratin

TABLE 4.
Volume percentages of keratin in spongy structures of green and blue barbs.

Blue spongy structure		Green spongy structure	
Determined from weighed areas	Determined from rod and channel diameters	Determined from weighed areas	Determined from rod and channel diameters
48.8	45.7	54.6	59.5
45.6	47.3	52.1	62.9
39.8	46.0	59.1	62.2

volume percentages were also calculated as $\frac{\text{rod diameter}^2}{\text{channel diameter}^2 + \text{rod diameter}^2} \cdot 100$ supposing that rods and channels in one type of spongy structure are on the average of the same length and using the average figures of Table 3. Calculated values are summarized in Table 4.

Table 4 shows that in blue spongy structures the volume percentage of keratin is 40–50 compared to 50–60 in green spongy structures. Values for blue and green spongy structures based on average diameters of rods and channels are 44.0 and 57.2 % respectively. The results obtained by the two methods agree fairly well; the weighing method tends to give the lowest values.

The keratin rods stain more densely with uranyl acetate plus lead hydroxide than does the main component of the cortex cells. Density of the rods is similar to that of the dense component of the cortex cells (fig. 5). The outer parts of the rods stain more densely than the rest; this is most evident after staining with lead hydroxide alone (fig. 13).

Some, but not all, transverse sections of green barbs show that the spongy structure of the most obverse medullary cell stains more densely than the spongy structure of the other medullary cells (fig. 9). Is was mentioned above (p. 14) that in some transverse sections of *green* barbs the most obverse medullary cells did fluoresce in ultra-violet light as did the obverse cortex. This coincidence in occurrence, restricted to green barbs, makes it likely that the denser staining observed in the electron microscopical pictures is due to this fluorescing substance. DRIESEN (1953) similarly found that in some cases the peripheral medullary cells of the yellow barbs of forehead feathers of the budgerigar (*Melopsittacus undulatus*) fluoresced as the cortex cells (although to a lesser extent). This dense staining was however also observed in sections of green barbs which were treated with pyridine and hydrochloric acid to extract the yellow pigment, even though the feathers did not fluoresce after this treatment (fig. 9). A close inspection of fig. 9 suggests that not only the keratin rods of the obverse cell stain more densely, but also the intervening epon-filled areas. This might be due to the staining solution having partly extracted the fluorescing substance.

Cell boundaries (fig. 5, insets). Where the cell boundary between two medullary cells containing the spongy structure is bounded by keratin on both sides it is of the same type as described above for cortex cells, but where only air is present on the sides, the central band between the cell membranes disappear and a gap (100–300 A wide) remains (fig. 5, right inset). The cell membranes on both sides become thicker (200–300 A), and appear dense compared to the keratin. The total cell membrane complex is thicker (450–650 A) than that present between cortex cells. In some instances where a cell boundary is bordered by keratin rods on both sides it may show a number of constrictions, some 100 nm apart, where the dark central band disappears (fig. 5, left inset).

Vacuoles. (figs. 7, 10, 17). KAWAMURA (1935) and AUBER (1941) showed that during keratinization of the medullary cells the nucleus disappears leaving an air-filled cavity, the nuclear vacuole. In sections these appear to be very regular, circular or slightly elliptical air-filled cavities with a median diameter near $5^1/_2\ \mu$, corresponding to a true value of c. 6 μ. The nuclear vacuoles of laterally compressed barbs tend to be laterally compressed also (fig. 7). No more than one great vacuole per cell was ever observed.

The vacuoles are limited by a layer of keratin rods arranged with their long axis mainly tangentially. The rods are separated by air-filled spaces so that there is continuity between the vacuoles and the air-filled space of the spongy structure. The arrangement of the keratin rods and air spaces is often less regular than in the spongy structure. In particular the rods may be narrow and irregular in form (fig. 17).

Small vauoles (c. 1.3 μ in diameter) are occasionally observed in the peripheral parts of the medullary cells, occasionally near the cortex (fig. 16). One of these contained a small (c. 1 μ) compact keratinous body (fig. 16, inset).

Melanin granules. These are of ovoid to rod-shaped form, the length varying between 0.8 and 1.7 μ and the width between 0.3 μ and 0.9 μ (figs. 13, 17). Width: length ratio is mostly 1:2 (probably near to 1:1 occasionally) in the most frequent type, the ovoid. In rod-shaped granules ratio may go down to 1:3 or less.

Apart from obvious artefacts the surface of the melanin granules is regular and smooth (figs. 14, 17). Some melanin granules also appear with a slightly irregular, wavy outline (fig. 17, inset).

The melanin granules consist of substances of varying density, where the most dense material may be arranged in a dendritic way (fig. 15).

The melanin granules are predominantly found around the nuclear vacuoles, especially on the outer sides of these, thus forming a layer between the peripheral spongy structure and the central vacuole-containing medulla (figs. 7, 10). The granules of this layer are mainly arranged with their longitudinal axis in a plane crosswise to the main direction of the barb and with the long axis pointing towards the nuclear vacuoles (figs. 7, 10). Where the barb is relatively broad (terminally in blue barbs) granules are also found between the vacuoles (fig. 10). The orientation of these granules is less regular. In longitudinal sections the melanin granules are seen to form

an almost continuous layer between the spongy structure and the vacuoles (fig. 16). In this layer the granules lie very close together (0.1–0.2 μ on longitudinal sectionsI) (fig. 14). In transverse sections (figs. 7, 10) the granules are found at somewhat greater intervals (0.0–1.8 μ, median value 0.3 μ) in a tangential direction.

Each melanin granule is found in a cavity formed by the surrounding keratin rods of the spongy structure (figs. 14, 17). The shapes of the cavities correspond closely to that of the granules, the keratin rods showing smooth regular surfaces on the sides facing the granules, contrasting with the more irregular outer surfaces (fig. 14). Where granules are lying close, a single keratin rod is usually found between neighbouring cavities, but they may also become partly fused (fig. 14). The dimensions of the cavities are 1.1–2.0 times greater than the granules which they surround. A few granules are found in the nuclear vacuoles, touching the surrounding layer of keratin rods (fig. 10). There are no traces of the granules being attached to the keratin rods by any special structures.

Barbules.

Plane of section is the same as used in light microscopy (p. 19).

a. Barbules of blue feathers.

Fig. 18 shows a typical cross-section of a barbule near the insertion on the terminal portion of a blue barb: the obverse part is rounded and broader than the reverse part.

The width varies mostly between 1 and 2 μ with a median value of 1.2 μ (halfway between obverse and reverse edges). It diminishes gradually from the point of insertion where the obverse and reverse widths may attain a maximum value of 3.3 μ and 1.8 μ, respectively, to the tip of the barbules with values down to 0.6 μ.

Cell boundaries surrounding an elliptical portion of another cell show that the cells may project as cornets into each other.

The dense material in the cortex cells is present also in the barbule cells (fig. 18) and to the same extent as found in the surface cells (comp. fig. 4). The barbule cells also resemble the surface cells of the cortex by the presence of an epicuticle (fig. 18).

b. Barbules of dark green back feathers.

Yellow pigmented parts. Barbules attaching terminally on green barbs are different in cross-section from those attaching on blue barbs by being both wider and higher and lacking the rounded edges obversely and reversely, the barbules instead tapering distinctly toward an acute obverse edge (fig. 21). Barbules are generally widest near the reverse part where the width varies between 2.1 μ and 5.0 μ with a median value of 3.8 μ.

The non-fluorescent yellow pigment is distributed fairly evenly in the entire barbule section (fig. 21). Obversely, a pigment network similar to that observed in

the obverse surface cells of green barbs (fig. 8) is found. Reversely, pigment is deposited mainly in an axial strand with irregular side branches. Along the epicuticle a 0.1–0.3 μ thick zone devoid of pigment is observed. On the barbule sections lying nearest the barb the lateral side of the barbule facing the barb is usually partly devoid of pigment, mainly reversely.

Melanin pigmented parts. The melanin pigmented parts of the barbules attaching terminally on barbs of dark green back feathers show a more irregular surface than the yellow pigmented parts, but they have the same general outline (fig. 19). The tendency of the barbules to have more irregular surfaces in their terminal portions is observed in blue barbs also.

The greatest width (near the reverse end) varies between 0.8 μ and 2.7 μ with a median value of 1.2 μ.

The melanin granules are of ovoid to rod-shaped form arranged with their longitudinal axis along the barbule so that in transverse sections the circular cross-sections (fig. 19) are observed. The diameter of these varies between 0.1 and 0.4 μ with a median value of 0.2 μ. Width: length ratio is mostly 1:2 and 1:3, but may go down to 1:7 (fig. 19, inset).

The melanin granules are fairly evenly distributed over the entire cross-section of the barbule, but most pictures suggest a tendency of the reverse end to be slightly less pigmented than the obverse end. In the transition zone between the yellow and black pigmented parts of the barbules, however, the melanin granules first appear reversely (see also text fig. 7,1). No yellow pigment is found in the melanized barbule portions (fig. 20).

The melanin granules in transverse sections mostly appear in small clusters (3–6), usually forming a row in which the individual granules almost touch each other (fig. 19). These rows of granules are mostly placed along strands of the dense material. No melanin granules are found in the outermost horny layer (mostly 0.1–0.2 μ thick), therefore they never touch the epicuticle.

The melanization of these barbules is not very intense. The volume of melanin granules was found to be 9 $^0/_0$ and 16 $^0/_0$ of the total barbule volume (in melanized parts) in two estimates. Barbules of other species may show much intenser melanin pigmentation (unpublished results).

c. Barbules of green belly feathers.

These are similar to the yellow pigmented parts of barbules of dark green back feathers described above.

Microspectrophotometry

Text fig. 14 shows reflectance curves of individual barbs (diameter of field of measurement: 14.3 μ).

All four curves show a distinct peak in the blue-green region, at 480–486 nm for blue barbs (curves 1 and 2), at 524 nm for a green barb from which the yellow pigment

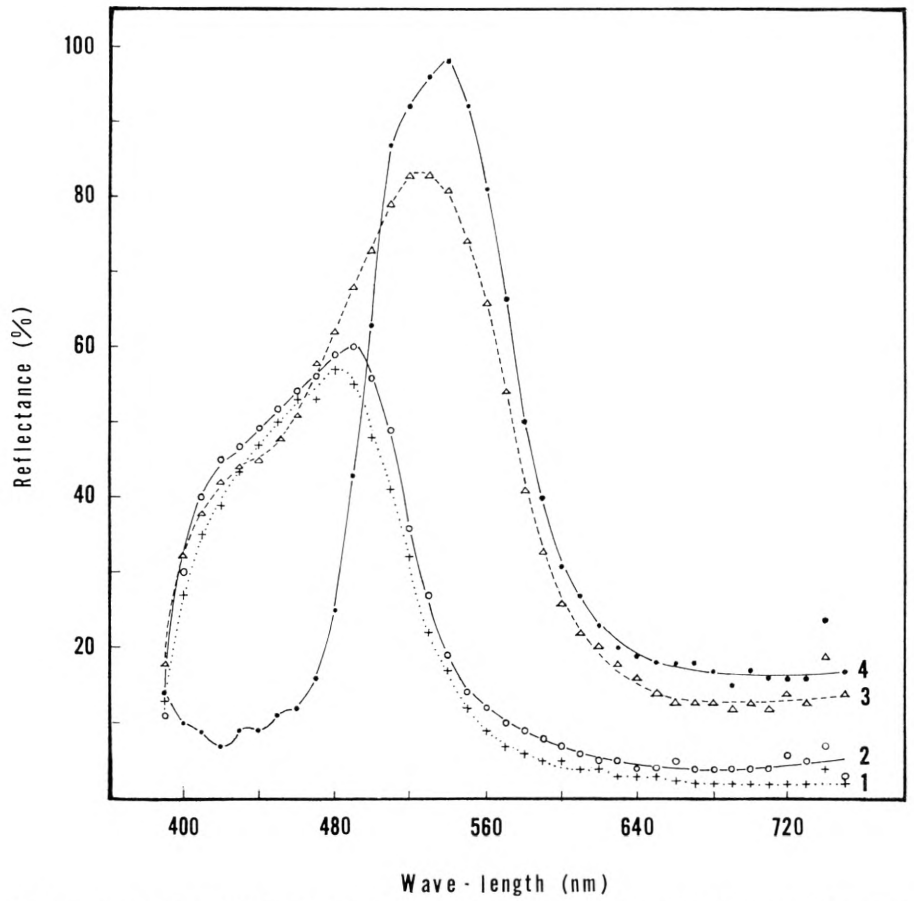

Fig. 14. Reflectance spectra of individual barbs. 1: blue barb laterally (barbules removed), 2: blue barb obversely, 3: green barb of belly feather with yellow pigment extracted, obversely, 4: green barb of belly feather, obversely.

has been extracted (curve 3) and at 538 nm for an intact green barb (curve 4). This latter curve differs from the three former in reflecting very little light of wave-lengths 390–470 nm (influence of yellow pigment). On curves 1,2 and 3 a shoulder is indicated at 420 nm.

The similarity of curves 1 and 2 demonstrate the constancy of the colour of the barb no matter whether it is observed laterally (curve 1) or obversely (curve 2).

Reflectance values of the peaks are considerably higher for green (80–100 %) than for blue (50–60 %) barbs.

The spectral distribution of light transmitted by the spongy structure was also determined (text fig. 15). For measurements 3.5 μ thick transverse, air-filled sections were used (diameter of field of measurement: 4.0 μ).

The transmittance curves of the spongy structures show a distinct minimum in the blue-green region roughly corresponding to the reflectance peak. Outside this

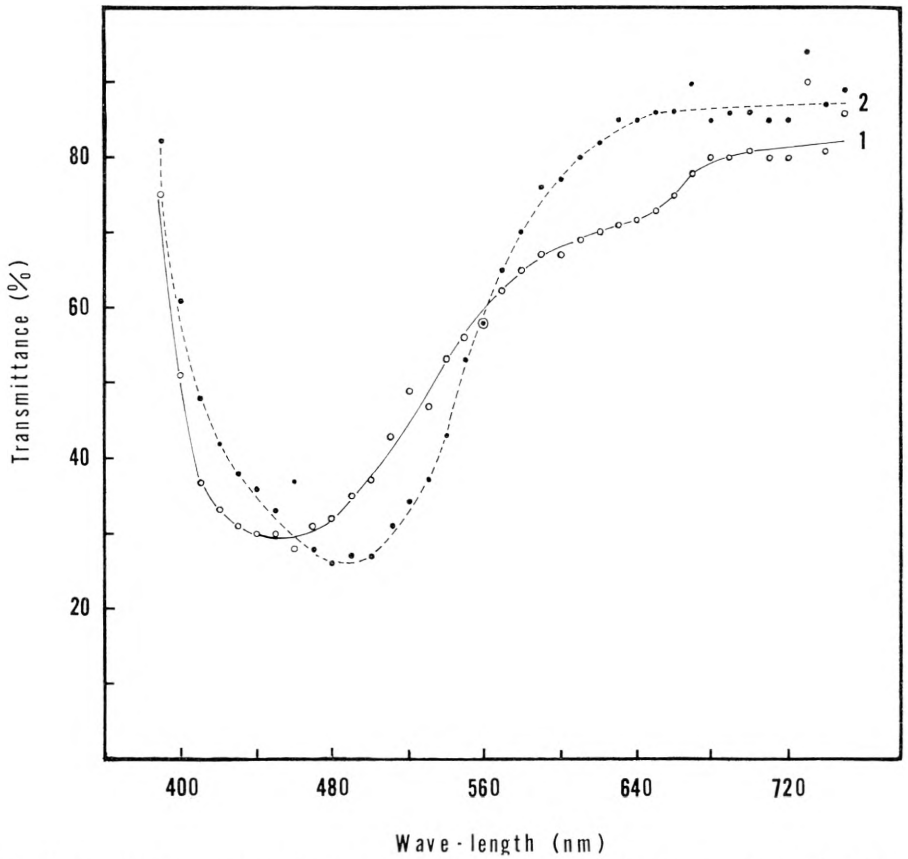

Fig. 15. Transmittance spectra of spongy structures. 1: blue barb, 2: green barb of belly feather.

minimum the transmittance is high, c. 80 % (in the violet and red ends of the visible spectrum). Transmittance values at the minimum are similar (25–30 %) for the two types of spongy structures.

The transmittance minimum of the green spongy structure occurs at a wavelength somewhat longer than that of the blue structure (Table 5). A similar difference

TABLE 5.

Reflectance peaks and transmittance minima (nm) of spongy structures of green and blue barbs.

Measurement	Blue barbs	Green barbs
Peaks of plumage reflectances (text fig. 2, curves 2 & 3)	452	482
Transmittance minima (text fig. 15)	457	488
Peaks of barb reflectances (text fig. 14, curves 1–3)	480–86	524

(c. 40 nm) is observed in the position of the reflectance peaks of blue and extracted green barbs (Table 5). The difference is also observed if comparing the "macro" reflectance curves of extracted green belly feathers and the blue rump (Table 5). Obviously this difference in the spectral distribution of reflected and transmitted light is due to the difference in the dimensions of the spongy structures of green and blue barbs demonstrated above (p. 24)

The difference in spectral composition of transmitted light of green and blue spongy structures is readily detected visually also, the structure of green barbs appearing more reddish than that of the blue barbs.

Whereas there is good agreement in the position of the transmittance minima and the reflectance peaks of the "macro" curves, the peaks of the "micro" reflectance curves are displaced c. 30 nm towards longer wave-length (Table 5). The reason is probably that light reflected in the direction of the incoming light is more long-wave than light reflected at greater angles to the incoming light. This explanation is supported by observations of a blue feather in unidirectional light: If the eyes are placed near a lamp the feather appears to be intensely turquoise-green coloured, whereas the feather appears much duller bluish violet if it is observed at right angles to or directly opposite the lamp.

For green barbs with intact yellow pigment the discrepancy in peak reflectance for the "micro" and "macro" curves is much less (538 resp. 530 nm).

