STUDIES ON
CERTAIN PHYSIOLOGICAL AND Biochemical Aspects of Vision In Some Arthropods

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The North-Eastern Hill University,
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CERTAIN PHYSIOLOGICAL AND Biochemical Aspects of Vision In Some Arthropods

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SUDIP DEY N.E.H.U
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CONTENTS

List of Tables

List of Figures

GENERAL INTRODUCTION

In the simplest way of definition, vision is a physiological phenomenon by means of which an organism sees the objects around it. Since, vision is a photo-physiological phenomenon, the two most important factors involved in the process are, (1) light and (2) photoreceptor, the organ, which is capable of absorbing light.

The electromagnetic radiation reaching the surface of the earth includes X-rays, ultra-violet, visible and infra-red light, micro-waves and radio-waves. The radiation travels in the form of quanta of energy. The energy is determined by the wave-length; the smaller the wave-length, the greater the energy (Dethier,1963).

Of the total radiant energy spectrum, only a very small segment, which is termed as visible light can be detected by organisms. The visible spectrum lies between about 300 to 800 m μ , although ultra-violet wave-lengths down to about 210 m μ can be detected by some organisms. (Dethier,1963).

To receive the radiant energy of this narrow band of the spectrum, the prime requisite for an organism is the visual pigments present in the photoreceptor organ. In the animal kingdom, there is a variety of photoreceptor organs. The most primitive type of photoreceptor is the eyemspot of Euglena, which is capable of detecting only the presence or absence of light. In metazoans, there are more complex receptor organs or eyes, which are capable of forming some type of

image on the photoreceptive surface.

The most highly developed organs of vision in the animal kingdom are the vertebrate eyes and the eyes of cephalopod mulluscs. These eyes have the refractive element, lens to focus light from an object, and it forms an inverted image on the retinal surface.

Among the photoreceptor organs of animals, the compound eye of arthropods has attracted many of the vision physiologists because of its unique structural organisation. Compound eyes of arthropods are image - forming eyes. They are capable of determining the plane of polarized light, and the animals can use such information for orientation or directed locomotion.

The compound eye consists of a number of structural and functional units, known as ommatidia. The number of ommatidia varies in different species. As for example, the worker of the ant, Ponera punctatissima has only one ommatidium, whereas in some Odonata the number is between 10,000 and 28,000 or more (Wolken, 1968).

An ommatidium consists of a cornel lens, a crystalline cone and retinula cells. The cornea is a transparent area of the cuticle and it forms the facet or lens of an ommatidium. It is more or less bi-convex and is cast off during each act of ecdysis (Imms, 1957).

Beneath the cornea is the transparent body known as crystalline cone.

Immediately behind the crystalline cone are the sensory elements or the retinula cells. The number of retinula cell is not constant in all species. In many ommatidia, the number of retinula cell is eight, e.g. Apis. In certain cases , the number is reduced to six or seven. In certain moths , the retinula cell are ten to twelve in number (Imms, 1957). Crustacean Ommatidia are also similar (Waterman,1961). The retinula cells collectively secrete an internal " light-trapping" rod-like structure known as rhabdom. The portion of the rhabdom , contributed by each retinula cell is known as rhabdomere. The rhabdomere is analogous in function to the retinal rod outer segment of the vertebrate eye (Wolken,1968).

In addition to these structures, the ommatidia of compound eyes contain a number of pigment cells. Surrounding the crystalline cone and corneagen layer are present a layer of densely pigmented cells, known as Primary iris cells. Primary iris cells and the retinula cells in turn are surrounded by a second group of elongated pigment cells, known as secondary iris cells. These pigment cells serve to isolate one ommatidium from the next.

The arrangement of these various components of the Ommatidium, however is not constant in all forms. Exner(1891) has described two distinct types of compound eyes in arthropod, on the basis od difference in their anatomy. They are the apposition and superposition eyes.

