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Colouration and Fading: How do Pigments Become Degraded or Altered by Light and their Environment

- Bob Stoddart

A. Introduction

Many natural history specimens show distinctive colours, which often prove challenging to preserve over long periods and may be very labile to drying, fixation or exposure to preservative solutions or air. In most instances, the instability of colour is the unavoidable consequence of the chemistry necessary for a pigment to be coloured in the first place. Similarly, the tendency of pigments to leach is also a reflection of their necessary chemistry and its consequences for hydrophobicity or amphoteric behaviour. Some colouration in natural history specimens is, however, essentially physical in origin, its being essentially a product of reflectance, refraction, interference or some combination of these, but such colouration can also be unstable and the reasons for this are considered first.

B. Interference

Interference patterns can arise where light passes through or is reflected from a *regularly* patterned surface, where the spacing of the pattern is sufficiently fine to produce an effect in the visible part of the spectrum. Examples of such colours are known from the wing-cases of many beetles, the bodies of some flies, wings of various insects, scales of many fish and shells of some molluscs: they often show a silvered or lustrous appearance. Such colours originate in the same way as those produced by interference filters. Similar interference effects can also occur where light passes through transparent films of appropriate thinness and reflects from their upper and lower surface as in thin films of oil on water or the coatings of lenses in optical instruments or spectacles. In each case, rays of light are produced, either by transmission or reflection, the phases of which are sufficiently 'offset; to cause interference and so lead to the deletion of particular wavelengths from visible white light, so giving colour. Such colouration is extremely sensitive to changes in the periodicity or regularity of the 'interference filters' from which they arise. An alteration of period *with retention of regularity* leads to a shift in the wavelengths deleted and, hence, a change of colour. *Loss of regular periodicity* abolishes colour. This has clear implications for the possible effects of desiccation or fixation upon some biological tissues, where either type of effect above could follow. Where interference is dependant upon total internal reflection at a boundary where there is a change of refractive index, it may also be very sensitive to any alteration in refractive index, such as that produced by immersing something normally viewed in air in a preservative fluid, or its converse – such as taking a fish out of water. The effect may be either the loss of colour or its being visible only from a different viewpoint.

C. Reflection and Refraction

Reflection and lensing effects occur on occasion. Many vertebrates show reflection from layers behind the retina when viewed 'head-on': typically they are species which hunt nocturnally or in poor light, such as cats, and the reflection affords a double-pass of light to the sensors in the retina. A few plants, such as the protonema of the moss *Schistostega*, have lens-like cell walls that focus light onto the chloroplasts, from which it is reflected, producing a shimmering gold appearance. Any change in the curvature of the lens or in the refractive index of the medium outside disturbs the optics, abolishes the effect and colour is lost. Hence, such apparent colour is extremely labile to drying or immersion in preservative fluids.

D. Pigmentation

Most colouration of natural history specimens arises from pigmentation. A few organisms may fluoresce if excited at visible wavelengths and many more are fluorescent under ultra-violet light but, in general, colour arises from the absorption of particular wavelengths from transmitted or reflected white light, so that the unabsorbed wavelengths give rise to the observed colour. Bioluminescence is a property of living cells and is not a phenomenon seen in dead tissues, except where it is the result of putrefaction and arises from the processes of decay – generally from a saprophyte.

When pigments absorb light they do so by taking up photons and promoting electrons from lower to higher energy levels: thus the pigment absorbs energy from light and stores it in the form of more energetic electrons than before. This energy can be dissipated by allowing the electrons to fall back to lower energy levels, usually in a series of steps, with the re-emission of energy in the form of photons of lower energy than those which caused the initial excitation. This is fluorescence. Alternatively, the energy can be dissipated in chemical reactions which will alter the pigment, or it can be transferred to other molecules which may, in

turn, fluoresce or undergo chemical change. It may be lost in the activation of water molecules and can eventually appear as heat by a variety of routes. However the energy is lost, the pigment cannot absorb again until its electrons have returned to a lower energy level.

The energy levels of the electrons in a molecule or atom are not continuously variable, but have precise, fixed values and transitions between them *must* occur by the absorption or loss of specific amounts of energy. Hence a pigment will absorb at specific wavelengths that correspond to the energies of these transitions. Most pigments in natural history specimens are organic chemicals; a few, such as haem or chlorophyll are organometallic compounds in which a metal (often a transition metal such as iron) interacts with an organic molecule. Pigmentation by metals alone is rare.

The energy levels of the electrons in a molecule reflect its chemistry. In most naturally occurring pigments the part of the molecule that absorbs visible light (the chromophore) is a structure extending over several atoms, such that some electrons can move across the domains of several atomic nuclei (ie. They occupy *molecular* orbitals, rather than being confined to *atomic* orbitals). The length of the system is the primary determinant of the wavelengths absorbed, but this is modified by the nature of and polarity of interacting substituent groups which may 'fine tune' the colour produced. Commonly pigments show extended chains of linkages which are usually represented as alternating single and double bonds between carbon atoms. The carotenoids (fig 1a) are a good example. As a group of pigments they share a common backbone of isoprene units in which the chromophore is composed of alternating double and single bonds and confers the ability to absorb with maxima at about 485, 455 and 425 nm, so generally giving a yellow, orange or red pigment. The exact shade of the colour is modified by variable, linked structures at each end of the chromophore.