The cause why green barbs reflect more than blue barbs is not quite clear. The difference might be due to a difference in specular reflection (gloss) from the outer surface of the barbs (seen under the microscope as irregular whitish stripes) due to differing orientation of barbs during measurements. This, however, cannot be the whole explanation, as gloss would tend to increase reflectance with the same amount throughout the whole spectrum and that is not the case for the spectrum of the green barbs. The difference is probably not attributable to the difference in the dimensions of the two types of spongy structure, since these show similar transmittance spectra and consequently similar reflectance spectra, as the relation: $^0/_0$ reflectance $= 100 \div {}^0/_0$ transmittance must be valid (absorption being negligible). The difference may perhaps be explained by the more intense melanization of the blue compared to the green barbs (compare figs. 7, 10).

Text fig. 16, curve 2 shows the transmittance spectrum of the yellow pigmented obverse cortex of a green barb. Transmittance is fairly constant (c. 70 $^0/_0$) between 520 and 750 nm and diminishes between 400 and 500 nm due to absorption by the yellow pigments with lowest transmittance at 430 nm. The spectrum is not detailed enough to show the absorption bands present as reflectance minima on text fig. 2.

Transmittance spectra of individual barbules (lying on their "flat" sides) are presented on text fig. 16 also. Thickness of the barbules measured is not known, but must be of the order 1–4 μ (compare values given on p. 29 and 30, here as widths). A whitish, transparent barbule from the terminal part of a blue barb transmits (curve 4) very nearly 100 $^0/_0$ at 500 nm and shows slightly falling transmittance towards both ends of the spectrum. A yellow pigmented barbule from a green belly feather shows

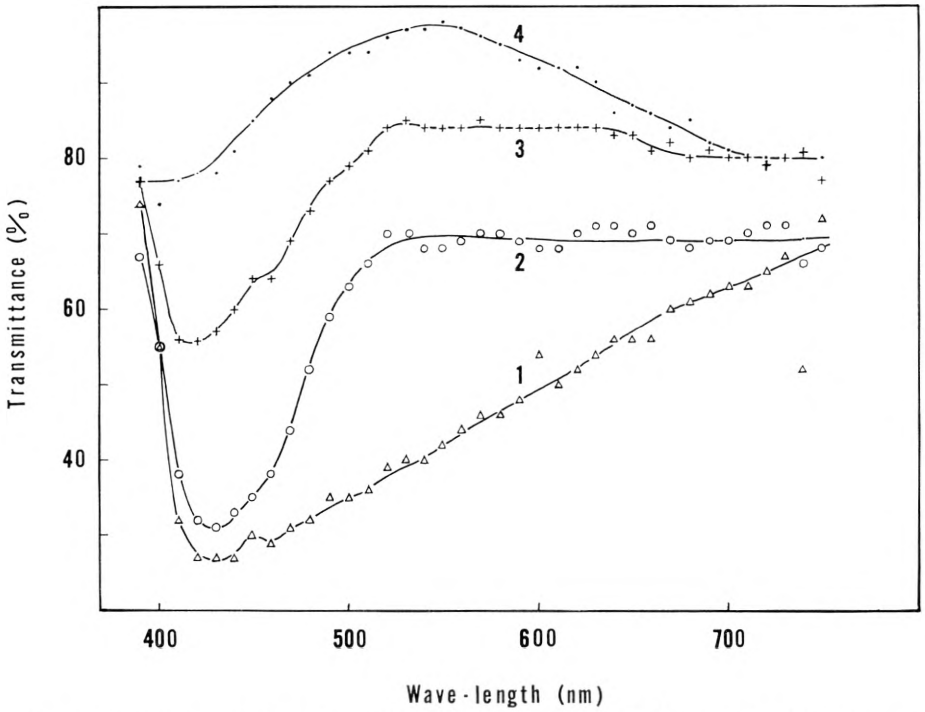

Fig. 16. Transmittance spectra: 1: melanized portion of barbule of dark green back feather, 2: obverse yellow pigmented cortex of barb of green belly feather, 3: Yellow pigmented barbule of green belly feather, 4: unpigmented barbule from terminal part of blue barb. Diameter of field of measurement 10 μ, except in curve 4, where it is 6.25 μ.

a transmittance spectrum (curve 3) similar to that of the obverse yellow cortex of a green barb. The only difference is that the transmittance in the entire spectrum is somewhat higher which indicates less yellow pigment than in the obverse cortex. This is also apparent from the electron microscopical pictures (figs. 8 & 21). Transmittance of the melanin pigmented part of a barbule of a dark green back feather decreases steadily with decreasing wave-length except for a high transmittance at 390–400 nm. This agrees well with black, grey and dark brown melanin pigmented plumage areas showing similar reflectance spectra (Dyck 1966), and the visual impression of the melanin granules being black (or dark brown). The increasing transmittance from 410 to 390 nm is not in accordance with melanin pigmented plumage areas which usually show a low reflectance in this region. This rise may be due to the well-known bluish fluorescence of keratin.

Spectrophotometry of yellow pigment

Text fig. 17 shows a transmittance spectrum of a solution of the yellow pigments in a mixture of pyridine and hydrochloric acid. Further on text fig. 17 a theoretical and an observed transmittance spectrum of the yellow pigments in keratin is shown.

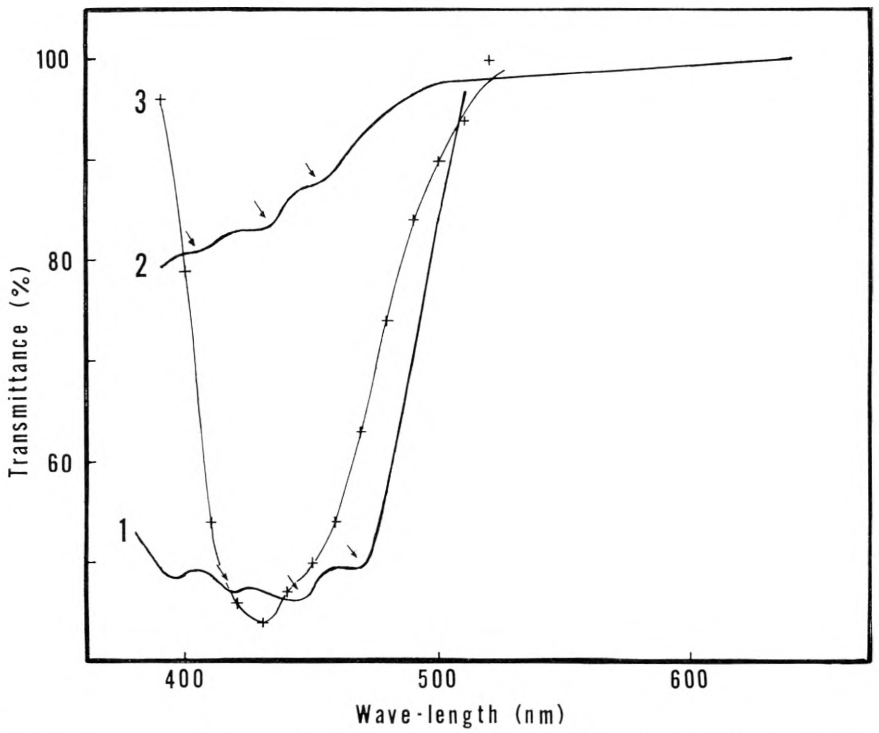

Fig. 17. Transmittance spectra of yellow pigments. 1: In keratin, calculated from reflectance spectra compare text, 2: Dissolved in pyridine-hydrochloric acid, 3: In keratin, observed spectrum (corrected compare text). Arrows point at absorption bands.

The theoretical spectrum was calculated from reflectance curves 3 and 4 (text fig. 2), supposing that reflection from green belly feathers (R_4) with yellow pigments intact is equal to $T^2 \cdot R_3$, where T is transmission of the yellow pigmented keratin and R_3 reflection from green belly feathers with yellow pigments extracted, hence $T = \sqrt{\frac{R_4}{R_3}}$ (calculated in the spectral region 380–500 nm only). Light scattering has not been taken into account. The real transmittance spectrum is the same as shown in curve 2, text fig. 16, only corrected so that transmittance in the region 500–750 nm is equated to 100 % instead of to 70 % as found, and transmittance in the remaining part of the spectrum corrected proportionately. This correction was made on the assumption that the lowering of transmittance from 100 to c. 70 % in the region 500–750 nm is mainly due not to absorption by keratin or yellow pigment, but to refraction caused by irregularities in the cortex, irregularities which presumably were much greater in the cortex used for measurement (it was simply pulled off the barb) than in the intact cortex. The correctness of this assumption is supported by the fact that these irregularities appearing as dark stripes almost disappeared when the isolated cortex was placed in a liquid with refractive index similar to that of keratin.

Text fig. 17 shows that there is a general resemblance between theoretical and observed transmittance spectra of yellow pigmented keratin. The observed spectrum does not show the absorption bands present on the theoretical curve, presumably

because of poor spectral resolution of the microspectrophotometer. The only major difference between the two spectra is the strong rise in transmittance between 410 and 390 nm observed, but not calculated. A possible explanation is, as for the melanized barbule, that this rise is due to bluish fluorescence of the keratin.

The general shape of the transmittance spectrum of the solution does not agree with the theoretical spectrum, presumably due to impurities in the solution. As already mentioned, I did not succeed in purifying the solution by transferring the pigment to another solvent.

The transmittance spectrum of the solution shows increasing absorption with decrasing wave-length and three inflections at c. 457, 430 and 409 nm (arrows). It seems reasonable to suppose that these inflections correspond to absorption bands at 468, 441 and 419 nm (arrows) on the theoretical curve (and to reflectance minima at same wave-lengths on reflectance spectra, comp. text fig. 2). This would mean that absorption bands are shifted 10–11 nm towards longer wave-lengths if the pigment is present in keratin instead of in solution. This appears likely as a similar shift was observed also for other feather pigments, e.g. carotenoids (Völker 1934, Dyck 1966), turacin (Keilin 1926) and non-fluorescent yellow and red pigments of parrots (Völker 1942).

The absorption bands are probably due to the non-fluorescent yellow pigment of the feathers, as parrot pigments of this type generally show one or several distinct bands (Völker 1942) whereas the fluorescent yellow pigments of parrots do not show such bands (Völker 1937).

The absorption of the fluorescing yellow pigment in the blue region, and thereby its influence on the reflectance spectra seems quite insignificant. This follows from 1) the observation that in unstained barb sections only a yellow colouring, corresponding to the distribution of the non-fluorescent pigment can be detected (comp. p. 14 and Völker 1937), and 2) the fact that green belly feathers of *Melopsittacus undulatus*, the colour of which closely matches that of green *Agapornis* belly feathers, does not contain fluorescing yellow pigments (Völker 1936, personal observation), yet the two types of feathers show almost identical reflectance spectra (Dyck 1966 and text fig. 2 this paper). The only significant difference between the two spectra is, that the *Melopsittacus*-curve shows increasing reflectance in the region 380–420 nm (Dyck 1966), which the *Agapornis*-curve does not show (text fig. 2). This indicates that the fluorescent yellow pigment of *Agapornis* absorbs somewhat in this region, whereas its absorption in the remaining part of the blue and blue-green spectrum is insignificant compared to that of the non-fluorescent pigment.

The yellow fluorescence of the green *Agapornis* feathers vanished if they were treated with the pyridine-hydrochloric acid mixture (cp. Schmidt 1961), and the solution did not fluoresce. It is therefore uncertain whether the solution contained the fluorescing pigment.

Reflection minima of green *Melopsittacus* feathers are closely similar to those of *Agapornis*, i.e. 469, 443 and 419 nm (Dyck 1966). This suggests that the same non-fluorescent pigment is present in both species.

Optical Properties and Structure

The former chapter presented details about structure and reflectance and transmittance properties. In the present chapter an attempt is made to combine the details from an optical viewpoint. How do the differently shaped and coloured barbs and barbules interact in order to produce the overall colour, represented by the reflectance spectra presented in text fig. 2? The question is considered by comparing 1) blue and green feathers, and 2) light and dark green feathers. Principally, the terminal parts of the barbs with their barbules where the structural differences are most pronounced, are considered.

The differences between green belly and blue feathers are summarized in Table 6.

TABLE 6.
Difference in structure and pigmentation between green belly and blue feathers.

Character	Feather type	
	Blue	Green belly
Barb Size and form of cross-section ..	Great, oval	Smaller, triangular to pear-shaped
Cortex: thickness	Thin, 2–5 μ	Thickened obversely, 10–15 μ
Cortex: pigmentation.....	Unpigmented	Yellow pigmented obversely and laterally
Spongy structure.....	Open, thin rods and wide channels, λ_{max} ~ 450–60 nm	Denser, thicker rods and narrower channels, λ_{max} ~ 480–90 nm
Barbule Size and form	Short, low and comparatively broad. Rounded obversely	Long, high and comparatively narrow. Tapering obversely
Pigmentation.....	Unpigmented	Yellow pigmented

The wave-length difference of reflectance peaks of blue and green feathers is c. 80 nm (peaks at c. 450 and c. 530 nm, text fig. 2). This difference is partly accounted for by difference in dimensions of spongy structures, which causes reflectance peaks of green barbs to be shifted c. 30–40 nm towards higher wave-length compared to those of blue barbs (Table 5). The remaining difference of c. 50 nm is due to influence of the yellow pigments. A calculation was made to check this.

Reflectance in an upward direction from a green barb is equal to reflectance of the spongy structure multiplied by the square of transmittance of the yellow pigmented obverse cortex (light scattering from cortex not taken into account). Percent reflection from the spongy structure of a green barb with good approximation is equal to $100 \div \%$ transmission of the spongy structure, assuming that the absorption by keratin is negligible. The "inverted" transmittance spectrum of text fig. 15 has been used (the thickness of the layer of spongy structure in the section used for measurement (3.5 μ) corresponds roughly to the thickness of the reflecting layer in the intact barb (3–5 μ)). Transmittance values of the obverse cortex corresponding to the corrected curve 3 (text fig. 17) were used. Text fig. 18 compares the calculated spectrum with the "inverted" transmittance spectrum.

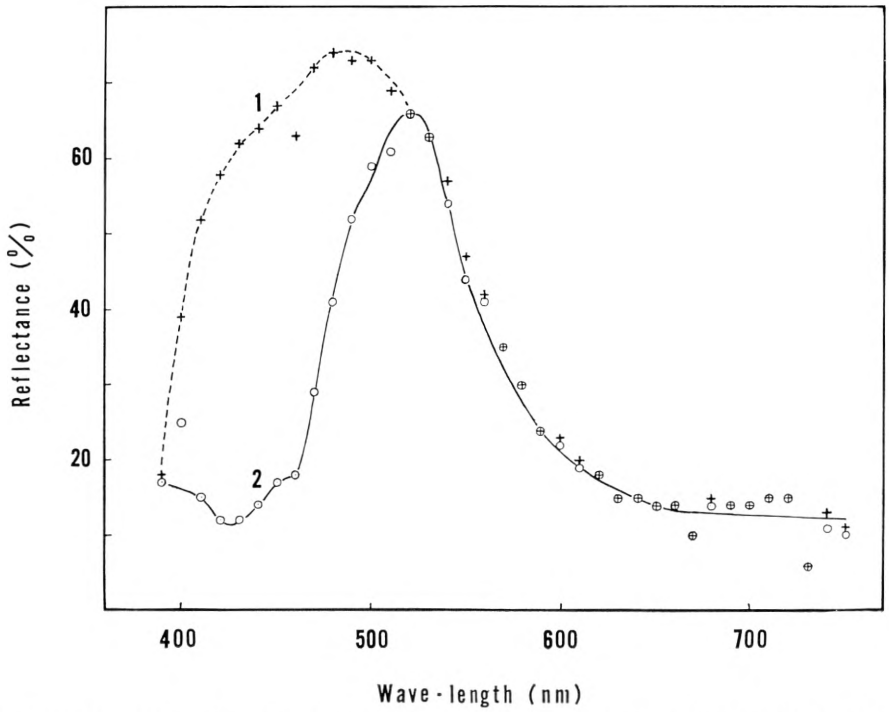

Fig. 18. Reflectance spectra of spongy structure of green barb without (1) and with overlying yellow cortex (2). Note that the yellow cortex shifts reflectance peak from 480–90 to c. 520 nm and lowers reflectance between 400 and 500 nm.

Text fig. 18 clearly shows the influence of the yellow pigment: reflectance between 4- and 500 nm diminishes greatly, and reflectance peak shifts c. 30 nm towards higher wave-lengths (from c. 490 to c. 520 nm). Thus the two factors (dimensions of spongy structure and yellow pigment) in a quantitative way account for the colour difference observed between blue and green barbs.

The influence of the barbules (their pigmentation, size and form) on the total overall plumage colour is more difficult to assess in a quantitative way, but some considerations are set forth. If one looks at a feather from above, areas of barbs and barbules corresponding to their horizontal projections are seen. Thus–as a first approximation–one may estimate the relative importance of barb colour compared to barbule colour by comparing the relative sizes of barb and barbule areas projected horizontically. Table 7 compares such values for the three types of feathers.

Values were determined from sections like those shown in text figs. 8 and 9. Values are given as relative areas (barb area + barbule area = 1.00).

Table 7 illustrates the dominating role of barb colour in blue feathers (relative horizontal barb area 0.54) compared to green feathers (relative horizontal area of barb of green belly feather 0.19) due to increased barb size and reduced barbule size in blue feathers.

TABLE 7.

Relative size of horizontically projected areas of barbs and barbules of green and blue feathers. Green belly feather: Barb 17 r (total length: 13.2 mm) with barbules 3.9 mm from tip, dark green back feather: Barb 10 l (total length: 10.5 mm) with barbules 3.2 mm from tip, blue feather: Barb 10 r (total length: 10.6 mm) with barbules 3.0 mm from tip.

Feather type	Horizontically projected area				
	Barb	Barbule			
		Unpigmented	Yellow	Melanized	Total
Green belly.....	0.19		0.81		0.81
Dark green back	0.20		0.35	0.45	0.80
Blue.....	0.54	0.47			0.47

Plumages show reflectance peaks which are reduced both relatively and absolutely compared to those of corresponding barbs (text fig. 19). How much of this reduction in reflection that is due to absorption by barbules is difficult to assess, because it is unknown what influence the complicated spatial distribution of the barbs and barbules, lying in several layers under each other, has on plumage reflection.