The apposition type of compound eyes are characteristic of diurnal insects. Among the crustaceans, this type of eye is found in diurnal, terestrial and littoral forms. In apposition eye, which is also known as photopic eye, the rhabdom is long and thin, and it lies directly beneath or against the crystalline cone. A double-layered sheath of pigment cells surround each ommatidium, and thus it is optically isolated from its neighbour. Light falling upon the lens of an ommatidium can only reach the rhabdomeres of that ommatidium. During light-dark adaptation, there is little or no longitudinal migration of the screening pigments. But in some cases there is radial movement of the screening pigments around the rhabdom due to the change in condition of illumination (Walcot,1974)

Superposition or scotopic eyes are those in which short and broad rhabdom lies some distance away from the crystalline cone. A marked longitudinal migration of the screening pigments during light - dark adaptation is a characteristic feature of this type of eye. During a condition of bright illumination, the pigment granules migrate inwards and extends the full length of the ommatidium. At dark, however, the pigments move upward and condense distally between the crystalline cones. The superposition eyes are found in nocturnal or crepuscular species (Dethies,1963).

To explain the functioning of the compound eye of arthropods, Johannes Muller (1826) formulated the classical theory

known as "mosaic theory". According to the theory, each ommatium receives light from its own lens. Light falling on the lense of one ommatidium cannot reach the adjacent one because of the presence of pigment-sheath. Thus, an image of the limited part of the visual field is produced by each ommatidium and the entire eye forms the image from the "reports" from individual ommatidium.

Since the time of Muler's "mosaic theory" (Muller, 1826), the compound eyes of arthropods have been extensively studied by different groups of authors from various disciplines to explain the mechanisms involved in photoreception in this group of animals. But, in spite of a large amount of work on the compound eyes, it seems that much research still remains before one can completely understand how compound eyes perform this complicated visual phenomenon.

It is seen from a review of previous works on the subject, that there is a need for some more information on certain aspects of arthroped vision.

To give an example, the lens, which covers the ommatidium of a compound eye is modified cuticle. It is well known that the nature of the cuticle of insects and other arthropods varies in different regions of the body, which perform various types of functions (Richards, 1972). It is also evident that the chemical nature of cuticles involved in various functions may vary (Wigglesworth, 1972). Since lens performs a special optical function of conducting light rays to the rhabdomes, besides playing a general defensive role, it is

expected that the lens -cuticle may differ in chemical nature and ultra-structure from the cuticle covering other parts of the body. But the chemical composition of the lens in relation to its special function has not been studied in detail. In the similar way, there are many other aspects in this field of research, worth-studying.

The migration of the screening pigments, for example, is one such area, which needs further investigations. The movement of pigment granules in the compound eyes during light-dark adaptation has been described by Exner(1899,1891) and has been reviewed by Parker(1932) and Turala(1954). Since that time, extensive amount of works have been performed on the subject. But inspite of that, we do not have a clear idea regarding the mechanism which controls the migration of the screening pigments. However, the phenomenon is more or less clearly understood in crustaceans (Fingerman 1959), but there is little information on the physiological control mechanism of eye-pigments migration in insects.

Another interesting aspect of arthropod vision is the fluorescence of the compound eyes. Many authors have detected fluorescence in the compound eyes <u>in vivo</u> (Hess 1920; Mercker 1929; Kay 1969). It has been observed that the compound eyes on irradiation with ultraviolet light in a darkened room give a blue or blue-green fluorescence. Hess(1920) has suggested that the U.V. sensitivity of the compound eye may be due to the fluorescence

set up in various tissues of the eye. This suggestion however, has been rejected by Walther and Dodt (1959) and Goldsmith(1961 a) Since that time, a great amount of controversy centres around the subject.

Another very important area, where there is a lacuna in our knowledge is the enzymology of the compound eyes. It is well established that certain enzymes, especially, adenosine triphosphatases (ATPases) are intimately associated with the process of photoreception (Langer, 1963, Drujan and Ali 1972) but inspite of an extensive investigation on the activity of this enzyme in the photoreceptors of various vertebrates and invertebrates, there is very little information regarding the study of ATPase system from the compound eyes of arthropods.

