Biological activity of the juvenile hormone active principle in *Attacus atlas* L.

Fractions	Total *Galleria* Units (GU)	
Ether extract	134,357	
First TLC	46,689	
JH-1/JH-2/JH-3 corresponding fractions		46,689
Rest of the TLC plates		0
Final TLC	61,855	
Fractions 1 and 2		0
Fraction 3		3,333
Fraction 4		7,692
Fraction 5		50,000
Fraction 6		830
Fractions 7 and 8		0

The bioactive material from this first TLC work-up was further separated into 8 very narow bands by a final TLC in order to localize the active principle more accurately. The total recovered bioactivity of 61,855 GU is close to that found in the first TLC work-up (Table). The detailed analysis of each fraction revealed the highest activity of 80.8% in fraction 5 (TLC Rf = 0.49). An activity of 12.4% was detected in fraction 4 (Rf = 0.52), 5.4% in fraction 3 (Rf = 0.55) and traces of activity corresponding to 1.3% were found in fraction 6 (Rf = 0.47). Thus, the main active ingredient of *A. atlas* was localized in fractions 4 and 5 (total of 93.2% activity), which co-chromographs with JH-3 (Rf = 0.51). The low activity observed in fraction 3 (5.4%), on the other hand, behaved similarly to the internal standard JH-1 isomer mixture (Rf = 0.56). The remaining fractions were devoid of any bioactivity.

The bioactivity containing fractions were subjected to further chemical analysis. The detection limit of the high resolution glass capillary GC system used in this study is approximately 1.5 ng per cm peak height, for all 3 hormones. The total volume of the GC solution was 25 µl in hexane. With an injection volume of 1 µl, approximately 40 ng of each JH must be present in the 25 µl hexane solution in order to obtain a 1 cm peak height. Based on the JH equivalent of 70 pg for JH-3, an amount of 538 and 3,500 ng equivalent was estimated to be present in fractions 4 and 5, respectively. Although the computed JH equivalent amount was far above the GC detection limit, no peak corresponding to the known JH-3 was detected in either fraction. Based on the JH equivalent of 1.6 pg for JH-1, a 5 ng equivalent only was biologically quantified in fraction 3, an amount which is below the GC detection limit. Considering the estimate of JH presence by means of the *Galleria* bioassay as semiquantitative due to its unavoidable error, as well as the given GC detection limit, the result presented as far as JH-1 and JH-2 is concerned cannot be conclusive. However, it can be stated from the GC results that the possible content of JH-1 and JH-2 in *A. atlas* cannot exceed the amount of 0.1 ng per g fresh weight.

Based on the results presented, it may be concluded that the ether extracts of *A. atlas* investigated in this study do not contain JH-1, JH-2 or JH-3 at a concentration corresponding to more than 0.1 ng per g fresh weight. The remarkable biological activity which co-chromatographs with JH-3 in the TLC systems employed, and which corresponds to a concentration of JH-3 far above the detection limit of the chemical analysis, could not be characterized chemically. Further investigations will be necessary to identify the JH-active principle in *Attacus atlas* L.

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STUDIORUM PROGRESSUS

Transparency in Organisms

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Summary. The occurrence in animal phyla of species having a relatively transparent body is noted and measurements of the transmittance of medusae made in a spectrophotometer are reported, but the approximate nature of the results obtained with a commercial instrument and the importance of the correct physical design of the measuring apparatus are emphasized. The application to invertebrates of the structural explanation of the predominant transmission of incident light by the vertebrate cornea is discussed and the role of other factors considered. Destructive interference of the scattered rays, sufficient to account for the transparency of the cornea, has been shown not to demand a completely regular arrangement of collagen fibres. The small diameter and regularity of the fibrillar components in the muscles of *Sagitta* may be adequate to account for their transparency.