Clear evidence of the influence of barbules on plumage colour is given by comparing dark and light green feathers. These two types seem to be identical as regards barb form and colour and barbule form. The only safe difference is the melanization of the terminal parts of the barbules. This reduces reflectance of the dark green back to slightly less than half the reflectance of the green belly (0.45–0.50 between 380 and 580 nm, 0.41–0.42 between 590 and 740 nm; text fig. 2). This agrees fairly well with the relative horizontally projected area of melanin pigmented barbules to be close to one half (exactly 0.45, Table 7), if reflectance of melanized barbules is set equal to zero, and their relative importance for plumage reflectance is set equal to their relative horizontally projected area (additive colour mixture, JUDD & WYSZECKI 1963).

It is strange that reflectance of the blue rump is so high compared to that of the barb in the short-wave region of the spectrum. A gradual decrease would be expected in concordance with the barb spectrum, but actually the spectra cross each other at 400 nm.

There is a difference in the attachment of the barbules terminally on the barbs, which is of importance for the interaction of barbs and barbules in the colour production. On blue barbs the transparent barbules are placed so far towards the reverse part of the barb and they are so small in cross-section that they interfere to a very small extent with light reflected laterally from the barb (text fig. 9). In contrast the yellow pigmented barbules of green barbs are placed in such a way that a fair amount of the light reflected from the lateral portions of the barb, where the yellow pigmentation is less intense than obversely, transverses the yellow pigmented barbules (text fig. 8).

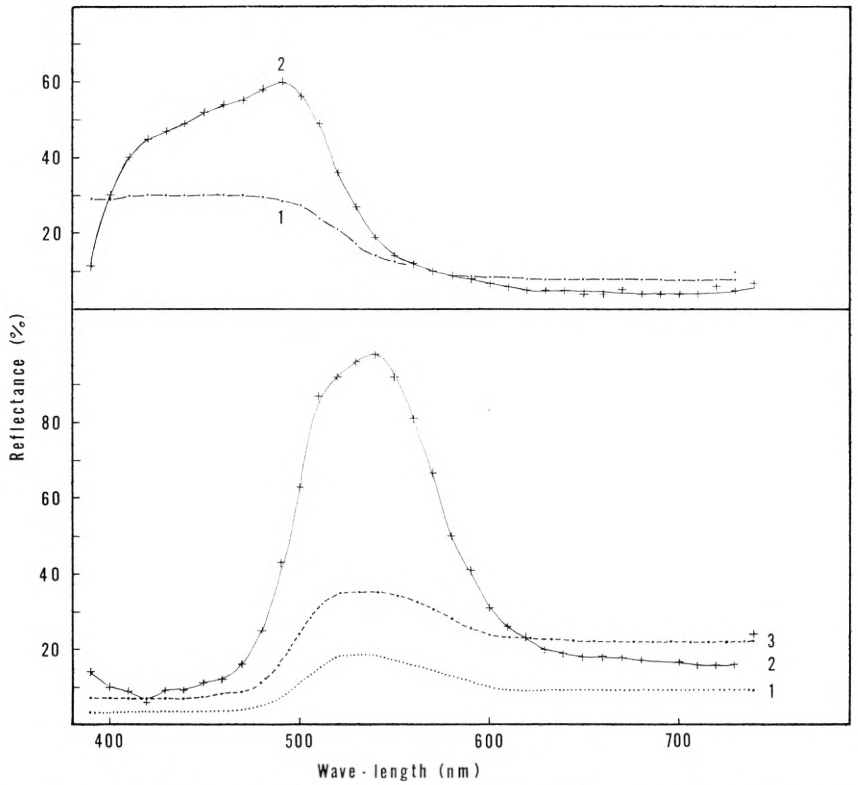

Fig. 19. Comparison of reflectance spectra of individual barbs and corresponding plumage areas. Above: 1: blue rump, 2: blue barb. Below: 1: dark green back, 2: green barb, 3: green belly.

The tapering obverse edge of yellow pigmented barbules contrast with the rounded obverse portion of unpigmented barbules of blue barbs. The tapering obverse edge of yellow barbules must result in a much less specular reflectance in an upward direction than from the rounded barbules of blue barbs (text fig. 20). This agrees with the visual impression (compare also figs. 3, 4 and 5 (colour plate)).

Another factor of importance for gloss is the twisting of the barbules so that these more or less lie on their flat (lateral) sides. A comparison of text fig. 8 and 9 suggests that such twisting is more pronounced for barbules of blue barbs than for barbules of green barbs, which enhances the difference in gloss from the two types of barbules.

Another fact which should be considered in relation to the production of the overall colour is the gradual decrease in yellow pigmentation from the distal to the proximal parts of green feathers (p. 14). To consider the barbs: Light which reaches the barbs of the proximal part of the feather has partly been transmitted through the yellow barbules of the distal part of the overlying feather. Hence this light is more

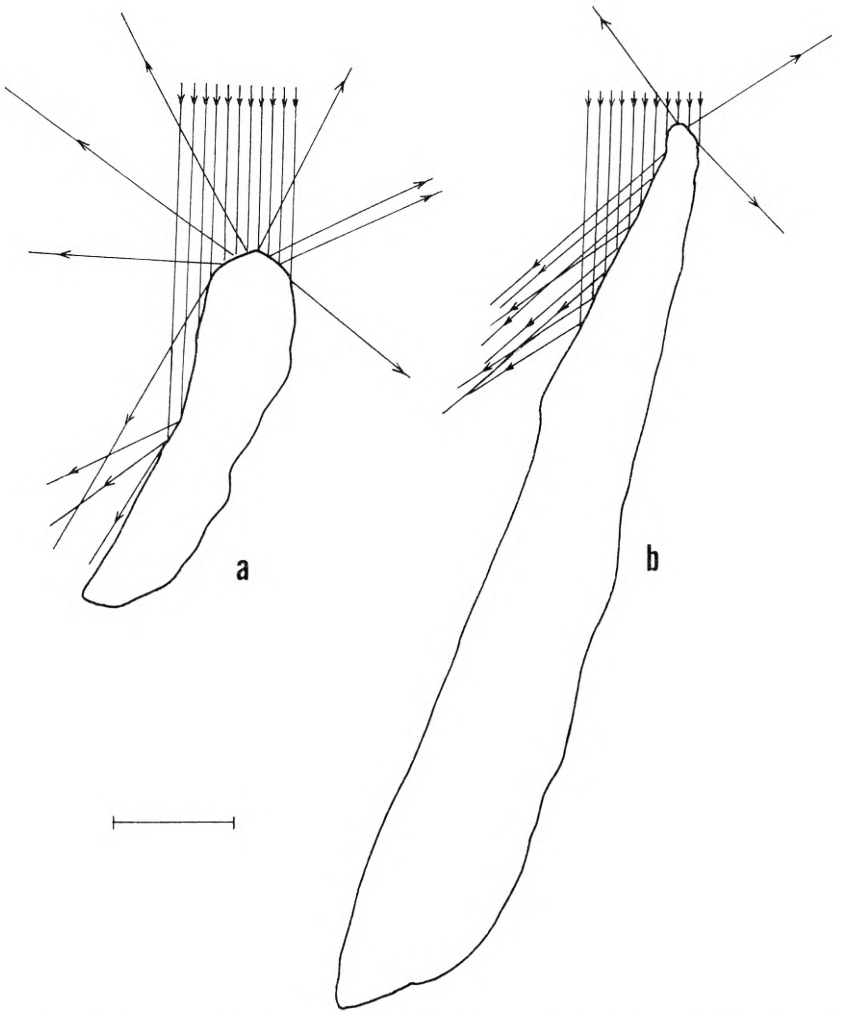

Fig. 20. Specular reflectance of barbules. a: cross-section of unpigmented barbule attaching terminally on blue barb, b: cross-section of yellow barbule of green belly feather. Note that the tapering obverse portion of the yellow barbule reduces specular reflectance in an upward direction. Scale: 1 μ.

yellowish than light incident on the barbs of the distal part of the overlying feather and consequently the underlying barbs should contain less yellow pigment in their cortex in order to reflect light of the same wave-length as the freely exposed barbs of the overlying feather. This reduction in yellow pigmentation is in qualitative accordance with what is actually observed, and it is therefore reasonable to assume that the reduction in yellow pigmentation proximally has a function in establishing colour constancy. A similar argument holds for the reduction in yellow pigmentation of barbules.

Still another fact should be mentioned in relation to the importance of underlying feathers for the overall colour, namely melanin pigmentation of medullary barb cells. It is sometimes stated (KNIESCHE 1914, AUBER 1941, 1957 b) that this underlying melanin pigmented layer is indispensable for the production of blue and green feathers due to their absorption of the yellow and red transmitted light. This is only partly correct: If a dry mounted transverse section of e.g. a green barb is observed in reflected light, the spongy structure reflects intensely bluish green, although the red light is not absorbed but transmitted through the microscope slide into the dark background. However, if the barbs of belly feathers of the Budgerigar lack medullary melanin granules partly or completely the belly looks yellowish green instead of intensely green as in the wild type with intact medullary melanization (STEINER 1932, AUBER 1941). This points to the role of medullary melanin pigmentation to be absorption of light coming in an *upward* direction through the barb, i.e. light reflected from underlying feathers rather than absorption of transmitted red light coming in a *downward* direction through the barb. In the case that medullary melanization is incomplete, a proportion of the upwardly reflected light from underlying feathers will be the red light transmitted through the spongy structure of overlying barbs, but as the relative horizontally projected area of the barbs is small this proportion will be small, so the major function of medullary melanization is evidently absorption of light transmitted through the yellow barbules and between them and reflected upwards again by underlying feathers. The situation is shown diagrammatically in text fig. 21. The correct interpretation of the function of the black pigmentation has been given by some of the early authors (HÄCKER 1890, p. 75 and BANCROFT *et al.* 1923). In the case that both spongy structure-containing cells and ordinary medullary cells are found in the same barb (e.g. *Loriculus* remiges, AUBER and MASON 1955) the importance of melanin pigmentation for absorption of the transmitted red light is obvious, however, since this light would otherwise be reflected into the spongy structure by the ordinary medullary cells.

Fig. 7 illustrates the efficiency of medullary melanin granules in absorption of upwardly directed light. It is seen that very nearly all light directed towards the central medulla is absorbed (forward scattering by the melanin granules has not been taken into account on fig. 7, and the situation therefore is not so simple, but probably light absorption is nearly as effective as suggested by fig. 7). Fig. 7 shows this for a green, comparatively narrow, barb. It is evident that in broad, blue barbs, where additional melanin granules are found in the central medulla, absorption is even more complete (compare fig. 10). It goes without saying that no absorption takes place for light transversing the lateral layer of spongy structure tangentially, and dark red zones on both sides of the central, melanized axis can be seen if the barb is observed in transmitted light.

Concluding this chapter it may be said that barbule pigmentation is of great importance for overall plumage colour. Not only the colour of the distal, freely exposed feather parts, but probably also of the proximal, underlying feather parts are of importance.

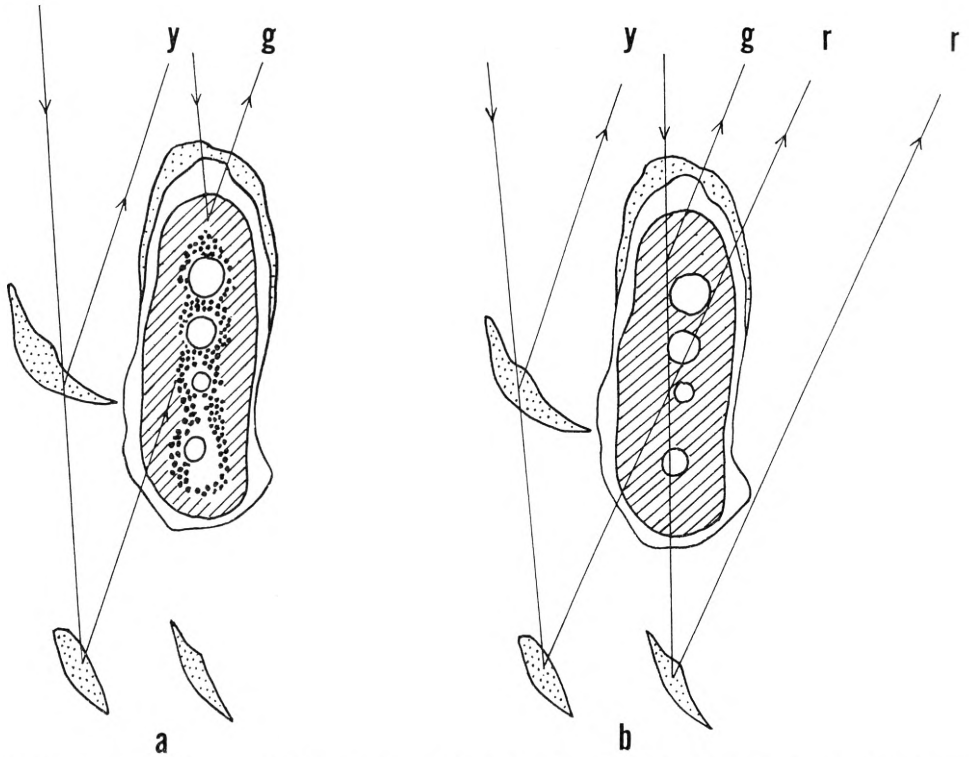

Fig. 21. Diagram showing effect of medullary melanization in a green feather. In (b) no absorption of upwardly directed light by melanin granules occurs and the reflected green light is partly masked by transmitted reddish light, the plumage thereby appearing more yellowish (reddish) than in (a). Yellow feather parts are dotted, spongy structure is hatched and melanin granules in (a) are dark dots. Colours of reflected light: g: green, r: red, y: yellow.

Structure and Mechanical Strength

In the preceding chapters feather structure was considered from an optical viewpoint, but it is clear that the feathers also have other functions in relation to e.g. heat transfer, water repellency (Rijke 1967), mechanical resistance and flight. A most peculiar function of the feathers of *Agapornis roseicollis* is that the feathers of the lower back and rump are used for transporting nesting material (Hampe 1938, Dilger 1960).

Undoubtedly most of the variation in form and size of barb and barbules described above functionally have an optical basis (compare Portmann 1963), but response to demands for mechanical strength is also apparent and will be briefly mentioned.

Terminally green and blue barbs of the middle part of the feathers show distinctly different cross-sections, but basally these are very similar (text fig. 5), being laterally compressed with the cortex thickened obversely and reversely (in the form of a reverse ridge). Such modifications have frequently been observed in feather barbs and are most pronounced in remiges (Spöttel 1914, Rutschke 1966). Undoubtedly,

they serve to increase mechanical strength, especially against bending in an obverse-reverse direction (RUTSCHKE *op. cit.*). In contrast to this, barbs from the distalmost part of the feather have no thickened cortex reversely (nor obversely in the blue barb) in their basal parts, but they show further increase in height and also lateral compression (text fig. 6). These latter barbs are fully exposed to view in almost their entire length (Table 2) and therefore respond to the demands of increased mechanical strength basally by increasing their general bulk which evidently does not affect colour production, whereas barbs placed more proximally where colour production is less critical respond by an increasing cortex thickness.

Discussion

Morphology of Barbs

The morphology of the green and blue barbs, as regards shape in cross-section and arrangement of spongy structure and pigment layers, of *Agapornis roseicollis* is very similar to that of other parrots as follows from the investigations of HÄCKER (1890), HÄCKER and MEYER (1902), KNIESCHE (1914) and FRANK (1939). The similarities with the feathers of the Budgerigar are particularly easy to establish due to the detailed investigations by STEINER (1932), KAWAMURA (1935) and AUBER (1941). Oval cross-sections are typical of blue (FRANK 1939) and violet barbs (AUBER 1941), whereas green barbs generally are more compressed with the obverse cortex thickened to a greater or lesser degree (KNIESCHE 1914, FRANK 1939). The obverse thickening of the cortex resulting in a more triangular cross-section of the green barbs of *Agapornis roseicollis* is more pronounced than in the parrot species illustrated by KNIESCHE, FRANK and AUBER (*op. cit.*). Broadening of the barbs in terminal direction from the basal parts appears to be the rule for blue barbs (STRONG 1902, FRANK 1939). In the Budgerigar AUBER (*op. cit.*) describes the same tendency for green and violet barbs and further mentions that the height of the barbs increases towards the basal end. As stated above (p. 16), this is true in *Agapornis roseicollis* for the distal barbs only; the proximal barbs are higher midway out than basally.

As in *A. r.* a ring-shaped layer of spongy structure is found in barbs of *Agapornis cana*, *Melopsittacus undulatus* (KNIESCHE 1914) and *Psittacula derbyana* (blue barb, FRANK *op. cit.*). In other parrot species the layer of spongy structure is absent reversely, this part of the barb being melanin pigmented or consisting of simple medullary cells without spongy structure (*Psittacula krameri* (*Palaeornis docilis*), *Pionites* (*Caica*) *melanocephala*, *Lorius roratus* (*Eclectus pectoralis*) and *Amazona* (*Chrysotis*) *amazonica*, KNIESCHE *op. cit.*).

Vacuoles. Vacuoles in the peripheral layer of spongy structure ("periaxial" vacuoles, AUBER *op. cit.*) are described for Budgerigar barbs by KNIESCHE and AUBER (*op. cit.*), but this appears to be the only parrot species in which they have been found. According to FRANK (*op. cit.*), they are not found in parrot barbs at all; in *A. r.* small, irregular, peripherally placed vacuoles are observed only occasionally (p. 28).

By some of the earlier authors (KNIESCHE *op. cit.*, STEINER 1932) the great, medullary vacuoles of *Melopsittacus undulatus* were considered spherical cells. KAWA-

MURA (1935) and AUBER (*op. cit.*) showed that this view was not correct, and found that the vacuoles were formed where the nuclei disappeared. The present observations on *A. r.* agree with this explanation.