Keeping all these in view, certain histochemical, biochemical and physiological studies have been performed on the compound eyes of some arthropods.

The animals which have been chosen for the present study are the house-fly, Musca domestica (Insecta); the honey-bee Apis-Cerana indica (insecta); the fresh-water prawn, Macrobrachium birmancium (Crustacea); and the marine prawn, Metapinneus sp. (Crustacea).

The aim of this research programme is to gather some more informations regarding certain physiological and biochemical aspects of vision in arthropods, with the available facilities.

Chapter - - - - I

HISTOCHEMICAL ANALYSIS OF THE LENS-CUTICLE: ACID MUCOPOLYSACCHARIDES.

INTRODUCTION

One of the most important structures, associated with the functioning of the photoreceptor in arthropods is the " Dioptric apparatus " or Lens. It is well-established that lens is a modified cuticle. It is more or less bi-convex in form and is cast off during each act of ecdysis (Imms, 1957). It is obvious that associated with the optical function performed by it, lens-cuticle should have certain specialities in comparison to the general body-cuticle. One of these specialities is definitely the transparency and in fact the cuticle covering the eyes and ocellus is very transparent. However, it is not very clear how this transparency is achieved in chemical terms. It is known that chitin is transparent in the visible range, so it may be possible that a transparent cuticle is formed with the added modifications of colourless cross-linking agents in the protein as suggested by Anderson (1971 b).

Goldsmith and Fernandez (1968) have reported that the corneal cuticle (e.g. in Musca) does not show any appreciable absorption in the visible or near UV region, but a peak occurs at 277 mμ. They have suggested that this property may be attributable to tryptophan and tyrosine residues in the cuticular proteins.

In Manduca sexta, carlson and Philipson (1972) have compared the absorption characteristics of the lens-cuticle and the crystalline cone - substances. They have reported that the cuticular lens is more transparent from 350 to 600 mμ but its absorption rises steeply from 310 to 290 mμ . Obviously this is to protect the underlying nerve cells from middle UV.

De Vries and Kuper (1958) have suggested that no change in intensity of transmitted light can be observed when the dioptric lens - apparatus of certain insect eyes is viewed through a rotating polaroid. Nevile (1975) has suggested that this is consistent with the helicoidal structure viewed along its optical axis. In Exner's theory of insect vision, it is suggested that in an ommatidial dioptric apparatus, the path of maximum refractive index falls off. As a result of this an inverted image is formed at the base of the lens. In Exner's theory, the concentric lamelae, which can be observed in longitudinal sections of dioptric lenses has been given a substantial amount of importance. Horridge (1969) has suggested that the concentric lamellae of dioptric lenses are due to alternating layers of different chemical constitution and hence of different refractive index.

However, Neville (1970) has rejected this view of Horridge and has suggested that difference in chemistry has nothing to do with lamellation. According to him it is the helicoidal orientation of chitin microfibrils which is responsible for such lamellations. In this context, it is of interest to mention that several theories are already in existance, which explain reflection of light by multi-layer structures (Huxley, 1968).

In the compound eye of arthropods, however, cuticle is not involved merely as a lens. In many other phenomenon of arthropod visual system, such as interference filters in eyes, reflection of specific wave-length from trachea behind the receptive cells, and in reducing reflection at the corneal surface, cuticle seems to play essential roles.

Bernard and his colleagues (1965) have reported that corneas of several insects show interference colours. In certain cases, as for example, in the horse-flies the interference colours are observed not only in living materials but also in isolated corneas. Electron microscopy reveals that the outer part of the corneal cuticle contains a series of layers, which reflect certain wavelengths of light. The wave-lengths, which are not reflected are transmitted to the underlying photoreceptor cells. Thus the corneal cuticle acts as a filter for specific wave-lengths.