Introduction. Light which is incident upon living organisms can be absorbed, scattered, reflected or transmitted. The visibility of an organism to an observer depends upon which fate befalls the incident light or which fate predominates. Absorption is enhanced by the presence of pigment contained in cells or tissues and its importance in nature needs no emphasis. Scattering in a medium results from inhomogeneities which, in organisms, may include the presence of fibrils, particles and intracellular membranes separating regions of different composition and hence of different refractive index. As an extreme case reflexion or light may occur at crystalline surfaces in certain cells e.g. those underlying fish scales and those in the tapetum of the eyes of nocturnal mammals. Transmission, resulting in transparency, occurs among planktonic organisms and in specific regions in many animals especially in the dioptric apparatus of the eye and may be regarded as a special condition in that it is dependent upon the reduction or elimination of light scattered by the inhomogeneities.

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The physical means by which some organisms or parts of organisms are rendered transparent has apparently been investigated only in relation to the vertebrate eye. It is the purpose of this article to enquire into the applicability of our understanding of the transparency of the cornea to the general problem of transparency in the invertebrates. It is also pertinent to note that, whereas the study of many biological aspects of pigmentation is well advanced, little if anything seems to be known of the role of transparency in the life of transparent organisms in nature.

Occurrence of transparency. Like the planktonic mode of life, and often accompanying it, transparency occurs sporadically among the phyla, although a large proportion of some sub-groups such as the medusae, siphonophores and Thaliacea are both planktonic and relatively transparent. Where such animals are pigmented at all they are generally coloured blue or violet, e.g. *Rhizostoma, Cyanea, Velella, Ianthina, Clio, Doliolum* although there are exceptions to this such as *Physophora, Chrysaora* and *Beroë*. Other colourless or very lightly pigmented genera include *Aurelia,* many hydromedusae e.g. *Polyorchis,* many siphonophores e.g. *Muggiaea, Hippopodius,* ctenophores such as *Pleurobrachia,* annelids such as *Tomopteris,* the chaetognath, *Sagitta,* the crustaceans *Cystosoma* and *Phronima,* the molluscs *Carinaria* and *Gleba* and the urochordates *Salpa* and *Pyrosoma.* Needless to say, transparency is a matter of degree but some species such as *Sagitta* and *Tomopteris* could be described as 'glass-like'.

It is generally believed that the colouration and pigmentation of animals are adapted to the amount and spectral composition of the light present in the zones which they inhabit. The greater penetration of blue light into medium depths of oceanic waters means that a blue tinted organism will contrast less strongly with its surroundings, whether seen by transmitted or reflected light,

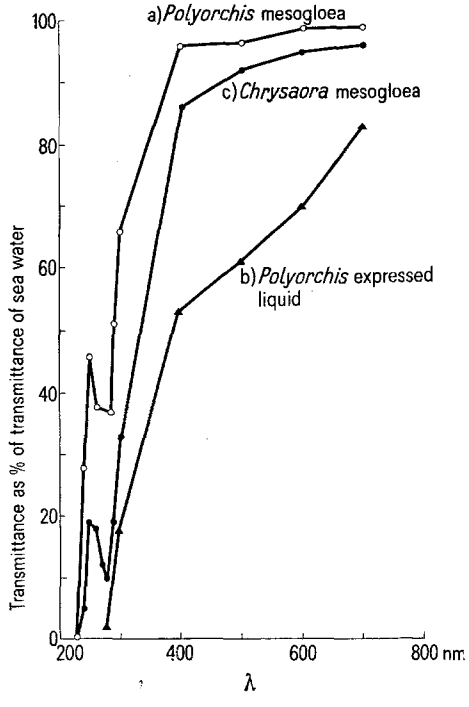

Fig. 1. Transmittance of mesogloea of a hydromedusan *Polyorchis* (a) and of liquid expressed from the mesogloea (b) and of the mesogloea of a scyphozoan *Chrysaora* (c), cut to fit the cuvette of a spectrophotometer, expressed as a percentage of the transmittance of sea water at various wavelengths.

than if it were coloured differently. In the dark abyssal zone organisms are generally heavily pigmented—often red or black. However the biological role of transparency of planktonic organisms inhabiting the uppermost layers appears to have received little attention. Presumably it confers a selective advantage such as by aiding approach to prey or avoidance of predators. An experimental investigation of this would seem to be possible using natural or artificially transparent or tinted specimens under laboratory conditions of controlled illumination.