Yellow pigments. FRANK (1939) states that yellow pigments are uniformly distributed in the cortex of green parrot barbs and STEINER (1932) states the same for the Budgerigar. However, KAWAMURA (1935) illustrates a distribution of the yellow pigment in the green barb of the same species which closely corresponds to that found for the non-fluorescent yellow pigment of *A. r.* (fig. 1), and AUBER (1941) gives a similar account, so probably the latter arrangement is the typical.

As regards the fluorescent yellow pigment earlier investigators (DRIESEN 1953, SCHMIDT 1961) conclude from light-, fluorescence-, and polarization microscopical results that this type of pigment is intimately associated with the keratin of the feather cells, no fine structure being discernible. The present study confirms this conclusion, since, not even with the electron microscope, could any fine structure associated with the pigment be found, only a slight change in staining properties of the keratin (p. 27). This agrees well with the observations by SCHMIDT and RUSKA (1965) on feathers of *Eupodotis senegalensis*. These feathers owe their fluorescence to a content of coproporphyrin in low concentration and neither here was it possible to detect the pigment with the aid of the light- or electron microscope.

Spongy structure. The spongy structure of several bird species has been investigated electron microscopically. The two other parrot species investigated (*Melopsittacus undulatus*, NISSEN 1958) and *Ara ararauna* (SCHMIDT and RUSKA 1962) show a spongy structure very similar to that of *A. r.* both as regards shape and dimensions; in some of the pictures, however, the spongy structure appears highly distorted with irregular empty areas. Very probably, these are artefacts resulting from incomplete embedding of the medullary cells, as also suggested by SCHMIDT and RUSKA (*op. cit.*). A special feature of the spongy structure of the blue wing coverts of the Jay (*Garrulus glandarius*) is the presence of fine keratin threads in addition to the usual rods (SCHMIDT and RUSKA, *op. cit.*); otherwise the spongy structure is closely similar to that of the parrot species. From the imperfect photographs of the spongy structure of *Pitta maxima* barbs (FRANK 1939), made in the first days of the electron microscope, it appears that this spongy structure is also of the parrot type. In *Cotinga cotinga* and *Tangara* (*Calospiza*) *mexicana* the air-filled spaces appear from the photographs of SCHMIDT and RUSKA (*op. cit.*) and FRANK (*op. cit.*) to be spheres rather than channels. This may, however, in some degree be an artefact, because one also gets the impression of spherical vacuoles if examining a thick section of the spongy structure of the parrot type (personal observation). As a matter of fact, at least one of the pictures by SCHMIDT and RUSKA (*op. cit.*) suggests a structure not very different from the parrot type. It seems that the spongy structure of these two species is rather variable, in some cases resembling that of *Agapornis*, in other cases being distinctly different. This corresponds with rather great variations in the diameter of the air-filled spaces (0.1 μ–0.5 μ, mostly 0.2 μ). SCHMIDT (1951) concluded from polarization microscopical observations that the rods of *Garrulus* barbs predominantly are oriented parallel to the surface of

the barb. The electron microscopical pictures (SCHMIDT and RUSKA 1962) of the *Garrulus* spongy structure do not, however, give the impression that this preferred orientation is very pronounced. In *A. r.* the pictures do not suggest a preferred orientation of the rods.

Surface cells. The division of the cortex into a single, outer layer of surface cells and interior cells belonging to the cortex proper was introduced by AUBER and APPLEYARD (1951). According to these authors, the surface cells are characterized by being flattened and polygonal as observed from the surface, contrasting with the long, spindle-shaped interior cortex cells. For the obversely thickened cortex of bluish green barbs of *Chlorophanes spiza* and *C. purpurascens* AUBER and APPLEYARD (1955) further mention that the interior cortex cells fluoresce blue-green while the surface cells do not fluoresce, remarking that this indicates a difference in type of keratin between the two types of cells. The present electron microscopical observations on the cortex cells of *A. r.* show (p. 20) that the surface cells are characterized by their greater content of an electron dense material compared to that of the interior cells. When the barbs are pigmented with a non-granular pigment a difference in pigment deposition in surface cells and cells of the cortex proper is usually observed (present investigation, p. 21 and SCHMIDT and RUSKA (1963 b)). It seems therefore appropriate to retain the distinction between surface cells and interior cortex cells (or cells of cortex proper) although the other criteria for surface cells given by AUBER and APPLEYARD (1951, 1955): flatness of cells, lack of blue-green fluorescence and polygonal rather than elongated shape do not apply to the barbs investigated at least not to the green ones (cp. fig. 8). As regards the elongated shape my pictures, however, do not allow a safe conclusion. The relation between surface cells and cells of the cortex proper may perhaps be considered analogous to those between cuticle and cortex cells of mammalian hairs (MERCER 1961). Barbule cells of *A. r.* should be regarded as surface cells (p. 29).

Cell membranes. SCHMIDT and RUSKA (1962) in their electron microscopical investigation of the spongy medullary barb cells of *Cotinga cotinga*, *Ara ararauna* and *Garrulus glandarius* were not able to find any cell membranes in the cell boundaries, and suggested that the spongy structure resulted in strong changes of the cell membranes. This view is probably erroneous; to maintain it, one should postulate specific differences, which appear unlikely to me. SCHMIDT and RUSKA (*op. cit.*) rather failed to observe them, because their sections were too thick. The same investigators postulated a continuous keratin brim in the medullary cells along the boundary between spongy structure and cortex cells. Such a brim has not been observed in *A. r.* (p. 20).

Melanin granules. The observations by KAWAMURA (1935) on the arrangement of melanin granules in green barbs of the Budgerigar agree closely with mine on *A. r.*, and according to AUBER (1941) a similar arrangement is found in violet barbs of the Budgerigar.

The electron microscopical studies of NISSEN (1958) and SCHMIDT and RUSKA (1962) have shown that also in the Budgerigar and *Ara ararauna* the melanin granules are found in cavities in the spongy structure as decribed in this study (p. 29).

Size and shape of melanin granules in barbs of *A. r.* agree with those of green

barbs of the Budgerigar (STEINER 1932, KAWAMURA *op. cit.*, NISSEN *op. cit.*). In the violet barbs of the same species, however, the melanin granules are rod-shaped, less than 0.3 μ wide and 1 μ long according to AUBER (1941). Similarly, in blue barbs of *Ara ararauna* the melanin granules figured by SCHMIDT and RUSKA (*op. cit.*) are rod-shaped, 0.3–0.4 μ in diameter.

Numerous investigations on the fine structure of melanin granules, particularly of mammalian origin, have revealed that these are not homogeneous, but consist of elements of different density (BIRBECK *et al.* 1956, BIRBECK and BARNICOT 1959, DROCHMANS 1963, SEIJI 1967). This applies also to avian melanin granules (CARR 1957, NISSEN 1958, SCHMIDT and RUSKA 1961 b, present study). FILSHIE and ROGERS (1962) however failed to recognize fine structures in granules of Black Australorp fowl barbs and suggested this to be a difference from mammalian granules.

Morphology of Barbules

In general outline and cross-section the barbules of the green feathers resemble those of greenish body feathers of other parrot species (CHANDLER 1914, AUBER 1941). The presence of well-developed hooklets on distal barbules from the distal portions of body feathers in species of *Agapornis* has been commented upon by SICK (1938). The arrangement with black melanin terminally and yellow pigment basally in barbules agrees with that of other parrot species (CHANDLER, AUBER, *op cit.*) as well as most other species which have barbules containing these two pigment types together (FRANK 1939).

The presence of smaller and more rod-shaped melanin granules (oriented longitudinally) in barbules compared to barbs has been noted also in the green rump feathers of the Budgerigar (AUBER *op. cit.*); in *Melopsittacus* barbules a higher proportion of the granules appear to be rod-shaped, however.

Optical Properties and Structure

In this chapter the colour-producing factors and their importance for plumage colour is discussed in relation to earlier views on the subject and in relation to the presumed function of the colours.

Agapornis roseicollis is an inhabitant of wooded grassland in south-western Africa (DILGER 1960). Undoubtedly, the function of the green colouration is camouflage in these, as it is supposed, predominantly green surroundings. Unpublished measurements by the author have shown that the spectral reflectance curves of the type presented in text fig. 2 are very similar to those of the green vegetation. The significance of the lighter underparts very probably is countershading (when illuminated from above the belly shows the same colour as the back if observed in a horizontal direction, due to the combination of being lighter and receiving less light (for discussion of the principle, see COTT 1940)). This explanation is strongly supported by the fact that the reflectance spectrum of the belly is nearly identical with that of the back multiplied by a constant factor, c. 2.2 (text fig. 2).

The vivid blue colour of the rump points to its function as a visual sign stimulus. It is probably not observed when the bird sits with folded wings. Its function may therefore be to show flight intention when the wings are unfolded and/or to act as a recognition mark during flight in this social species. DILGER (1964) considers that the blue rump may have evolved in connection with specializing in tucking nest material amidst the feathers of the back.

Before entering into a discussion it may be useful to summarize earlier findings as regards production of green colour in *A. r.*: The reflection maximum in the green part of the spectrum is due to the barbs with their spongy structure showing a reflection maximum at 480–490 nm overlaid by a cortex containing two types of yellow pigments. The extent of black and yellow pigmentation in the barbules determines, to a great extent, the reflection level (lightness) of the plumage.

The possibility that the spongy structure of green barbs may reflect light of a different colour from that of blue barbs never seems to have been definitely stated. STEINER (1932), however, suggested that some changes in barb colour observed in some Budgerigar varieties were caused by a change in the spongy structure with a corresponding wave-length shift. FATIO (1866) seems to be the only author who has described the colour difference between transmitted light of sections of green and blue barbs. HÄCKER and MEYER (1902) and KNIESCHE (1914) both mention that the medullary cells of olive and green barbs may reflect a more greenish light than those of blue barbs, but both authors ascribe this to a partly imperfect spongy structure, and evidently at least KNIESCHE (*op. cit.*) is of the opinion that in bright green barbs the spongy structure is identical with that of blue barbs. Thus the result of this investigation, that green barbs contain a highly efficient spongy structure with a reflectance spectrum different from that of blue barbs is new and does not agree with the view previously held.

The triangular to pear-shaped cross-section of the green barbs corresponds to the typical form in feather barbs (generalized configuration of AUBER 1957 b). The function of the thickened obverse cortex is partly to accomodate the yellow pigment, but obviously its thickness in *A. r.* is greater than required by the presence of yellow pigment (fig. 8). Probably, its function is also to increase strength (SPÖTTEL 1914), and gloss from this type of barbs presumably is rather low.

In many green barbs the obverse cortex is still more thickened and with an acute edge. FRANK (1939) ascribes a twinkling effect (Glitzer-struktur – great difference in light intensity if the barb is viewed from above or from the side) to this form. In *A. r.* such an effect is not apparent.

There has been some controversy as to the importance of the barbules for the green colour. The importance of yellow and black pigmented barbules for the overall green colour was realized by HÄCKER (1890), HÄCKER and MEYER (1902) and AUBER (1941, 1957 b), whereas KNIESCHE (1914) considered them unimportant. The present data on *A. r.* show their great importance in this species, the relative projected area of the barbules being c. 4 times as great as that of the barbs (Table 7). The halving

of reflection resulting from the substitution of yellow pigment with black melanin terminally in barbules (p. 39) is also noteworthy, in particular because melanization in the barbules is not very intense (p. 30). Intenser black melanization may reduce reflection much more than observed in the back of *A. r.* (actually, other species of *Agapornis* are considerably darker green than *A. r.*, which indicates intenser black melanization in these species). Probably, the variation in reflection level (lightness) which can be achieved by variation in pigmentation of barbules amounts to a factor 4 or the like. The influence of the yellow and black barbules on hue (dominant wave-length) is less important. This follows from the fact that olive-green plumage areas may be produced from feathers showing a mixture of black and yellow pigmented feather parts, but lacking a well-developed spongy structure (apposition colours, Häcker 1890, Stubbs 1910, Frank 1939, Auber 1957 a, Auber *in* Moreau 1957, Dyck 1966). In *A. r.* the substitution of yellow pigments by black melanins changes the hue very little (Table 1). Frank (*op. cit.*) states that melanin pigmented barbules result in a darker and more olivish colour. This, however, can only be the case when the barbules are brown, not black pigmented, as a change from a green to a more olive colour corresponds to an increased dominant wave-length (Dyck *op. cit.*).

Thus the data on *A. r.* leave no doubt as to the great importance of the barbules for modifying colour (in particular lightness) in green plumages.

The orientation of the barbules so that only the acute obverse edges are visible from above is the typical constitution in contour feathers (Frank 1939) and undoubtedly serves to minimize gloss (see p. 40) from the feather surface. In *A. r.* gloss reduction probably has the function to maximize the cryptic effect of the green plumage, most natural backgrounds being characterized by their lack of gloss (Duntley 1964).

The significance of the fluorescent yellow pigment is difficult to understand as its importance on the reflectance spectrum seems to be small (p. 36). Its function may be to reduce reflectance in the spectral region 380–420 nm (p. 36) and perhaps at lower wave-lengths also as reduced reflectance in this region will tend to make the reflectance spectrum of the bird more similar to that of the green vegetation (unpublished results), but admittedly the influence of this change as to the total colour may be very small (compare p. 36).

It is also to be expected that the emitted greenish yellow light would slightly increase reflectance in the corresponding spectral region. This emitted light is not included in the reflectance spectra of text fig. 2, as the bird was illuminated with monochromatic light during measurement, but unpublished measurements on a green plumage area of *Psittacula krameri* containing a yellow fluorescent pigment has shown that the change in reflection properties due to emitted light is negligible.

It is noteworthy that the *Agapornis* form which fluoresces most intensely (*A. personata nigrigenis*, Völker 1940) is the form which from other criteria appears to be the most recently evolved (Dilger 1960).

The blue colour of *A. r.* feathers is due mainly to the spongy structure of the barbs with its reflectance maximum at 450–460 nm, no pigments being present in

barbs or barbules which may change the hue (dominant wave-length) of the feathers to any noteworthy extent. The consequence of this in *A. r.* as in many other species, is a broadening, possibly also a torsion, of the barb distally, often accompanied by a reduction of the barbules so that the blue area of the feather increases. In many species this tendency is far more marked than in *A. r.* (see FRANK 1939, for examples).

The broadening of the barbs also results in increased gloss from the upper surface giving an "enamel' effect (FRANK *op. cit.*, AUBER 1957 b). Gloss often enhances visibility (DUNTLEY 1964) which may be of importance in plumage areas functioning as visual sign stimuli.

The influence of the barbules on the blue colour varies much in different plumages. In cases where the barbs are broadened to such a degree that they almost touch each other (see FRANK *op. cit.*, p. 499) the influence of barbules on overall colour becomes, of course, negligible. Their rôle in *A. r.* is not clear as mentioned above (p. 39). Although in *A. r.* the barbules attaching terminally on the barbs are reduced, their relative horizontally projected area still amounts to one half (Table 7). The rounded obverse surface of these barbules may have some function by further increasing gloss in addition to that of the rounded barbs (text fig. 20). The black, melanized, well-developed barbules attaching basally on the barbs may have some function by decreasing reflection (comp. FRANK *op. cit.*) but to what extent is unknown.

The rôle of the barbules is much more evident where blue barbs work together with red barbules to produce a lilac colour (AUBER 1941).

Is the colour of the spongy structure produced by Tyndall scattering?

The statement of HÄCKER and MEYER (1902) and BANCROFT *et al.* (1923) that the blue colour of the medullary barb cells is due to Tyndall scattering following Lord Rayleighs equation for scattering from small, transparent, optically isotropic particles has been generally accepted and quoted by nearly all subsequent authors (KNIESCHE 1914, GOWER 1936, FRANK 1939, AUBER 1941, 1957 a, b, 1964, SCHMIDT & RUSKA 1962 and DYCK 1966). RAMAN (1936) appears to be the only worker to suggest that this explanation is erroneous.

The term most frequently found in modern, ornithological reference books for the process by which the blue colour of the spongy structure is produced, is Tyndall scattering (FOX & VEVERS 1960, RAWLES 1960, VEVERS 1964). In so far as modern reference books on light scattering (KERKER 1969) deal with both Tyndall blue for scattering in accordance with the Rayleigh equation and higher-order Tyndall spectra for the scattering from greater particles, the use of this term is not very appropriate. In accordance with KERKER (*op. cit.*) it seems better to use the term *Rayleigh scattering* whenever it is inferred that a blue colour is produced by scattering in accordance with Rayleighs equation (scattering dependent upon the inverse fourth power of the wave-length).

Before entering the discussion it may be useful to summarize the facts which have to be taken into consideration:

1. Dimensions and shape of the spongy structure (figs. 11, 12 and Table 3). In particular the fact that channels are wider and rods narrower in blue than in green barbs.
2. Spectral measurements, in particular values of λ_{max} of reflectance spectra (Table 5). Principal attention is given to values obtained by microspectrophotometry as these give the best information about the light reflected directly backwards.
3. When a blue feather is observed in unidirectional light it appears to be intensely turquoise-green when the eyes are placed near the light source; it appears much duller bluish violet (a reddish component is sometimes apparent too) if it is observed at right angles to or directly opposite the light source. Similar observations have been made by RAMAN (1935) on feathers of *Coracias benghalensis* (*indicas*).

   Under the microscope in reflected light the colour of a blue *Agapornis* barb viewed perpendicularly changes from a greenish to a more bluish one as the angle of incidence of the incoming light increases.
4. If a drop of a liquid (water, acetone, xylene or carbon disulfide) is placed on an *Agapornis* barb section, greatly reduced reflection and a shift to colours of longer wave-lengths (yellowish green to red) occur; the higher the refractive index of the liquid (xylene, carbon disulfide) the more pronounced the effects. Similar observations have been made by HÄCKER and MEYER (1902), BANCROFT *et al.* (1923) and RAMAN (1935). The latter mentions the possibility that the presence of the liquid makes the cavities swell, but this seems very unlikely to me, as the colour change is observed instantaneously and with different liquids.
5. Birefringence is hardly noticeable when the spongy structure is observed in reflected light. This has been established with the "Wild" accessory for reflected light to microscope M 20 (normal incidence, illumination directed through the objective). This is in accordance with RAMAN (1935) who found polarization effects hardly noticeable with *Coracias benghalensis* (*indicas*), while SCHMIDT (1951) and SCHMIDT & RUSKA (1962) found the spongy structure of *Garrulus glandarius* to be birefringent when observed in transmitted light. BANCROFT *et al.* (1923) found the polarization of the laterally scattered light to be "unmistakeable, though far from complete".
6. Viewed in reflected light the spongy structure is not uniform but appears to consist of shining spots and stripes separated by a network of dark stripes (colour plate, fig. 6). The shining spots and stripes are best observed when the direction of the incoming light approaches perpendicular incidence. When the angle of incidence is great the barbs appear more homogeneously coloured. The diameters of the spots and the widths of the stripes are of the order of magnitude 4–600 nm.