A detail study regarding the function of this interference filters has been made by Bernard (1971) on the flies of the family Dolichopodidae. The compound eye of these insects contain alternating rows of corneal facets with different interference colours. Bernard (1971) has measured the reflectance spectra for each colour of facet by using living cornea, and he has found that a reflectace peak in a given facet coincides with a minimum in a neighbouring facet and vice versa. In addition to this, ultra-structural studies made by Trujilocenoz and Bernard (1972) on Symycnus and Condylostylus have showed that in the underlying superior central cells, rhabdomeric microvilli are oriented differently in ommatidia with red and yellow corneas. In the former type, orientation is vertical and in the later, it is horizontal. These differences are paralleled by structural differences in the lamina and medulla of the brain, and this gives a direct evidence that corneal interference filters have a visual function.

Another very important example of the involvement of cuticle in the visual system of arthropods is the corneal nipples. The corneal nipples are submicroscopic protuberances of uniform height (200nm) present and periodicity (200 nm) present in the anterior surface of the cornea of certain nocturnal Lepidoptera (Bernhard, et al., 1965). The important

examples of insects which posses corneal nipples are Vanessa, Prodenia, Cerapteryx, Sphynx, Endromix, (Lepidoptera); Myrmeleon (Neuroptera); Phryganea (Trichoptera), and Aedes, Culex and Anopheles (Diptera).

Electron microscopy of the vertical and tangential sections reveal that the corneal nipples from a series of cones with hexagonal spacings. Gemne (1966, 1971) has studied the development of this regular pattern of nipples on the corneal surface in Manduca sexta. He has found that in pupal stage of this insect, the corneal nipple arises as double layer of outer epicuticle.

Initially it forms caps over each microvillus projecting from the epidermal cells which secrete the cornea. Thus corresponding to each microvillus there is one cap and hence one nipple. The function of corneal nipple is to increase transmission through the lens-cuticle of nocturnal insects.

From all these reports, it appears that involvement of eye-cuticle in the visual system of arthropods is extensive and varied.

It is well-established that the nature of the cuticle of insects and other arthropods varies in different regions of the body which performs various types of functions (Richards, 1972).

It is also evident that the chemical nature of the cuticles involved in various functions may vary (Wigglesworth, 1957). Since lens-cuticle performs some special optical functions, it is

expected that it differs in chemical nature and ultra-structure from the cuticle covering other parts of the body.

Neville (1970) has found that lens-cuticle does not contain pore-canals whereas adjacent interommatidial cuticle is rich in pore-canals(Home,1973). Neville(1970) has reported that in Lampyris, dioptric apparatus is lamellate throughout its thickness and there is no daily growthlayers. He has suggested that this feature may be associated with vision. Home(1973) has reported that in beetles the lens is"helicoidal", whereas the surrounding cuticle is having"polywood" structure. Neville(1970) has found that in the non-image forming median Ocellus of Schistocerca greagaria, daily grouwth-layers are present.

This proves the suggestions that light sensitivity may change with age(Neville, 1963 b). Cassier(1965) has suggested that light sensitivity of ocelli decreases with age as the cuticle covering them thickens.

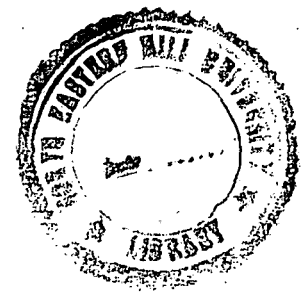
Thus, there are some significant differences in the ultra-structure of the lens-cuticle and the cuticle covering other parts of the body. But inspite of the fact that there are some informations regarding the ultra-structure of the lens-cuticle, very little is known on the chemistry of eye-cuticle in relation to its special function.

Anderson(1971 a) has analysed the total amino-acid content of several cuticles and he has found that the corneal cuticle of compound eye is intermediate in composition between hard and soft cuticles. It is known that hard cuticle contains a low amount of polar amino acids and high alanine content,

whereas the soft cuticle contains a high polar amino acid content and low amount of alanine (Anderson 1971). In the corneal cuticle, Anderson(1971) has found that both the polar amino-acid and alanine content is high. He has also found that hardness of the corneal cuticle lies between those of typically hard or soft cuticles.