Assessment of transparency. The assessment of transparency by measurement of the 'direct transmission factor' (transmittance) cannot accurately be carried out in a spectrophotometer although this instrument is commonly used for the purpose[2]. Many errors are liable to be present in a simple estimation of this kind made in a commercial instrument. These errors may arise from several causes the chief of which is forward scattering which results in the receiving aperture playing a critical role in the measurement of apparent transmitted light. However, a spectrophotometer would appear to provide a rough and ready means of assessing the transparency of organisms and some results obtained by its use are mentioned below.

Conditions giving rise to transparency. A number of conditions have to be fulfilled if incident light is to be transmitted freely through an object. Coloured or pigmented compounds must be absent. The material would be transparent if it consisted of a single component or phase e.g. a crystal, a solution of a crystalloid or possibly a gel which has a high transmittance in the visible spectrum. If the transparent material consists of two or more components these would normally be expected to have the same refractive index so that scattering or dispersion of light at the component boundaries is eliminated. This condition is unlikely to apply to any animal body, which is marked by division into cells, each bounded by a membrane, and within which submicroscopic structure is highly developed. However, many of the submicroscopic structures are of a size far below the 500 nm wavelength of visible light and may therefore be expected to be less important as scattering or refracting agents than structures which are of dimensions greater than the wavelength of light.

Effect of solutes on transparency. The solute concentration of many marine animals is similar to that of sea water, in ions at any rate, although their composition may differ somewhat. It is also a feature of planktonic animals that their density is close to that of sea water[3] and that this is partly achieved by their possessing a low concentration of organic materials. There is not a great deal of variation in the refractive index of solutions of different organic compounds[4]. In general 1 g/100 cm^3 produces a change in η of only 0.0018.

Uniformity of composition. While no animal body is of uniform composition some transparent structures such as the swimming bells of siphonophores and the bells of medusae consist of relatively thick regions of material in which clearly defined structural components are few and are generally in the form of fine fibres. Cells are confined to a layer on each side of the bell and are of a thickness measured in μm as compared with millimeters for the mesogloea.

Uniformity of refractive index. Uniformity of refractive index is also of importance (and was at one time thought

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[4] R. BAHER, *Weighing cells with the Microscope* in *Tools of Biological Research* (Ed. H. J. B. ATKINS; Blackwell, Oxford 1959), p. 122.

to explain the transparency of the cornea). If the refractive index increment of a 1% solution of protein is 0.0018 it is unlikely that the refractive index of a medusa is altered by this amount overall since its organic concentration is low. The percentage of protein in *Aurelia* is about 0.67%[5] and my determinations of that in *Polyorchis* (following T.C.A. fixation and dialysis) gave values of only 0.16%. The refractive index increment is liable to vary only between, say 0.00018 and 0.0018 both of which are small fractions of the refractive index of sea water which is about 1.338. However the organic material cannot be regarded as uniformly distributed and, being in the form of fibres, will produce some small refractive discontinuities. It follows that these will be greater the fewer and more concentrated fibres there are. But this in turn will diminish the number of refractive discontinuities.

Measurement of transmittance. There appear to be no measurements in the literature of the amount of light transmitted by coelenterate tissue. Attempts were therefore made to estimate the transmittance of medusae in as natural a state as possible by determining the transmittance of samples of material cut to fit cuvettes. These were used in a Hitachi-Perkin Elmer spectrophotometer which covered the range 200–1000 nm. In all cases the transmittance is expressed as a percentage of the transmittance of sea water and is plotted graphically in Figure 1. The transmittance of the expressed juice from mesogloea is shown for comparison. In Figure 2 is shown the transmittance of a specimen, similar to the others but cut so as to include the subumbrella cell layers which were placed with their plane at right angles to the light path. From these transmittance curves it will be seen 1. that the transmittance is high in the visible spectrum but 2. falls, relative to that of sea water, in the UV range, 3. that the expressed juice has very similar but generally lower transmittance properties and 4. that the subumbrella cell layers, which are thin relative to the total light path through the tissue, are nevertheless responsible for producing a considerable reduction in transmittance.