   RAMAN (1935) speaks about a granular structure of the cells and notices that the different granules in a cell usually are of the same colour.

Rayleigh scattering. The intensity of light scattered by a small dielectric sphere when the incident light is unpolarized is given by (KERKER 1969, p. 35):

$$I_u = \frac{8\pi^4 a^6}{r^2 \lambda^4}\left(\frac{n^2 \div 1}{n^2 + 2}\right)^2 \quad (1 + \cos^2\theta),$$

where a is the radius of the sphere, n is the ratio between the refractive index of the sphere and that of the surrounding medium, r is the distance from the sphere to the point of observation, λ is the wave-length in the surrounding medium and θ is the angle between the direction to the point of observation and the proceeding beam. The $\cos^2\theta$ term in the parentheses refers to the component of the scattered light whose electric field vector lies in the plane defined by the incident and scattered beams, and the unity term refers to the component of the scattered light whose electric vector is perpendicular to this plane.

Under fixed observation and illumination conditions the equation reduces to:

$$I_u = \frac{C}{\lambda^4}$$

where C is a constant. A further necessary supposition is, that the refractive indices are independent of wave-length. As keratin shows no strong absorption bands in the visible spectrum (Dyck 1966, text fig. 16 this paper, and unpublished observations) there can be no great variations in its refractive index, so evidently this supposition holds with approximation.

If the spongy structure is considered a turbid medium for which the Rayleigh equation holds, we shall therefore expect reflectance to be inversely proportional to the fourth power of wave-length. Since the reflectance spectra of individual barbs show a distinct maximum, it is evident that they do not agree with the Rayleigh equation, and thus that colour production in barbs of *A. r.* can not be explained by light scattering in this way, as was done by Häcker & Meyer (1902) and Bancroft *et al.* (1923) for blue and green barbs in general. Text fig. 22 compares a theoretical spectrum calculated from the Rayleigh equation putting $C = 2.64 \cdot 10^{10}$ nm^4 (corresponding to a reflectance of 36 % at 520 nm as found in one of the reflectance measurements on a blue barb) with actually measured spectra. The discrepancy is obvious.

However, some earlier reflection measurements have showed good agreement with the Rayleigh equation (Häcker & Meyer 1902, Peiponen 1963 and Dyck 1966). When Häcker and Meyer made their measurements on a blue plumage area of a *Malurus sp.*, their apparatus was restricted to wave-lengths between 480 and 660 nm. If we suppose that *Malurus* barbs have properties similar to those of *Agapornis* barbs, we expect their reflectance spectrum to have had a maximum below 500 nm, and it would therefore not have been possible for them to detect it. In the region from 500 to 600 nm they found good agreement with the Rayleigh equation, in accordance with text fig. 22 (cf. curves 2 and 3). The measurements on blue throat feathers of *Luscinia svecica* (Peiponen 1963) and on blue wing coverts of *Garrulus glandarius* (Dyck 1966) both show steadily increasing reflectance with decreasing wave-length

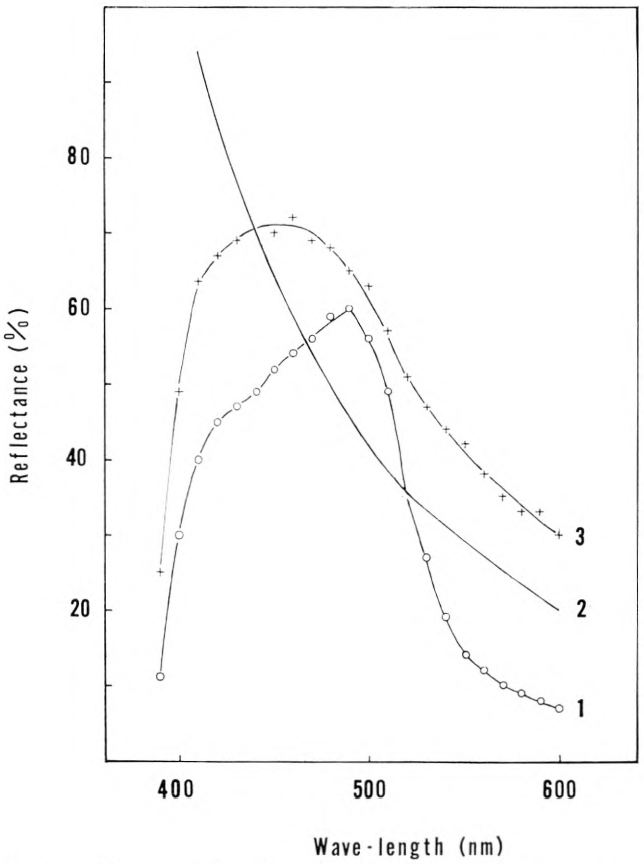

Fig. 22. Comparison of theoretical (curve 2) and measured reflectance spectra (curves 1, 3) of a blue barb. Theoretical spectrum according to the Raleigh equation, see text. 1: blue barb obversely, 3: 'inverted' transmittance spectrum of blue spongy structure. Note lack of agreement between theoretical and observed spectra.

(down to 330 nm in *Luscinia*, to 400 nm in *Garrulus*) in approximate agreement with the Rayleigh equation although the rise in reflectance is not so steep as has to be expected. Viewed in reflected light under the microscope the barbs appear very much like those of *A. r.*, and most probably their spongy structure is basically equal to that of *A. r.*; actually SCHMIDT and RUSKA (1962) found a spongy structure in *Garrulus* barbs nearly identical with that of *Agapornis*. If there is no difference in the spongy structure why does not the reflectance decrease at c. 400 nm? The measurements on *Garrulus* and *Luscinia* were both made on intact plumage areas, and it will be remembered that also the blue plumage area of *A. r.* showed an astonishing high reflectance at 400 nm compared to that of the blue barb alone (text fig. 19 above). So probably the high reflectance at this wave-length is not caused by the spongy structure. The only explanation I can find is that this reflectance is produced by short-wave scattering due to e.g. irregularities in the keratin.

The size range of spheres for which the Rayleigh equation generally is considered valid is usually given as $\frac{a}{\lambda} \lesssim 0.05$ (KERKER 1969, section 3.9.1). If we consider the channels and rods of the spongy structure, we obtain for their half diameters $\frac{a}{\lambda} \approx 0.1$. The length of the channels and rods corresponds to greater $\frac{a}{\lambda}$ values. From considerations of size we should therefore not expect the Rayleigh equation to be valid for the spongy structure, and it is actually found that it is not. On the other hand, calculations of specific turbidity have shown (KERKER *op. cit.*) the Rayleigh equation to be a reasonable approximation in the size range corresponding to the widths of the rods. It is therefore difficult to ascertain whether Rayleigh scattering may play a role (if only a minor one) in the case of the spongy structure. It seems a fair guess that the indicated shoulder at 420 nm on the "micro" reflectance spectra (text fig. 14) is due to Rayleigh scattering.

I will next discuss other possible explanations for colour production by the spongy structure.

Other explanations. The spongy structure may (from an optical point of view) be considered to consist of keratin cylinders with diameter d and length approximately $2^1/_2 \cdot d$. The scattering of light incident perpendicularly on dielectric cylinders of infinite length has been treated by FARONE *et al.* (1963). They find that the intensity of light scattered directly backwards ($\theta = 180°$) varies strongly with $\alpha = \frac{\pi \cdot d}{\lambda}$. The values of α for which intensity backwards is maximum (in the following referred to as values of α_{max}) depend on the refractive index of the cylinder and to a lesser degree on the state of polarization of the incident light. For the present analysis values of α_{max} corresponding to $n = 1.34$, 1.38 and 1.54 have been extra- and interpolated from the values given in the paper. Two set of intensity curves are given, i_1 and i_2. i_1 is defined as $\frac{\pi^2 \cdot r}{\lambda_0} \cdot \frac{I_1}{I_0}$, where I_1 is the intensity scattered in the direction θ and at a distance r from the cylinder when the electric vector of the plane polarized incident radiation (of intensity I_0) is parallel to the cylinder axis; λ_0 is the wave-length in the medium surrounding the cylinder. i_2 is defined correspondingly, only is the electric field vector perpendicular to cylinder axis. In the region of α of interest i_1 is 2–3 times i_2. The values of α_{max} differ slightly for the two cases. The values used in the following are average values interpolated according to the ratio $i_1 : i_2$. Text fig. 23 shows examples of intensity curves corresponding to case 1 (i_1) for $n = 1.40$ and 1.56. The i_2-curves show similar shapes.

Two types of cylinders are considered; one type corresponds to the keratin rods with diameters equal to widths given in Table 3. The refractive index of keratin is

taken as 1.54 (BANCROFT *et al.* 1923). The other type is considered to be a hollow keratin cylinder with an air-filled core (text fig. 24). In axial, longitudinal section it appears as two keratin rods with an air-filled channel in between. The diameter of the cylinder is thus equal to 2 × width of rod + width of air-filled channel. For convenience of calculation it is equated to a cylinder of same diameter but of constant refractive index. This constant refractive index has been calculated assuming that it is reduced compared to that of keratin in the ratio of the diameter of the air-filled core to the total diameter. We obtain for the two types of spongy structure, using the widths from Table 3:

$$n_{\text{blue}} = \frac{114.0 \cdot 1.00 + 202.0 \cdot 1.54}{316.0} = 1.34$$

$$n_{\text{green}} = \frac{103.6 \cdot 1.00 + 238.8 \cdot 1.54}{342.4} = 1.38$$

Table 8 shows the values of $\lambda_{\max}$ for light scattered backwards from the two types of cylinders, as calculated from values of $\alpha_{\max}$ and d. Values of $\alpha_{\max}$ higher than c. 2 are not considered as these correspond to wave-lengths shorter than those of visible light.

The values in Table 8 are to be compared to the values of $\lambda_{\max}$ obtained by microspectrophotometry, *i.e.* 480–486 and 524 nm for blue and green barbs respectively (Table 5). It is clearly seen that these agree very well with those obtained for hollow cylinders corresponding to values of $\alpha_{\max}$ equal to c. 2, and less well with those obtained for simple cylinders and $\alpha_{\max}$ equal to c. 1.

Values of $\alpha_{\max}$ change only little if light scattered obliquely backwards ($\theta = 135°$) is considered instead of light scattered directly backwards ($\theta = 180°$) (FARONE *et al.* 1963).

In addition to considering perpendicular incidence we also have to consider oblique incidence with tilt angle φ. In this case the scattered radiation is propagated along the surface of a cone whose axis is the cylinder axis, whose vertex is at the intersection of the incident ray and the cylinder axis and whose apical angle is (180°

TABLE 8.

Values of $\lambda_{\max}$ calculated from values of $\alpha_{\max}$ given by FARONE *et al.* (1963) and diameters of cylinders corresponding to Table 3. Compare text.

Colour of barb	Cylinder		First	$\lambda_{\max}$	Second	$\lambda_{\max}$
	d (nm)	n	$\alpha_{\max}$	(nm)	$\alpha_{\max}$	(nm)
blue	101.0	1.54	0.895	354	1.92	165
green	119.4	1.54	0.895	419	1.92	196
blue	316.0	1.34	1.06	937	2.15	*462*
green	342.4	1.38	1.10	979	2.095	*514*

Note: $\lambda_{\max}$ is found as $\frac{\pi \cdot d}{\alpha_{\max}}$

$\div 2\varphi$) (KERKER 1969, section 6.1.4). Thus, the more oblique incidence is, the more scattering becomes restricted to the forward direction. LIND & GREENBERG (1966) have calculated extinction efficiences (equal to scattering efficiencies if absorption is considered negligible) of infinite cylinders of refractive index 1.6 for various tilt angles and values of α. For not very oblique incidence (φ = 25°, 45°) scattering increases rather regularly with $\alpha(0 \lesssim \alpha \lesssim 2)$. At high obliquity the scattering by a dielectric cylinder resembles that of a dielectric needle at perpendicular incidence (KERKER 1969, p. 301). For this it holds that the intensity of the scattered radiation varies inversely as the cube of the wave-length (KERKER 1969, p. 267). When tilt angle approaches end-on incidence scattering efficiency shows distinct peaks at a few values of α; at $\varnothing$ = 89° these occur at α = 0.7 and 3.2. For the keratin rods α = 0.7 corresponds to λ_{max} = 452 and 532 nm for blue and green spongy structures respectively, in good agreement with measured values. These values correspond only to cylinders of infinite length, however. For cylinders of finite length scattering peaks are at higher values of α, thus for a cylinder $2.8 \cdot \lambda$ long at α = 1.3, which means that there is no scattering peak in the visible region. For cylinders with length equal to $2^1/_2$ times width (as the rods may be compared to) we should therefore not expect any peaks in the visible region. Thus only for such rods in the spongy structure which may be considered of infinite length will there be selective scattering of light with $\lambda_{max} \approx 500$ nm. Part of this forwards scattered light could be reflected at the boundary surface where the rods end or bend sharply and would thus contribute to backwards reflected light. As most rods are far too short it appears unlikely to me, however, that the contribution from light incident end-on the rods is of great importance (if of any at all) for the backwards reflected light.

Backscattering from the air-filled channels is considered next. The channels may be considered to be immersed in keratin, thus their relative refractive index is 1.00 : 1.54 = 0.65. The influence on backscattering of diminishing refractive index of cylinders (text fig. 23) is an increase in α_{max}, which corresponds to a decrease in λ_{max}. The same applies if refractive index becomes less than unity (calculations on spheres, KERKER 1969, figs. 4.38 & 4.40). For keratin cylinders with n = 1,54 and diameters similar to those of the channels we found values of λ_{max} around 350–400 nm (Table 8). For the channels these values would have to be reduced further (probably by at least 25 %); it thus becomes very unlikely that there will be any λ_{max} in the visible region due to backscattering from the air-filled channels.

This is made further unlikely by the fact that the channels of the green spongy structure are narrower than those of the blue one (Table 3), which means that we should expect λ_{max} of the former to be lower than that of the latter in case backscattering by the channels was of importance. This is in contradiction to the observed values of λ_{max} (Table 5).

As regards the scattering of light incident end-on the channels RAMAN (1935) mentions the possibility that backward scattering may be greater than forward scattering in this case in contrast to the scattering of the rods. I have been unable to find

calculations or experiments which answer this problem. It therefore remains unknown what the colour of the light possibly scattered backwards would be, but it is perhaps again reasonable to assume that the wider channels of the blue spongy structure would result in an intensity maximum at a higher wave-length than that resulting from the narrower channels of the green spongy structure, in contradiction to the observed tendency.

Thus it appears that of the various possibilities for colour production discussed above back-scattering of light incident approximately perpendicular on keratin cylin-

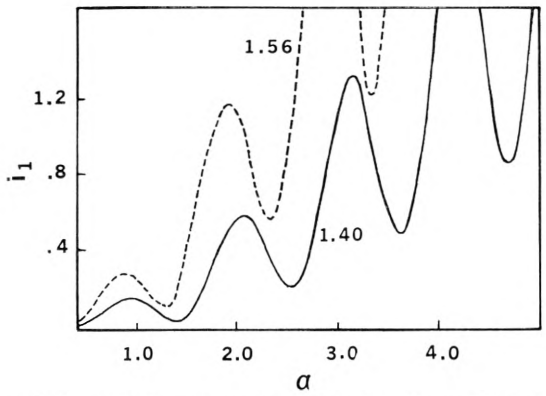

Fig. 23. Backscattering by infinite cylinders at perpendicular incidence. Variation of i_1 with α at $\theta = 180°$ for $n = 1.40$ and 1.56 (after FARONE *et al.* 1963). For further explanation, see text.

ders is the only one of importance. Now which of the two cases tabulated in Table 8 may be considered the most important, the simple cylinders corresponding to $\alpha \sim 1$ to or the thick ones corresponding to $\alpha \sim 2$? Two facts point to the latter being the principal one: (1) The calculated values of λ_{max} for the thick cylinders agree better with those observed than is the case with the thin cylinders (p. 55). (2) The proportion $\lambda_{max,\ green}$: $\lambda_{max,\ blue}$ calculated for thick cylinders (1,11) agress better with the one observed (1.08) than is the case with the thin cylinders (1.18).

The hollow cylinder model. Several approximations and sources of error have to be taken into consideration, however, before accepting this model of colour production.

The approximations are:

1. The diameters used are measured from electron microscopical pictures. The reproducibility in magnification is given in the manual as $\pm 3\ ^0/_0$. Diameters could be measured on the photographs with an accuracy of c. 0.5 mm $\approx$ 10 nm.
2. The inclusion of measurements on obliquely sectioned channels and rods tend to make the measured widths somewhat too large.
3. The value of refractive index for keratin used has been taken from the litterature. It has not been determined for this particular species.