Sannasi(1970) has detected the occurence of the colourless structural elastic protein, resilin in the lens-cuticle of fire-fly, Photinus pyralis by staining the histological preparations with toluidine blue-light green and - by autofluorescence of the lens in the UV light with a maximal intensity at 420 mμ and also by studying the swelling properties with various reagents. With all these in view, histochemical and biochemical studies have been performed on the compound eyes of the house-fly, Musca domestica, honey-bee, Apis Cerana indica, fresh-water prawn, Marcrobrachium birmancum and the marine prawn, Metapinneus.sp.

Special attention has been given to study the mucopolysaccharides from the compound eyes, because of certain very interesting properties of the substances, which it has been thought may have bearings on the visual phenomenon of arthropods.



MATERIALS AND METHODS

Histochemistry: The eyes are separated from the alive animals and fixed in 7% neutral formaldehyde untill they are used. A few animals are fixed in alcoholic Bouin's and Carnoy fluid. Histological preparations are made by paraffin embedding and sections are taken with a rotary microtome at 10 u thick.

To differentiate the layers of the lens-cuticle, the histochemical methods customarily used in arthropod cuticular studies have been employed. These are Mallory's tripple stain (Mallory, 1938) Masson's trichrome (Pantin,1948); and Heidenhain's iron haematoxylin stain (Lillie,1954).

Detection of Mucopolysaccharides:

The histological and histochemical techniques employed for studying mucopolysaccharides in the sections of the compound eyes, are those reviewed by Curran (1954).

Fixation of the tissue : It is known that common fixations denature and precipilate proteins and mucopolysaccharides but the reaction is often reversible. Thus, there are doubts about the ability of any fixative to retain all mucopolysaccharides (curran 1964). However, different authors have given importance to different fixatives for studying mucopolysaccharides, although each of the fixatives have certain advantages and disadvantages.

With this view, we have tried a number of fixatives for studying the acid mucopolysaccharides. However, it has been

observed that 5% neutral formaldehyde gives the best result.
Staining methods: For detection of mucopolysaccharides, the stains used are Basic fuchsin, Aldehyde fuchsin and Toluidine blue.

Basic fuchsin: The stain is prepared by making 0.1% basic fuchsin in 0.05 N Hcl. The sections are placed in the staining solution for 7-10 minutes and are washed in several changes of distilled water.

Aldehyde fuchsin: This stain is prepared by adding paraldehyde to an acidified solution of Basic fuchsin.

Toluidine blue: Tuluidine blue has been used as 0.5% aquous solution and also as 0.1% solution in 30% ethanol. The staining time varied from 15 to 20 minutes.

Examination of the stained sections: The " metachromatic " reaction given by cationic dye, such as Toluidine blue is distinguished by its ability to survive alcoholic degradation (Curran, 1964). However some authors have advocated the examination of the sections in hydrated state. To study the effect of dehydration on the " metachromasia" some sections have been examined wet and other have been immersed in ethanol for periods ranging from ½ to 30 minutes.

Preparation of the Mucopolysaccharide-extract: For the analysis of mucopolysaccharide the method of extraction employed in this study is that followed by Bera et al (1955)

The experimental material has been treated with 5% sodium hydroxide for 24 hours. After such treatment the material ceased to stain with toluidine blue. Presumably the mucopolysa-

saccharide was removed from the lens by alkali-extraction. The alkali-extract was then neutralised with 1 N Hcl, and Zinc hydroxide was added to a concentration of 5 gm/ 100 ml. Protein was removed from the extract by Amyl alcohol and chloroform mixture, in the proporation of 1;1. The polysaccharide was later precipitated using a volume of ethanol equal to three times that of the extract.