Structure of this type will show all the reactions expected of this class of molecule (ie. all the reactions of olefinic structures). Any such reaction which interrupts the alternating sequence of conjugated double bonds in the chromophore will alter or abolish colouration. If the interruption is near the end of the chromophore the colour may not be wholly lost, but absorption will change to a shorter wavelength and so the colour will alter. Interruption near the centre of the chromophore will shift absorption out of the visible range of wavelengths and colour will be lost. If an addition occurs which extends the chromophore, colour will also change, as the wavelength absorbed will be longer, provided that it remains in the visible range.

Carotenoids are susceptible to reducing agents, which convert – CH = CH – to - CH2 – CH2 - and so interrupt the chromophore. Oxidising agents which form epoxides with the double bonds can split the chromophore and so also cause loss and change of colour. Many types of addition reaction are possible, such as halogenation by chlorine or bromine, so that solutions of hypochlorite can cause bleaching. Sensitivity to oxidation – reduction state should particularly be considered when considering pigments that are chemically related to redox carriers such as the pteridines and carotenoids. Reduced or oxidised, colourless derivatives of pigments may remain even when a specimen has apparently faded.

Some pigments, such as the porphyrins (fig 1b), have chromophore systems which are conjugated in such a way that they form large loops. In systems of this kind, the loss of one double bond does not interrupt the chromophore completely and, while colour may be modestly altered, the molecule as a whole will still absorb light. If the large ring is broken, however, the molecule will become a more or less linear tetrapyrrole (as in the conversion of haem to bile pigments), the length of the chromophore will be increased and the colour of the pigment will alter.

A further change will also occur. Chromophores absorb light most efficiently when light waves are aligned with the axis of the chromophore and least efficiently when they are at right angles to it. Thus a linear chromophore absorbs effectively only when in a particular orientation. In contrast, a molecule such as haem can absorb effectively in a much wider range of orientations and, because a solution of a pigment will have molecules in all orientations within it, a solution of (for example) a porphyrin will have a larger molar extinction coefficient than (for example) will a carotenoid. Hence the conversion of haem to a bile pigment will lead to less efficient absorption of light.

Where tetrapyrrole structures, such as porphyrins, are conjugated to metals, as in haem or chlorophyll, some of their absorption of light is attributable to their interaction with the metal. If the metal is lost, colour will change, as in the chlorophylls. Occasionally, the presence of metal shifts a very intense absorption band from the near ultra-violet into the visible range, generating very deep colouration, as in the manganese-

porphyrins of some avian feathers. If the metal is removed by weakly alkaline solutions, massive loss of colour occurs.

The effects of polar substituents upon the colour of pigments is well illustrated by the anthocyanin pigments of red, purple and blue flowers (fig 2) in which the particular shade of colour is highly sensitive to the number and position of – OH and - O methyl substituents upon the core structure and to the nature of the sugar to which the anthocyanidin is glycosidically linked. Hydrolysis of the glycoside affects both the colour of the pigment and its water – solubility. The anthocyanidins are also sensitive to pH and their colour depends upon whether they are present in charged, uncharged or quinonoid forms. Litmus is a mixture of anthcyanidins and the colour change is exploited in indicator paper. These changes are reversible, but have implications for changes in preservative fluids and atmospheric acidity.

The phenolic hydroxyl groups of anthocyanidins, in common with other phenols, can form adducts with ferric ions to yield deeply coloured coordinaton complexes. This change can occur in old specimens in fluid collections over time and may be followed by oxidative damage catalysed by the metal ions.

Many plant phenols show a tendency, over time, to undergo oxidative polymerisation, with attendant formation of larger chromophores and deeper and more red-brown or blackish colour. This process occurs naturally during lignification and on wounding of many plant tissues and is catalysed by polyphenol oxidases which can be relatively resistant to denaturation and may persist in preserved specimens for an extended period. Their action is the basis of tea fermentation, in which polymeric derivatives of catechins, gallocatechins, epigallocatechins and epigallocatechin gallates are oxidised; the result is the range of colours of tea, especially of black tea.

The chemistry of many classes of pigment also confers upon them characteristic patterns of solubility and susceptibility to leaching in specimens stored in preservative fluids. The linear, rather hydrophobic carotenoids and the bile pigments are sparingly soluble in water, but more soluble in alcohol and are readily absorbed into fat. Porphyrins, the chlorophylls and related pigments tend to be soluble in polar organic solvents, especially in the presence of a little acid or alkali. Anthocyanins and the smaller plant phenols are rather soluble in water and, in some cases, in aqueous alcohol, but the larger phenols are insoluble in most solvents. All of this and the pH-sensitivity of some pigments has obvious major implications for the design of preservative fluids.

No form of preservative is wholly suitable for all pigments and some preservatives and fixatives may interact with them chemically. Picric acid can interact with DNA by its insertion between the bases of the double helix to form a charge-transfer complex and it can, at least in principal, show similar behaviour with pigments that have flat ring structures, especially if they contain basic groups. This can produce large shifts of colour. Formalin is a reducing agent and, over time and in the presence of oxygen, it can alter colours. Alcohol, acetone, chloroform and may other solvents can leach some pigments. However, the 'signatures' of the pigments are not wholly lost and their colourless derivatives may remain in the specimen, or the fluid around it, and be capable of identification by modern microanalytical methods. This should be considered when assessing whether specimens have deteriorated beyond use or salvage.