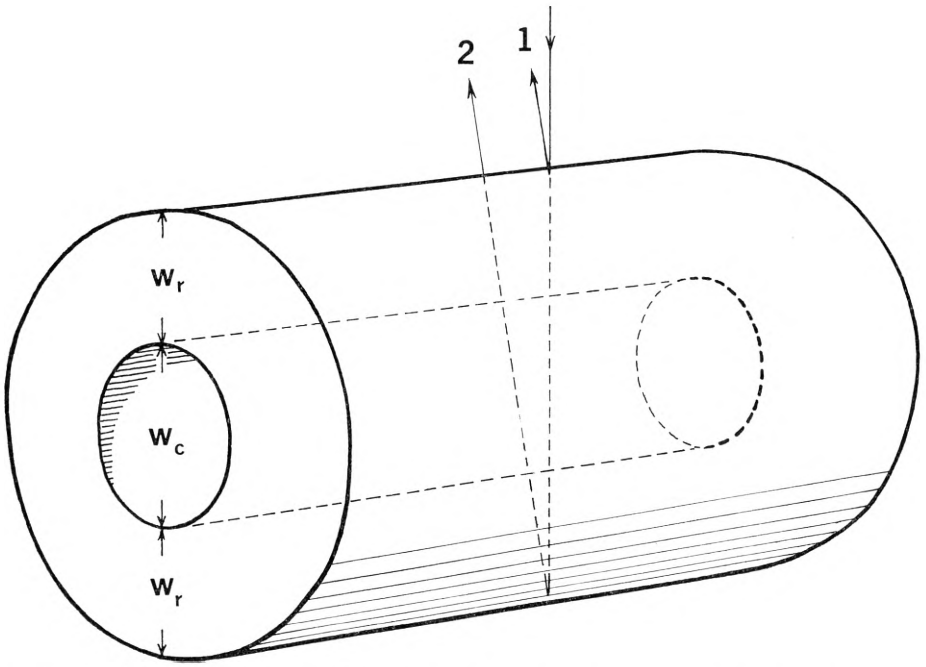

Fig. 24. Hollow cylinder model of colour production by spongy structure. Colour is due to interference between the two reflected, axial rays (1 & 2). Diameter of hollow cylinder = $2\,w_r + w_c$, where w_r = width of keratin rod and w_c = width of air-filled channel.

4. The spongy structure is equated to straight cylinders of constant diameter and infinite length. As mentioned below (p. 61) backscattering from cylinders can with good approximation be regarded as being produced by interference between two axial rays, one reflected from the front surface of the cylinder and one reflected from the rear surface after having transversed the cylinder (text fig. 24). It is evident that in this case it becomes of minor importance that the cylinders of the spongy structure are neither straight nor of infinite length. The principal thing is that they are of fairly constant diameter which is demonstrated in Table 3. Lind & Greenberg (1966) from microwave-scattering experiments conclude that the extinction cross-section of a cylinder of finite length at normal incidence is fairly well represented by the infinite cylinder calculations.
5. The thick hollow cylinder is equated to a cylinder of same diameter with constant, but lower refractive index. If backscattering is treated as mentioned under the preceding point, it becomes likely that this approximation is a permissible one. Evans *et al.* (1964) found that scattering from a hollow cylinder with inner:outer diameter = 0.10 is practically indistinguishable from that of a homogeneous cylinder.
6. Extrapolations were used for determining values of α_{max} corresponding to $n = 1.34$ and 1.38.

Qualitative estimates and the views expressed in the preceding points in my opinion make it unlikely that the approximations used are so crude as to invalidate this model of colour production.

The model is based on the dimensions and shape of the spongy structure and predicts the observed values of λ_{max}. How does the model fit with the other observations summarized on p. 51 (points 3–6)?

Variation of colour with direction of observation (point 3): The curves of Farone *et al.* (1963) for light incident perpendicularly on cylinders and those of Lind & Greenberg (1966) for oblique incidence both show that for scattering in other directions than directly backwards the overall tendency is an increase of scattering with increasing values of α in the region of α under consideration. This means that scattering is more intense the shorter the wave-length. This agrees with the bluish violet colour of the barbs when observed at right angles to or directly against the incident light. The change of colour towards the short-wave region of a blue barb viewed perpendicularly when the angle of incidence of the incoming light is increased, may be regarded as the usual dependence of interference colours on angle of vision.

The curves of Farone *et al.* (1963) also show that scattering by a single cylinder is much more intense in the forward than in the backward direction. This does not agree with the observations in unidirectional light which show the intensity to be greatest in the backward direction. The difference stems partly from the presence of several "layers" of cylinders whcih increases backscattering at the expense of forward scattering and partly from absorption by medullary melanin granules.

The reddish component sometimes observed probably is due to the transmitted light.

Change of colour and intensity when the air of the spongy structure is replaced by a liquid (point 4, p. 51): The decrease in intensity of the reflected light when the air is replaced by liquids of higher refractive index is readily explained by the smaller difference in refractive index between keratin and the medium surrounding it (cp. text fig. 23). The increase in wave-length resulting from the substitution is explained by the increased refractive index of the hollow cylinders when the refractive index of the core is increased, resulting in increased optical length across the cylinder.

The lack of birefringence in reflected light (point 5, p. 51): The intensity of light scattered in the backward direction by an infinite cylinder depends on the polarization of the incoming light. From the curves of Farone *et al.* (1963) the ratio $i_1 : i_2$ can be estimated as approximately 2.5. With perpendicular incidence on a cylinder the state of polarization is not altered by scattering (Kerker 1969, p. 263). Thus, when the incident light is natural, unpolarized, the light scattered backwards is partially polarized with the electric field vector parallel to cylinder axis. In a system of cylinders randomly oriented in a plane perpendicular to the direction of the incoming light the polarizations of the light scattered by the individual cylinders will cancel each other out and so the overall effect is that the light scattered backwards by the system will be unpolarized when the incoming light is natural, but the system will appear birefringent.

Thus the lack of birefringence observed is in contradiction to the model. This may be due to the fact that the above holds for infinite cylinders while the real cylinders are short ones. The polarization of light scattered from short cylinders is less that of infinite cylinders (GREENBERG *et al.* 1961).

The appearance of the spongy structure in reflected light (point 6, p. 51): The width of the shining spots and stripes of the spongy structure (400–600 nm) is in good agreement with our model which involves hollow cylinders of diameters 300–350 nm taking into account the resolution of the microscope, 300–400 nm for blue light.

Hence, with the exception of the lack of birefringence, the observations described in points 3–6 are in qualitative agreement with the hollow cylinder model. The model is shown diagrammatically on text fig. 24 and cross-sections of hollow cylinders are shown on fig. 12.

Multiple scattering. In the foregoing sections the scattering of the spongy structure has been regarded simply as the sum of the scattering by the individual cylinders, the scattering of which have been considered independent of the presence of other cylinders. This is not correct. "When the particles are very close to each other, their polarization fields interact, and it is necessary to match the electromagnetic fields at both particle boundaries simultaneously. This results in different scattering functions from those of the isolated particle.

Even when the particles are more than several diameters apart so that mutual polarization is absent, some of the radiation scattered by a particular particle will be incident upon a second particle, which then rescatters it. This is multiple scattering" (KERKER 1969).

The influences of these phenomena on the colour production of the spongy structure are unknown, but one fact should be considered in this connection: Backscattering from simple cylinders. Table 8 shows that we should expect λ_{max} at 419 nm, respectively 354 nm, for green, respectively blue keratin rods. From the curves of FARONE *et al.* (1963) it is estimated that the scattering intensity of the simple cylinder is approximately 60 $^0/_0$ that of the hollow cylinder. If backscattering by the spongy structure is simply the sum of the backscattering by the individual cylinders we should therefore expect the backscattering by the simple cylinders to be at least as intense as that of the hollow cylinders, as there are more simple cylinders than hollow ones. But the reflectance spectrum of the green spongy structure shows no intense maximum corresponding to backscattering by simple cylinders. Evidently the scattering by the simple cylinders is eliminated somehow, probably because of destructive interference.

It is thus clear that the scattering by the spongy structure is more complicated than can be described by the simple hollow cylinder model. It could be argued that the colour produced by the spongy structure is the result of a complex interaction of many different scattering possibilities and that it is not a valid approximation to ascribe the colour to only one type of geometrical configuration. Calculations are needed to decide whether this is a reasonable objection. But in my opinion a number of facts

strongly suggest that the hollow cylinder is a good model of what is actually happening. The principal facts are, 1) the good agreement between calculated and observed values of λ_{max} and, 2) that the spongy structure in reflected light appears similar to the electron microscopical picture, the only difference being the greater width of the shining stripes compared to the keratin rods. This indicates that the scattering of the spongy structure can be regarded as the sum of scattering by relatively independent units as our model demands. If the colour was the result of a complex interaction of many different scattering possibilities, the spongy structure would probably appear more homogeneously coloured.

Scattering or interference. Considered next is the question as to whether the colour produced by the spongy structure should be classified as an interference or a scattering phenomenon as discussed by RAMAN (1935). Scattering is generally used (when dealing with animal colours) for structural colours which are not iridescent; i.e. the colour does not vary with the angle of view. Interference is used for iridescent colours produced by thin laminae; the succession of colours as angle of vision changes being as in Newton's rings (FOX & VEVERS 1960).

The regular oscillating character of the cylinder curves (text fig. 23) indicate that the backscattering can be regarded as a simple interference effect, the maxima corresponding to constructive interference and the minima to destructive interference. I will show, using FARONE *et al.*'s (1963) data, that the backscattering from the cylinders may as a first approximation be considered as produced by interference between two axial rays, one reflected from the front surface of the cylinder and one reflected from the rear surface after having transversed the cylinder.

A dielectric cylinder with diameter d and refractive index n is considered. The difference in optical path length between the two above mentioned axial rays is $2 \cdot d \cdot n$ and the phase difference δ. The two rays will exhibit constructive interference with wave-length maximum λ_{max} given by

$$2 \cdot d \cdot n = \left(m \pm \frac{\delta}{2 \cdot \pi} \right) \lambda_{max} \quad (m = 0, 1, 2, \ldots .)$$

Putting $\alpha_{max} = \dfrac{\pi \cdot d}{\lambda_{max}}$, we obtain:

$$\alpha_{max} = \left(m \pm \frac{\delta}{2 \cdot \pi} \right) \frac{\pi}{2 \cdot n}.$$

The phase difference can be considered constant and so we have to expect maxima of i_1 and i_2 to be separated $\dfrac{\pi}{2 \cdot n}$ along the α-axis. Table 9 shows the positions as found from the curves of FARONE *et al.* (1963) putting $n = 1.54$, corresponding to $\dfrac{\pi}{2 \cdot n} = 1.02$.

The agreement with the expected separation of the peaks is seen to be good, in particular for i_1.

TABLE 9.

Positions of intensity peaks for backwards scattered light as a function of $\alpha = \frac{\pi \cdot d}{\lambda}$. Here i_1 is incident plane polarized radiation with the electric field vector parallel to the zylinder axis, and i_2 correspondingly, with the electric field vector perpendicular to the cylinder axis. Values have been taken from the curves given by FARONE *et al.* (1963).

	α_{max}			
i_1	0.88	1.94	2.93	3.95
i_2	0.95	1.93	2.74	3.73

The finding, that backscattering from cylinders can as a first approximation be calculated in a simple way using geometrical optics, is in agreement with studies on spheres (including bubbles) and on dielectric-coated, infinite cylinders (cited in KERKER 1969).

We then return to the question of whether the colour of the spongy structure should be classified as an interference or a scattering phenomenon. From the above said it is evident that backscattering from the individual cylinders has to be regarded as an interference phenomenon. That the colour of the barbs nevertheless does not appear iridescent is of course related to the fact that the spongy structure has the scattering cylinders randomly oriented. Thus when the feather is observed in diffuse light backscattering occurs in all directions with the result that the colour appears constant and diffuse, while in unidirectional light the feather appears much more brilliantly coloured when viewed along than when viewed at right angles to or against the incoming light.

Regarding the colour as being produced by interference is in agreement with RAMAN (1935) who stated: "Only in the case of simple spherical cavities would we be justified in regarding the colours as a diffraction (scattering – J.D.) effect. With elongated or flattened cavities, the phenomenon should be assimilated with those of interference".

It should be pointed out in relation to Raman's statement that it is the dimensions rather than the shape of the cavities that determine whether interference colours may result. Also for spheres with radii sufficient great to be comparable with the wavelength brilliantly coloured scattering due to interference may be observed (Tyndall spectra of higher orders, KERKER *et al.* 1966).

Interference colours of animals are often produced by multilayers consisting of a succession of homogeneous layers of alternately low and high refractive indices (e.g. sunbird feathers (DURRER & VILLIGER 1962)). In case the two kinds of layers are the same optical thickness, systems of very high reflectivity result. Is there any evidence for such a system in the spongy structure? Two neighbour hollow cylinders may be considered separated by an air-layer of thickness corresponding to diameter of an air-filled channel. Since the diameter of the hollow cylinder is greater in the green than in the blue spongy structure we should also expect the channel diameter

to be greater in the former than in the latter type. Actually the reverse is the case, the channel diameter of blue and green spongy structures being 114.0 and 103.6 nm respectively, so that the spongy structure cannot be described as an multilayer system of the above-mentioned type.

To summarize, it may be said that the blue and blue-green colours produced by the spongy structure of *A. r.* barbs are due principally, not to Rayleigh scattering, but to the interference of light. The interference is probably due principally to back-scattering from the numerous hollow, randomly oriented keratin cylinders (fig. 12) of which the spongy structure may be considered to consist.

Since most investigated species show a spongy structure very similar to that of *Agapornis* (p. 45) this probably applies to most instances of blue and green barbs.

The range of colours which may be produced by spongy medullary cells probably is not to be limited to blue and bluish green. Thus the colour of violet barbs is probably due to a spongy structure with dimensions differing from those of blue and bluish green barbs (Auber 1941, 1957 b). It is possible that other barb colours are produced by varying the dimensions of the spongy structure and observations indicate this possibility too: If a green barb of *A. r.* from which the yellow pigment has been extracted is observed in reflected light most medullary cells appear bluish green, but considerable colour variation between individual cells is observed and blue, green and even yellowish green cells are found. The colour of the individual cells is constant, independent of illumination variations and tilting and rotating of the barb, so there can be little doubt that these colour variations are due to variations in the spongy structure between different cells.

Raman (1935) has observed a similar variation in cell colour in a barb from a turquoise wing covert of *Coracias benghalensis* (*indicas*) where even some orange cells were found.

Nomenclature. A few words may be added on the nomenclature of the colour-producing structure of the medullary cells, in this paper referred to as spongy structure. The structure was by one of the earliest workers (Häcker 1890) considered to be the thickened, porous walls of the medullary cells (Schirmzellen) and this term has repeatedly been used up to the present time (Strong 1902, Kniesche 1914, Bancroft *et al.* 1923, Kawamura 1935, Frank 1939, Rawles 1960). It is evident that this term is inadequate, as the structure, in fact, has nothing to do with cell walls; it is a constituent of the cell interior as is the keratin of the cortex cells. Auber (1957 b) called the structure "cloudy medium"; this term also does not seem adequate to me, since one associates it with separated particles, not with connected channels and rods as actually found. Other more descriptive terms as "thick keratin containing sub-microscopic air spaces" (Fox and Vevers 1960) are not practical and I therefore suggest use of the term used here, namely spongy structure.

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Færdig fra trykkeriet den 30. juni 1971.

PLATES

Key to Labelling:

B: Barbule
Ba: Base of barbule
C: Cortex
Ca: Cavity containing melanin granule
CB: Cell boundary
Ch: Air-filled channel in spongy structure
Cl: Cilium of barbule
CP: Cortex proper
DM: Electron-dense component in keratinized cells
E: Epicuticle
FC: Fluorescing cortex
H: Hooklet
KR: Keratin rod
L: Ledge for barbules
M: Melanin granule(s)
MC: Medullary cell
MP: Melanized portion of medulla
o: Obverse part of barbule
P: Pennulum of barbule
Pv: Peripheral vacuole
R: Reverse ridge of barbule
S: Spongy structure
SL: Surface layer
V: Nuclear vacuole
Y: Non-fluorescent yellow pigment

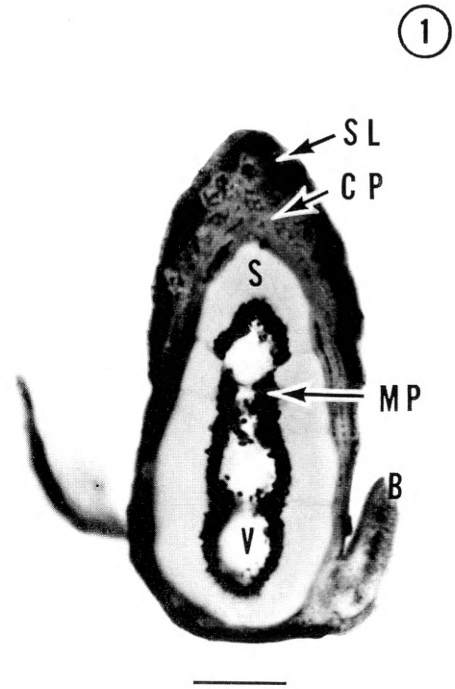
PLATE I.

Fig. 1. Transverse section of green barb terminally. Cortex consisting of heavily staining surface layer and less staining cortex proper. Medulla with ring of spongy structure, melanized portion and vacuoles. Scale: 10 μ. Toluidine blue.

Fig. 2. Transverse section of blue barb terminally. Scale: as fig. 1. Toluidine blue.

Fig. 3. Transverse section of green barb terminally illuminated with ultra-violet light. Note fluorescing areas in cortex divided by narrow non-fluorescent zones (cell boundaries?). Scale: as fig. 1.