Chromatography:

The extracted mucopolysaccharide was hydrolysed with 6 N Hcl acid at 100°C for 24 hours. The acid hydrolysate was then evaporated to dryness. The dried residue was then dissolved in .5 ml. of glass distilled water. The hydrolysate was spotted on Whatman No.1 filter paper, and circular paper chromatogram run using butanol : acetic acid: Water(4:1:1:Vol/Vol) as solvent (Giri and Nigam,1954). The chromatogram was developed with silver nitrate (o.1 ml of saturated solution of silver nitrate in 20 ml. of acetone) and sodium hydroxide(0.5 gm NaOH in 25 ml of rectified spirit), as suggested by Trevelyan, et al (1950).

Electrophoresis: The electrophoresis has been performed by applying the crude mucopolysaccharide extract sample on the paper strip (Whatman No.1). The buffers used are:

(1) 0.05 M and 0.001 M phosphate with a range of pH from 5.5 to 8.5.

(2) 0.15 - 0.2 M Zinc Sulfate.

(3) 0.15 - 0.2 M Zinc acetate.

(4) 0.1 M Barium acetate buffer, pH 6.6

(5) 0.1 M Cupric acetate, ph 5.3

The time of the electrophoretic run and the potential gradients applied in different buffer- system are as follows:-

(1) Phosphate buffer - 4 V / cm is employed for 8 - 10 hours.

(2) Zinc sulfate or Zinc acetate:- In this buffer system, the voltage applied is 4 V / cm for 16 - 20 hours.

(3) Barium acetate:- The voltage applied is 50V and the time of the run is 3 - 4 hours.

(4) Cupric acetate :- The voltage applied is 100 V and the time is 1.5 hrs. to 2.5 hrs.

The electrophoretic movement pattern of the extracted mucopolysaccharides from the compound eye of the house-fly, Musca the honey bee ,Apis, the fresh-water prawn, Machrobrachium and the marine prawn, Metapinneus have been compared with several standard acid mucopolysaccharides such as chondroitin-4-sulfate, chondroitin-6-sulfate, Keratan sulfate, and heparan sulfate.

After removal from the electrophoresis apparatus, the paper strips are dried at room temperature and stained with toluidine blue (0.04% toluidine blue in 80% acetone).After the staining , the strips are rinsed 2-3 times with 0.1% acetic acid, and then 2-3 times with distilled water.After washing with distilled water the stips are dried at room temperature.

OBSERVATIONS

Histochemistry:

Lens-cuticle of the house-fly, Musca domestica: The lens-cuticle of house-fly, Musca is composed of an outer epicuticle and an inner pro-cuticle.(Fig.1) The inner pro-cuticle comprises an outer colourless "hayline" exo-cuticle. When the sections of the eye are stained with Mallory's tripple stain, it has onserved that the region below the corneal layer, the so-called crystalline cone shows affinity towards acid fuchsin, corresponding to the meso-cuticle of other insects. When the sections are subjected to Sudan black-B and Nile blue reagents tests, the outer portion of the epicuticle gives intensely positive reaction, indicating the presence of lipids in it. However, this layer gives negative reaction to Biuret, Xanthoproteic and Millon's test, indicating the absence of protein. In contrast to this, the inner fuchsinophil layer of the epicuticle is positive to Xantho-proteic and Millon's test indicating the presence of proteins. Sudan black-B and Nile blue also gives some positive reactions to this layer.

When the sections are stained in 0.1% basic fuchsin, it is observed that the entire width of the cuticle(excluding the outer epicuticle) becomes intense red in colour. Similarly, when the sections are stained with Aldephyde Fuchsin the same region becomes purple in colour, i.e. the region is metachromatic. With this stain, some metachromatic reactions are also observed in the "cone" region. When stained with Toluidine blue, the entire

procuticle becomes purple in colour (metachromatic). The region of the rhabdome is orthocheomatic i.e. blue in colour. The cone region is again metachromatic (see Table 2).