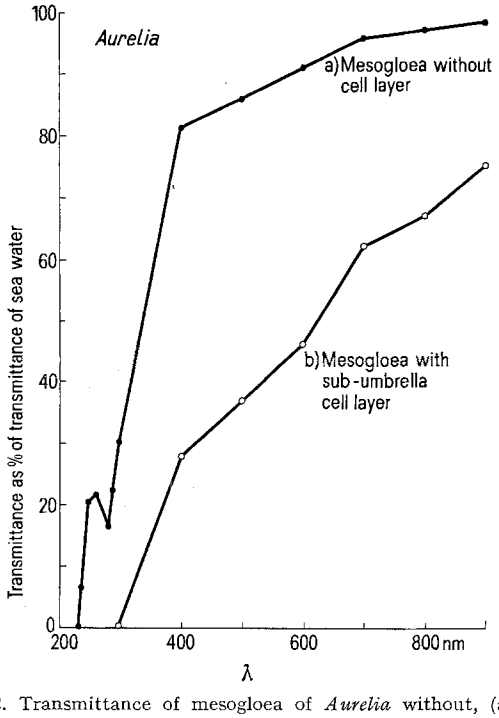

Fig. 2. Transmittance of mesogloea of *Aurelia* without, (a) and with, (b) a cell layer placed at right angles to the light path (as % transmittance of sea water).

Effect of the arrangement of components. In the outstandingly transparent part of the eye, the cornea, a high proportion (some 20%) of the wet weight is collagen. This has a refractive index of 1.55, as compared with the matrix in which the collagen fibrils are embedded, which has a value for η of 1.335[6]. Less than 1% of light incident on the cornea is scattered. This was explained by MAURICE[6] who considered that the collagen fibrils are of such dimensions in size and arrangement that light scattered by each interferes destructively with that scattered by the others in such a way that only the direct ray passes on. He demonstrated that disturbance of the critical and regular spacing of the fibrils by mechanical strain or adsorptive swelling would have a marked effect on the transparency and light scattering. More recently MAURICE's premises have been re-examined and it has been shown that a regular spatial arrangement is not necessary for complete destructive interference of light. It was shown that the collagen fibrils of rabbit cornea are neither uniform in diameter nor regular in arrangement and that the transmittance calculated from measurements and spatial distribution of the fibrils agreed with the measured values. Calculations showed[7] that the type of fibril arrangement present, which is described as quasi-ordered – quasi-random would give rise to a transmittance high enough to be regarded as transparent. COX et al.[8] also considered that a greater randomization of the collagen fibrils would decrease the transmission of light but they also point out that, provided the diameter of the collagen fibrils is a small percentage (about 4%) of the wavelength of the light, they may vary in size considerably without modifying normal transparency. Thus it would not appear that perfect geometrical regularity of fibres is a necessary condition for destructive interference to give rise to transparency provided that the fibrils involved are of small diameter compared with the wavelengths of light.

In the highly transparent arrow worm *Sagitta* the radius of the myosin filaments in the muscle, which makes up the bulk of the body, is about 9 nm compared with the 15 nm for the collagen fibres of mammalian cornea. That of the actin filaments is smaller. These parallel, uniform fibrils of two sizes are arranged in the familiar lattice when seen in cross section. Hence muscle fibres in general might be expected to be transparent and those with a minimum of connective tissue and absence of myoglobin particularly so. It seems likely that the explanation of the transparency advanced by MAURICE[6] and modified by COX et al.[8] to explain the transparency of the cornea is applicable to *Sagitta* at least in part.

Conclusion. Of the three possible ways in which transparency can be achieved (uniformity of composition, uniformity of refractive index and critical and regular arrangement of components) it seems likely that all these contribute in different degrees in different animals. It is suggested that the transparency of many planktonic animals is brought about by the relatively small fraction which is occupied by cells (e.g. in medusae) and the relative absence of internal scattering surfaces. In those organisms which are more highly organized in cellular terms e.g. annelids and chaetognaths, the great regularity of the muscle fibrils plays a part similar to that of the regularly arranged collagen fibrils in the mammalian cornea. This is enhanced by the small size of the myosin fibril

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