Fig. 4. Transverse section of cortex of blue barb terminally. Note flattened cells, irregular network of electron-dense material in surface cells, epicuticle on outer surface and convoluted cell boundary between surface cells (CB, left arrow). Scale: 0,5 μ. Uranyl acetate + lead hydroxide.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MP
B
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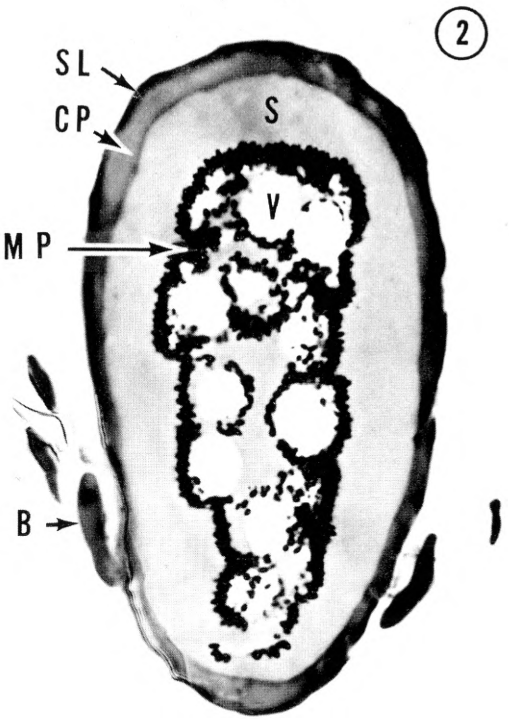
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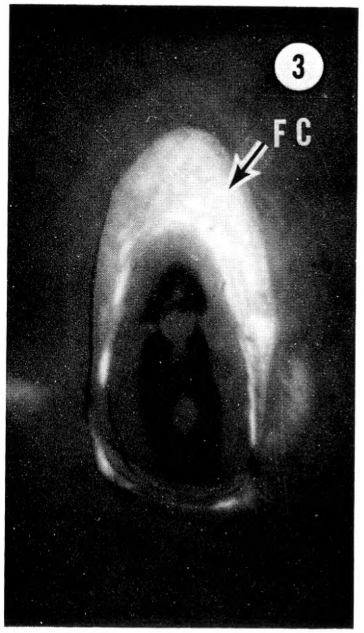
3
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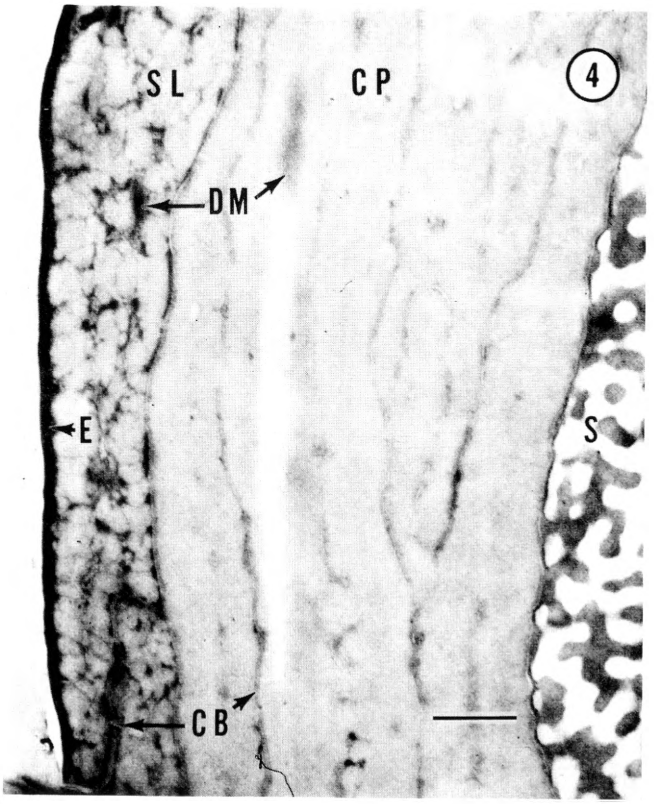
SL
CP
4
DM
E
S
CB

PLATE II.

Fig. 5. Transverse section of cortex and adjacent medullary cells of blue barb basally. Note the highly irregular cell boundaries where a barbule inserts, the dark central band between cell membranes (CB arrow), narrow bands of electron-dense material in cortex cells, spongy structure in medullary cells. Insets show boundaries between medullary cells at higher magnification. Left inset: Note frequent constrictions of cell boundary with central dense band disappearing (arrows). Right inset: Note thickened, very dense cell membranes and absence of central dense band between them, where cell boundary is bordered by air spaces on both sides (arrows 1, 2), contrasting with typical configuration, where cell boundary is bordered by keratin rods (arrows 3, 4). Scale of main picture: 0,5 μ, of insets: 0,2 μ. Uranyl acetate + lead hydroxide.

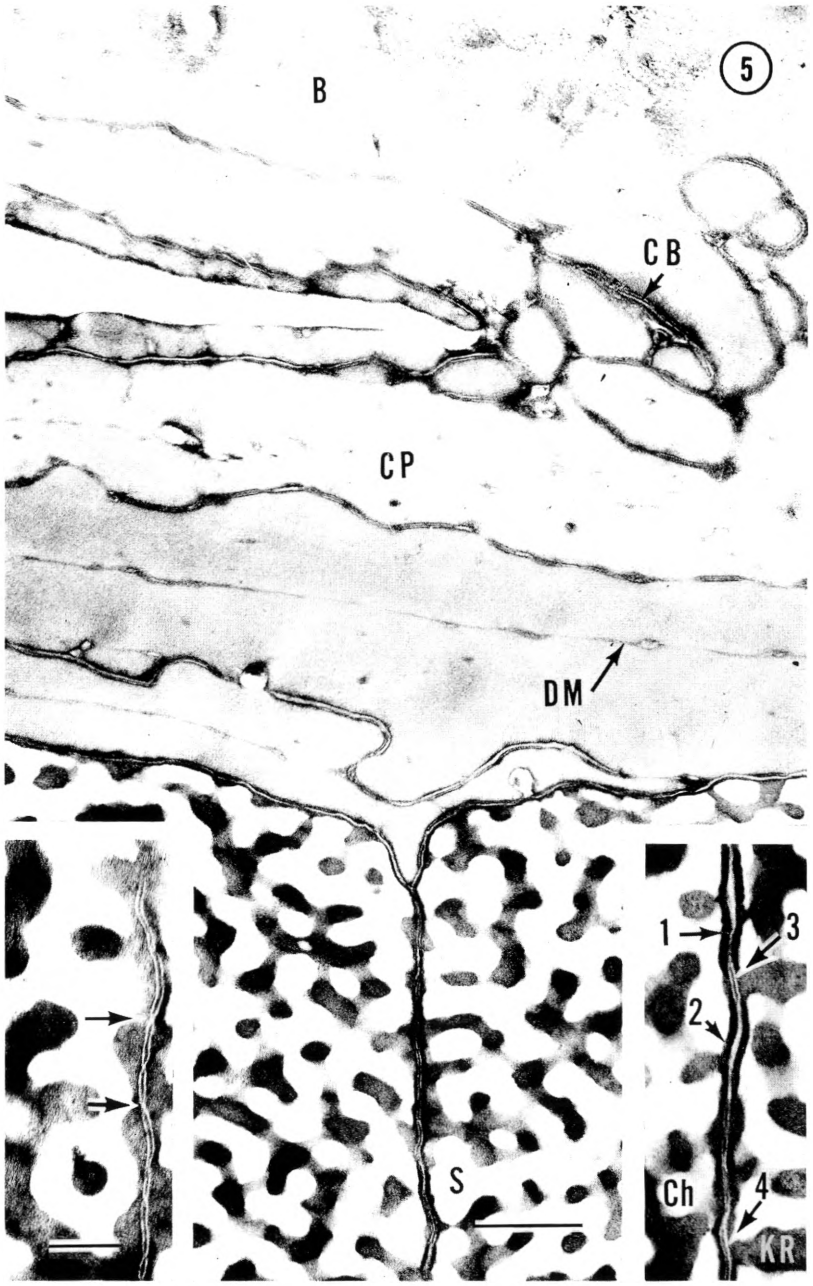
5
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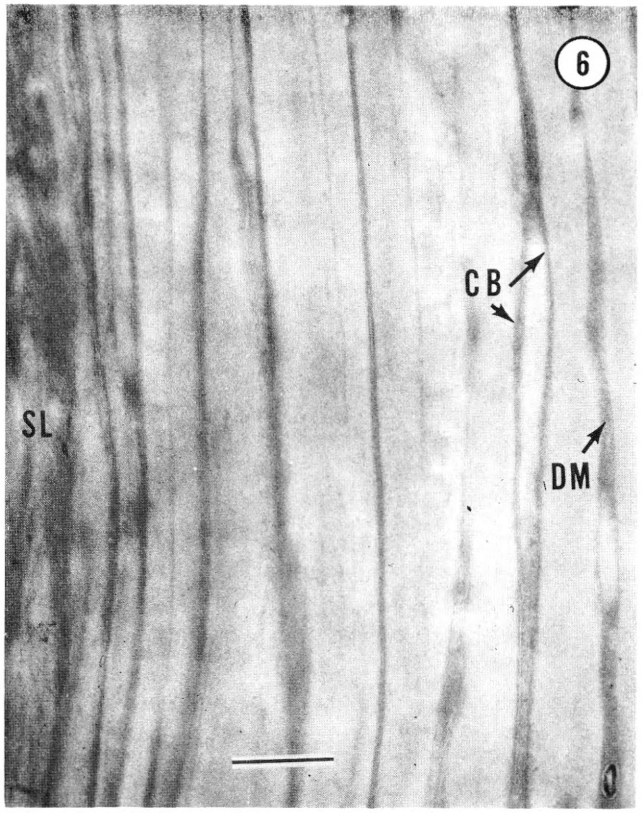
PLATE III.

Fig. 6. Longitudinal section of cortex of blue barb basally. Note elongated, spindle-shaped cortex cells with longitudinally oriented bands of electron-dense material. Scale: 0.5 μ. Uranyl acetate.

Fig. 7. Transverse section of barb of dark green back feather terminally. Note central vacuoles surrounded by only one layer of melanin granules and absorption of upwardly directed, transmitted light by melanin granules (comp. text p. 42). Scale: 5 μ. Lead hydroxide.

Fig. 8. Transverse section of thickened obverse cortex of barb of dark green back feather terminally. Note high concentration of non-fluorescent yellow pigment in surface cells contrasting with low concentration in cells of cortex proper. Scale: 2 μ. Uranyl acetate + lead hydroxide.

Fig. 9. As fig. 8, but after extraction of non-fluorescent yellow pigment. Note contrast between densities of spongy structures of obverse and lateral medullary cells. Scale: as fig. 8. Uranyl acetate + lead hydroxide.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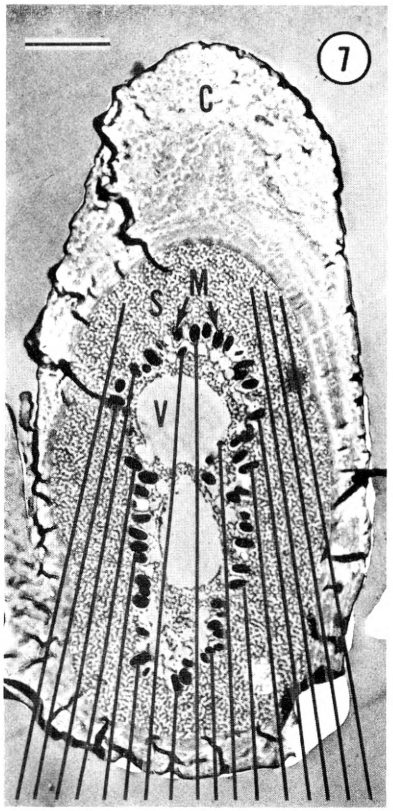
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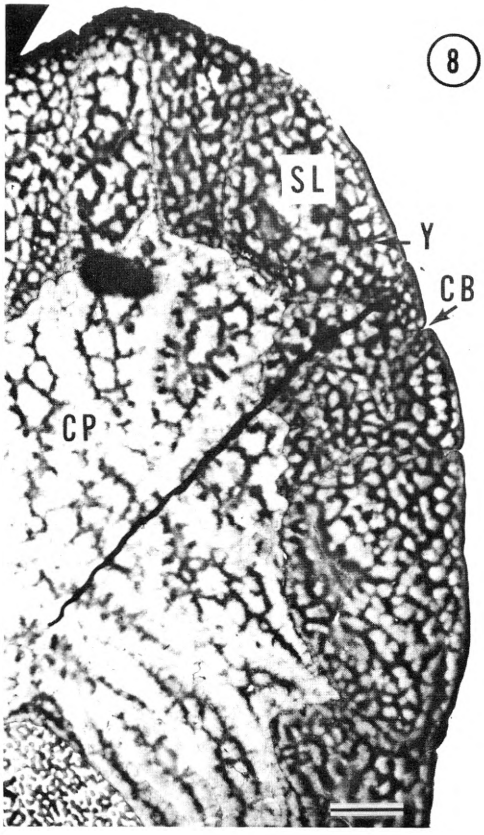
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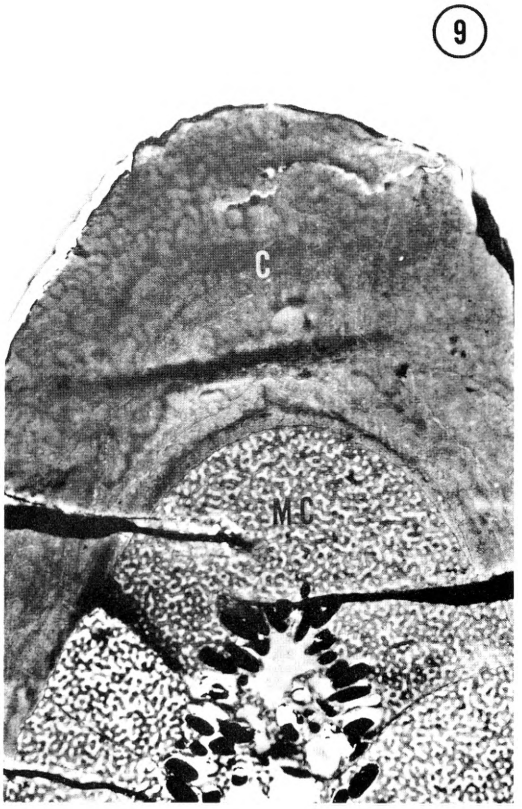
9
C
MC

PLATE IV.

Fig. 10. Transverse section of blue barb terminally. Note melanin granules surrounding central medulla with great, nearly circular nuclear vacuoles. Scale: 5 μ. Uranyl acetate + lead hydroxide.

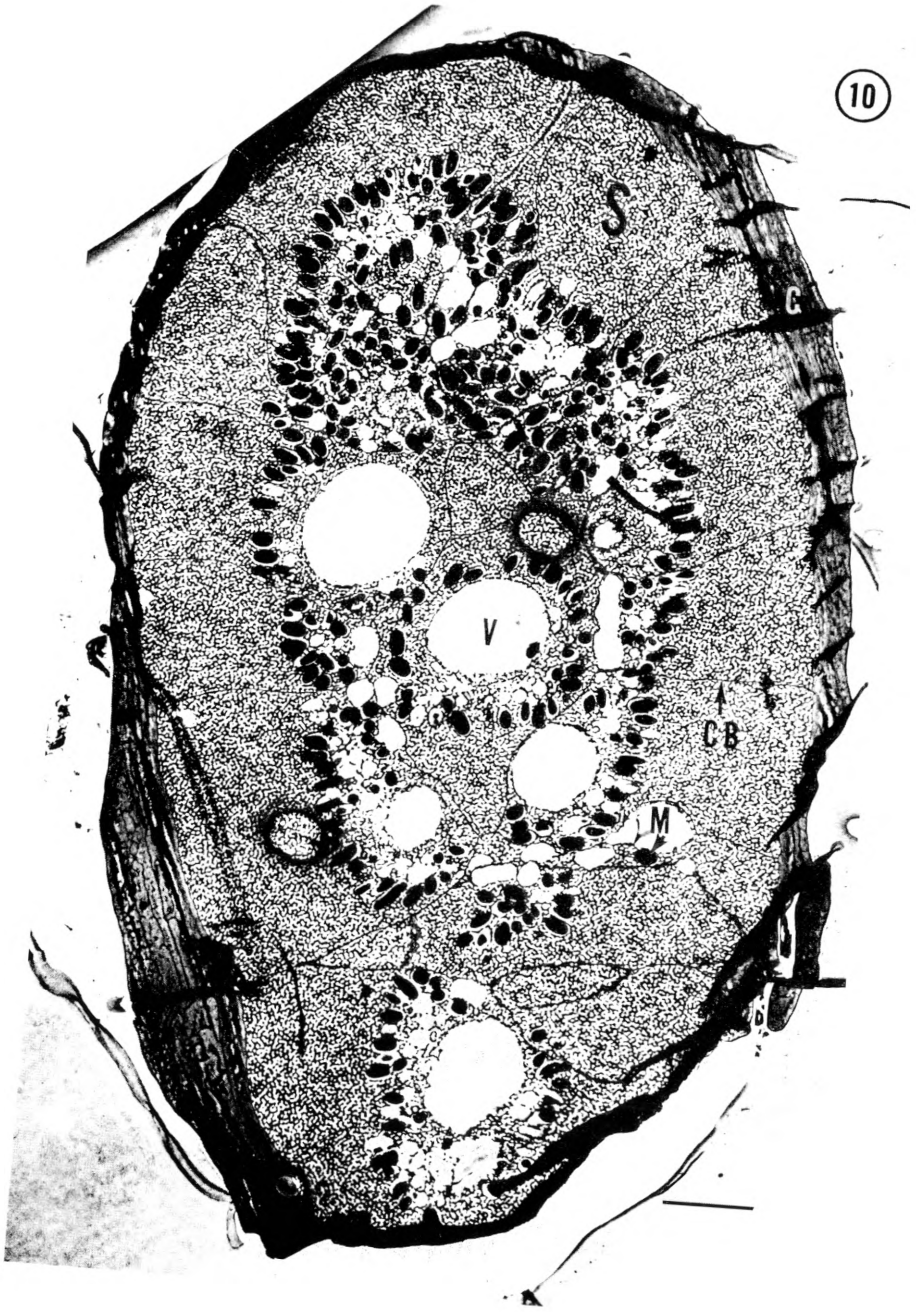
10
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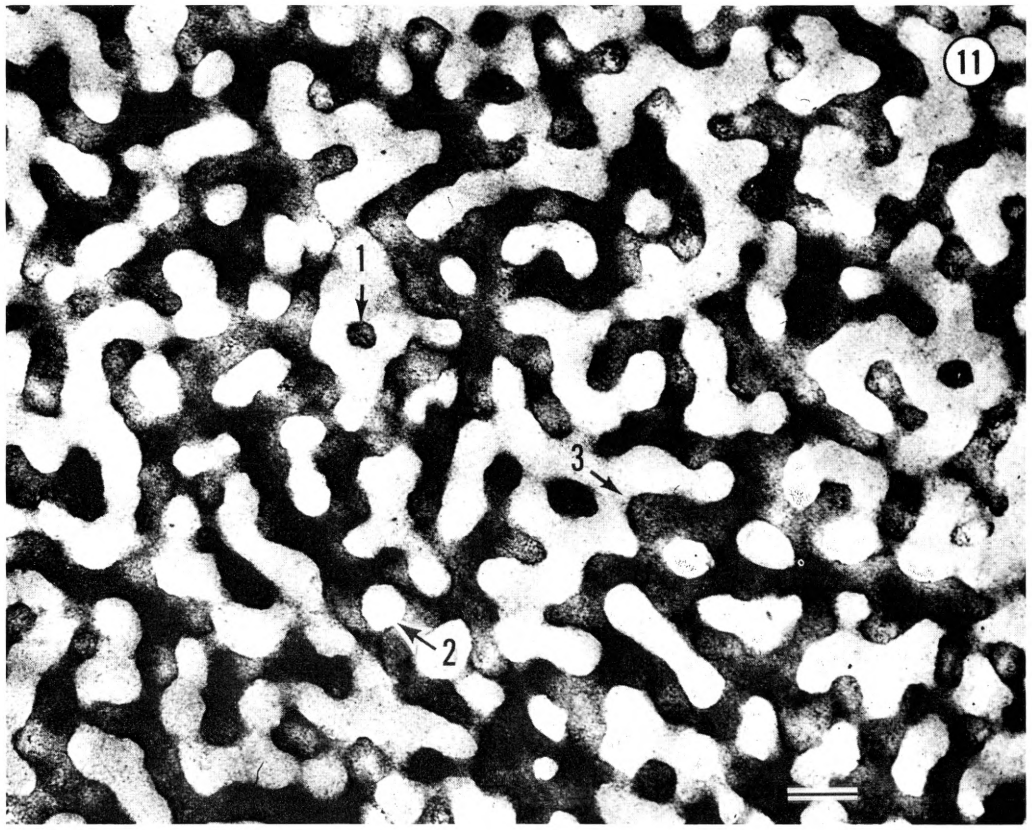
PLATE V.