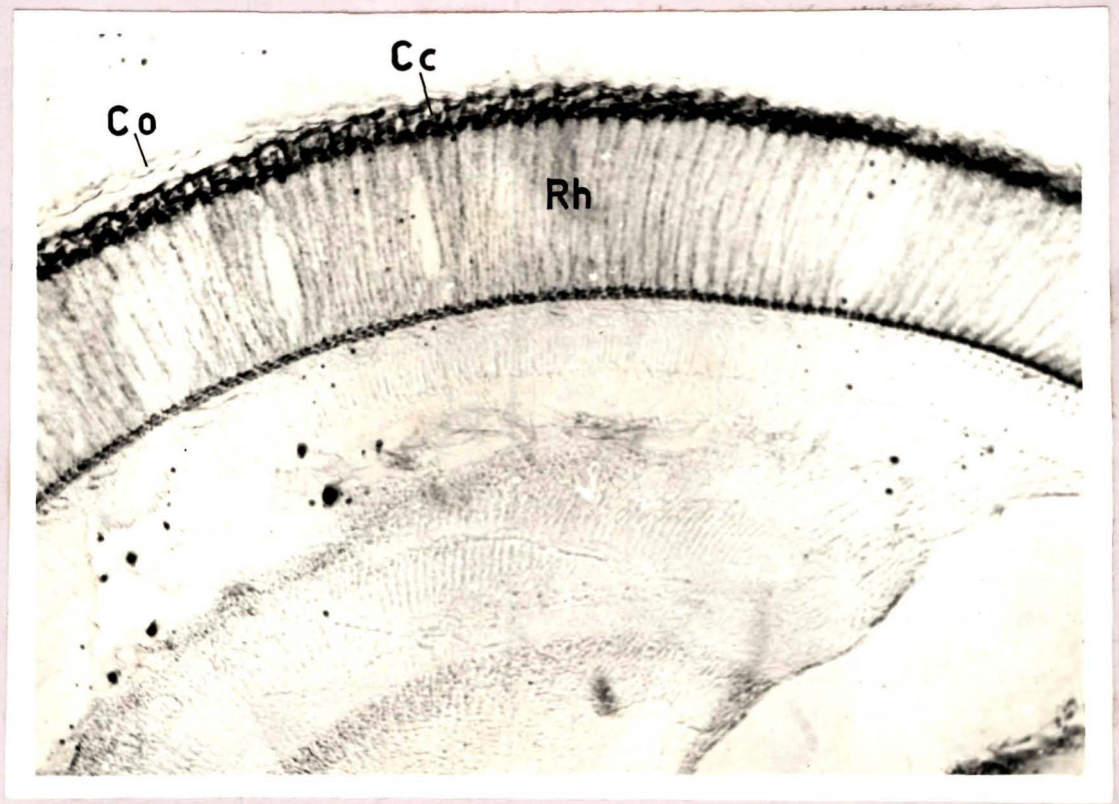
EXPLANATION TO FIGURES

Fig. 1. Transverse section through the eye of the house fly , Musca domestica . 10 x 10 , after staining with Mallory's stain.

Co , Cornea.
Cc , Crystalline cone.
Rh , rhabdom.

Fig. 2. T/S through the eye of the housefly, Musca domestica 10 x 40., after staining with Toludine Blue.

Co , Cornea. (metachromatic)
Cc , Crystalline cone,(metachromatic)
Rh , Rhabdom(orthochromatic).