Fig. 11. Transverse section of spongy structure of blue barb terminally. Note circular cross-section of isolated keratin rods (arrow 1) and air-filled channels (arrow 2), many sharp bends of rods and channels (arrow 3) and the very constant dimensions of rods and channels. Scale: 0.2 μ. Uranyl acetate + lead hydroxide.

Fig. 12. Transverse section of spongy structure of green barb terminally. Note wider keratin rods and narrower air-filled channels compared to spongy structure of blue barb (fig. 11). Encircled: Cross-section of what is considered to be the 'functional unit' in colour production, namely a hollow keratin cylinder with an air-filled core. Scale: as fig. 11. Uranyl acetate + lead hydroxide.

Fig. 13. As fig. 12 but stained only with lead hydroxide. Note keratin rods staining denser on their surfaces than inside. Scale: as fig. 11.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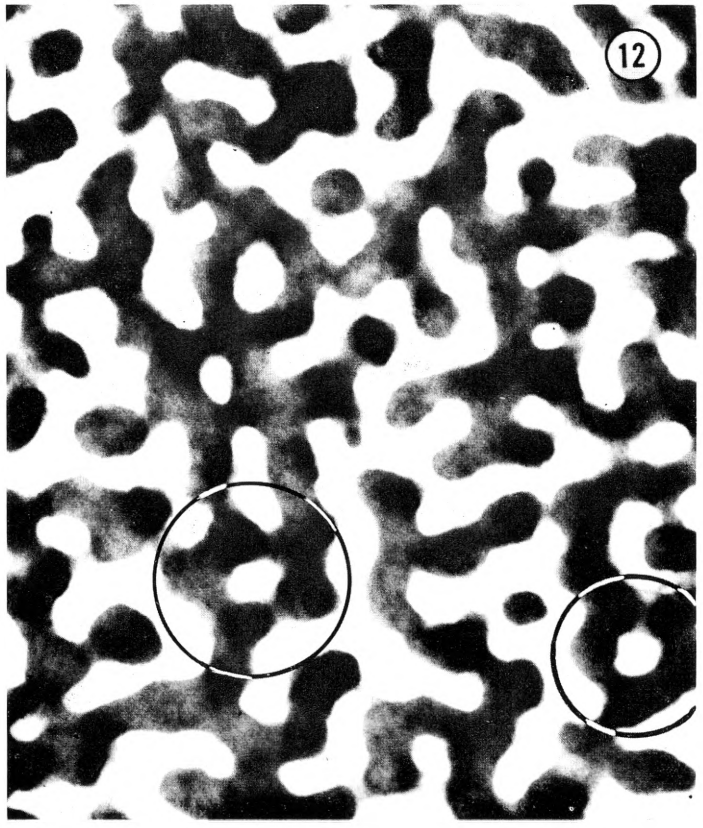
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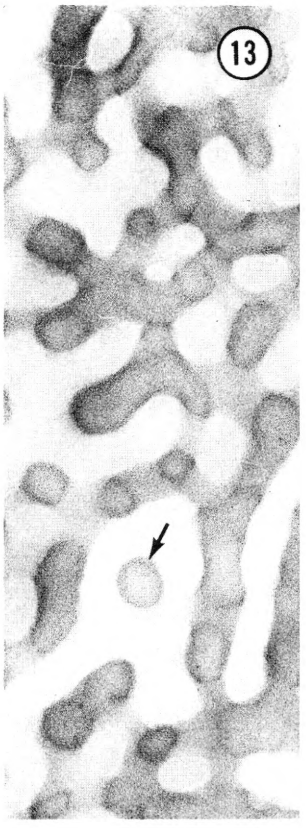

13

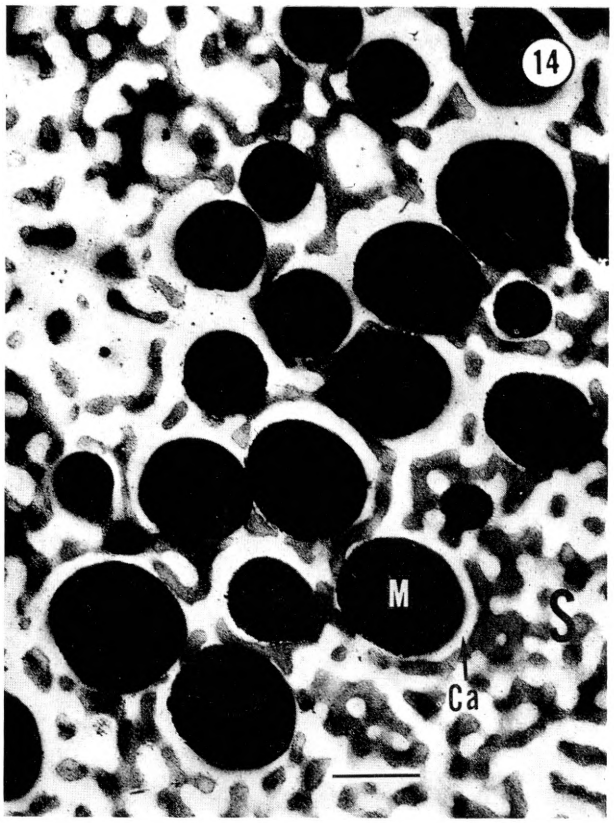
PLATE VI.

Fig. 14. Longitudinally section of blue barb basally. Melanin granules in spongy structure. Note regular, circular outlines of transversely sectioned melanin granules situated in well-fitting cavities with smooth inner and irregular outer surfaces. Scale: 0.25 μ. Uranyl acetate + lead hydroxide.

Fig. 15. Transverse section of medullary melanin granule. Note dense, dendritic material. White dots are probably artefacts. Scale: 0.1 μ. Lead hydroxide.

Fig. 16. Longitudinal section of blue barb basally. Note regular, polygonal outline of medullary cell and presence of a small, peripheral vacuole. Inset: Keratinuous body in peripheral vacuole. Scale: 0.2 μ. Uranyl acetate + lead hydroxide.

Fig. 17. Transverse section of blue barb terminally. Part of wall of nuclear vacuole with adjacent melanin granules. Note more varying dimensions of keratin rods of nuclear vacuole wall compared to those of spongy structure. Inset: Melanin granule with wavy outline. Scale: as fig. 14. Uranyl acetate + lead hydroxide (inset: lead hydroxide only).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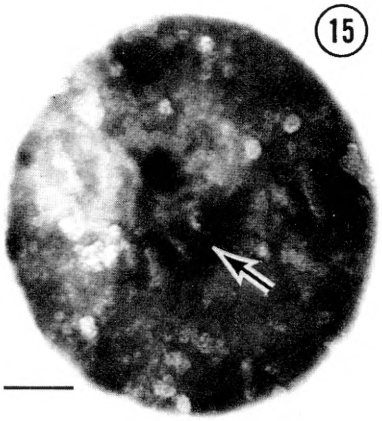
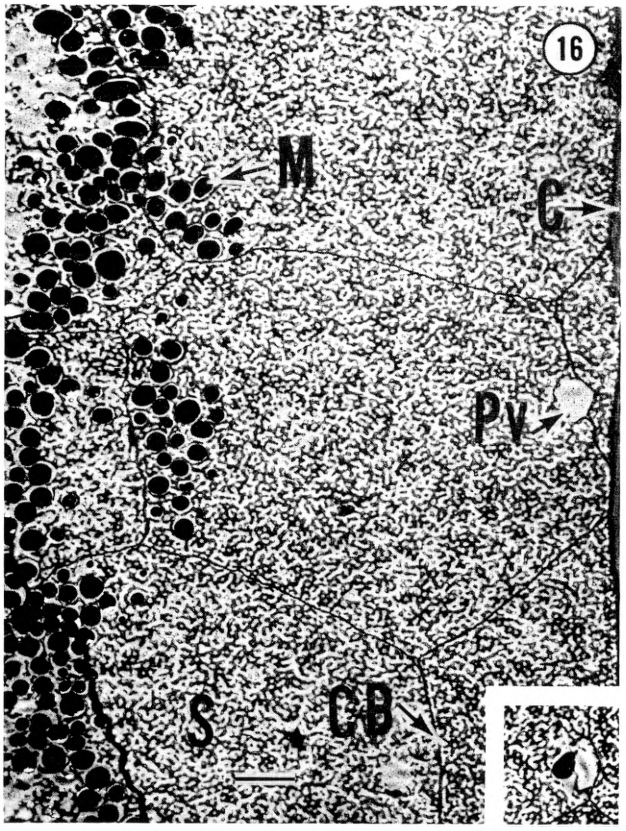

15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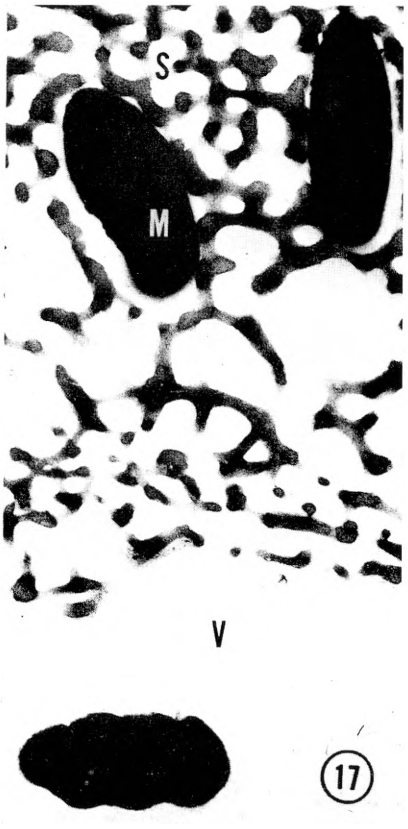
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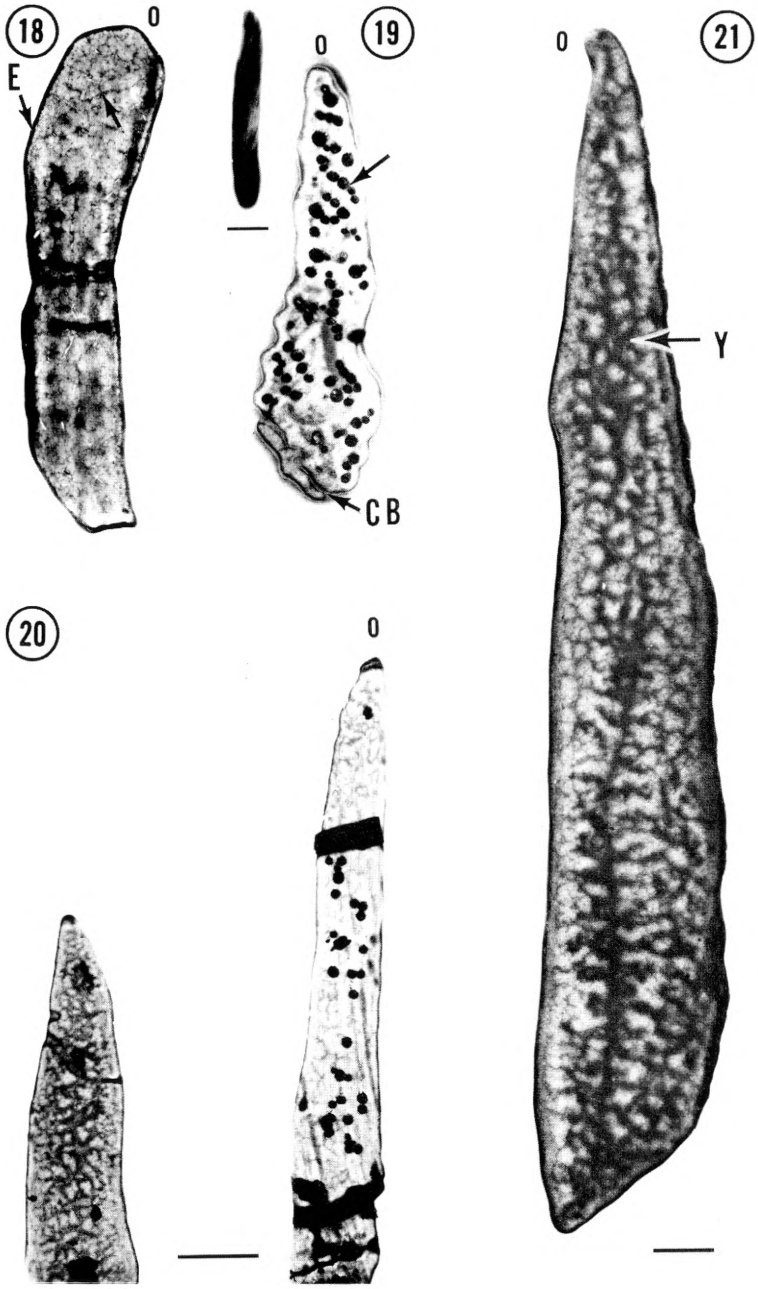
PLATE VII.

Fig. 18. Transverse section of barbule attaching terminally on blue barb. Note form of barbule and network of dense material (arrow). Scale: as fig. 21. Uranyl acetate + lead hydroxide.

Fig. 19. Transverse section of melanin pigmented part of barbule attaching terminally on barb of dark green back feather. Note wavy surface of barbule and circular cross-sections of melanin granules found in small linearly arranged clusters (upper arrow). Inset: Rod-shaped melanin granule in longitudinal section. Scale: barbule as fig. 21, inset: 0.2 μ. Osmium + lead hydroxide.

Fig. 20. Transverse sections of yellow (left) and melanin pigmented (right) portions of barbules attaching terminally on barb of dark green back feather. Note lack of non-fluorescent yellow pigment in melanin pigmented part of barbule. Scale: 2 μ. Uranyl acetate + lead hydroxide.

Fig. 21. Transverse section of yellow pigmented part of barbule attaching terminally on barb of dark green back feather. Note form of barbule and distribution of non-fluorescent yellow pigment. Scale: 1 μ.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20
O

PLATE VIII.

Fig. 1. Upper parts of *Agapornis roseicollis* showing contrast between dark green wings and blue rump. Enlargement: × 1.3.

Fig. 2. Feathers from different plumage regions of *Agapornis roseicollis*. Upper left: Dark green back feather, Upper right: Green belly feather, Lower left: Dark green back feather, reverse side exposed, Lower right: Blue rump feather. Enlargement: × 1.

Fig. 3. Green belly feather. Terminal portion of green barb with attached yellow barbules. Enlargement: × 48.

Fig. 4. Dark green back feather. Green barb with attached yellow and black barbules. More basally on barbs than fig. 3. Enlargement: as fig. 3.

Fig. 5. Blue rump feather. Terminal portion of blue barb with attached white (and more basally partly black) barbules. Note glossy appearance of barbules, especially those attaching most terminally on barb. Enlargement: as fig. 3.

Fig. 6. Transverse section of barb of dark green back feather in reflected light. Barb 10 l (length 10.5 mm) cut 7.1 mm from terminal tip. Note the strongly reflecting spongy structure that appears to consist of separated shining spots and stripes. 'Wild' accessory for reflected light, Epi-Achromate 40 x, NA: 0.65; light directed through objective (perpendicular incidence). Blue filter. Enlargement: × 600.

Det Kongelige Danske Videnskabernes Selskab

Biologiske Skrifter

Biol. Skr. Dan. Vid. Selsk.

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kr. ø.

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Bind **18**

(*uafsluttet/in preparation*)

1. HANSEN, GEORG NØRGAARD: On the Structure and Vascularization of the Pituitary Gland in some Primitive Actinopterygians (*Acipenser, Polyodon, Calamoichthys, Polypterus, Lepisosteus* and *Amia*). 1971 ..... 45.–
2. DYCK, JAN: Structure and Spectral Reflectance of Green and Blue Feathers of the Roce-faced Lovebird (*Agapornis roseicollis*). 1971..... 45.–

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