Cc
Co
Rh

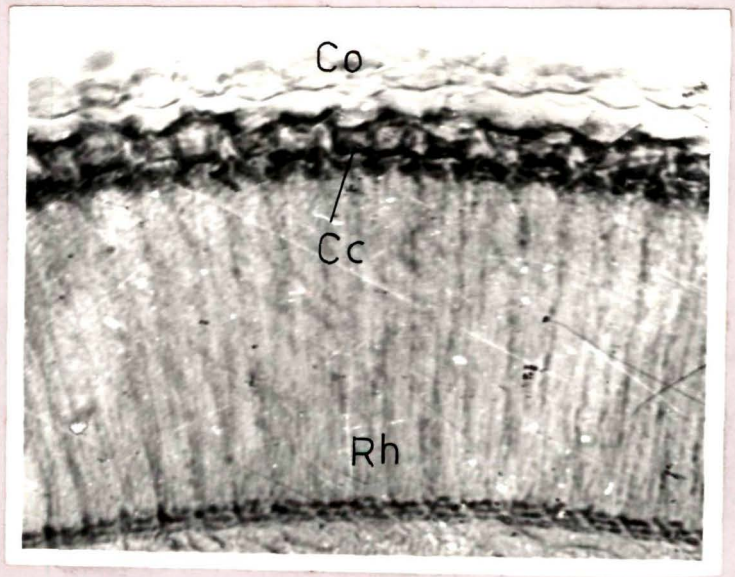

Co
Cc
Rh

Table: 1 Results of staining reactions and histochemical tests obtained with the lens - cuticle of the house-fly, Musca domestica.

Serial No.	Stains and tests	References	Regions		
			Outer epicuticle	Inner epicuticle	Procuticle
1.	Mallory's tripple stain	Mallory (1938)	Blue	red	Blue
2.	Masson's trichrome test	Pantin (1948)	Green	red	Green
3.	Heidenhain's haematoxylin	Lillie (1954)	Black	brown	brown
4.	Biuret test	Faeron (1946)	-	+++	++
5.	Xanthoproteic test	Pearse (1961)	-	+++	+
6.	Millon's test	Bensley and Gersh (1933)	-	+++	-
7.	Sudan black B	Baker (1956)	+++	+	-
8.	Nile blue	Cain (1954)	+++	+	-

Key :- +++ intensely positive ; + slightly positive;
- negative.

Table 2. Results of staining reactions and histochemical tests for the detection of acid mucopolysaccharides, in the compound eye of house-fly, *Musca domestica*.

Sl No.	Stains and tests	References	Regions		
			Corneal lens	Crystalline cone	Rhabdome
1.	Basic fuchsin	Stempien (1962)	bright red	red	reddish brown
2.	Aldehyde fuchsin	Gomori (1950)	Purple(deep) (strongly metachromatic)	Light purple	blue (Orthochromatic)
3.	Toluidine blue	(Pearse, 1961)	+++	+	-
4.	PAS test	(Mamanus and Cason, 1950; Hotchikiss (1948)	+	+++	-
5.	Alcian blue	(Steedman 1950)	+	+	-

Key :- + + + intensely positive ; + slightly positive ;
- negative.

Lens-cuticle of the honey-bee, Apis cerana indica:-

The regions of lens-cuticle of the honey bee can not be distinctly differetiated (by using the customary stains used for cuticular studies, such as Mallory's tripple stain, Masson's trichrome stain and Heiden-hain's iron haematoxylin stains) as compared to the house-fly. However, the two layers, the outer epi-cuticle and inner pro-cuticle are more or less distinct (Fig.3). The outer epicuticle gives negative reaction towards Xanthoproteic test, Millon's test, Bieuret test.

When the sections are stained with basic fuchsin the pro-cuticle is coloured red indicating the presence of mucopolysaccharides. The more intense reactions have been observed towards the crystalline cone.

The paraldehyde fuchsin stain gives a positive metachromatic reaction in the epicuticle as well as pro-cuticle and an intense metachromasia in the cone region (Table 3).

When the sections are stained with Toluidine blue; the epicuticle, the pro-cuticle as well as the crystalline cone become purple in colour, showing the presence of mucopolysaccharides. The rhabdom region however, gives a blue colour reaction in presence of Toluidine blue i.e. the region is orthochromatic (Table 4.)

Lens-cuticle of the fresh-water Prawn, Macrobrachium birmanicum :- Histological preparation of the lens-cuticle of the fresh-water prawn, M. birmanicum after staining in Mallory's

EXPLANATION TO FIGURES

Fig. 3. T/S through the eye of the honey bee, *Apis cerana indica*. 10 x 40 , after staining with Mallorys Tripple Stain.

Co , Cornea

Cc , Crystalline cone,

Rh , Rhabdom.

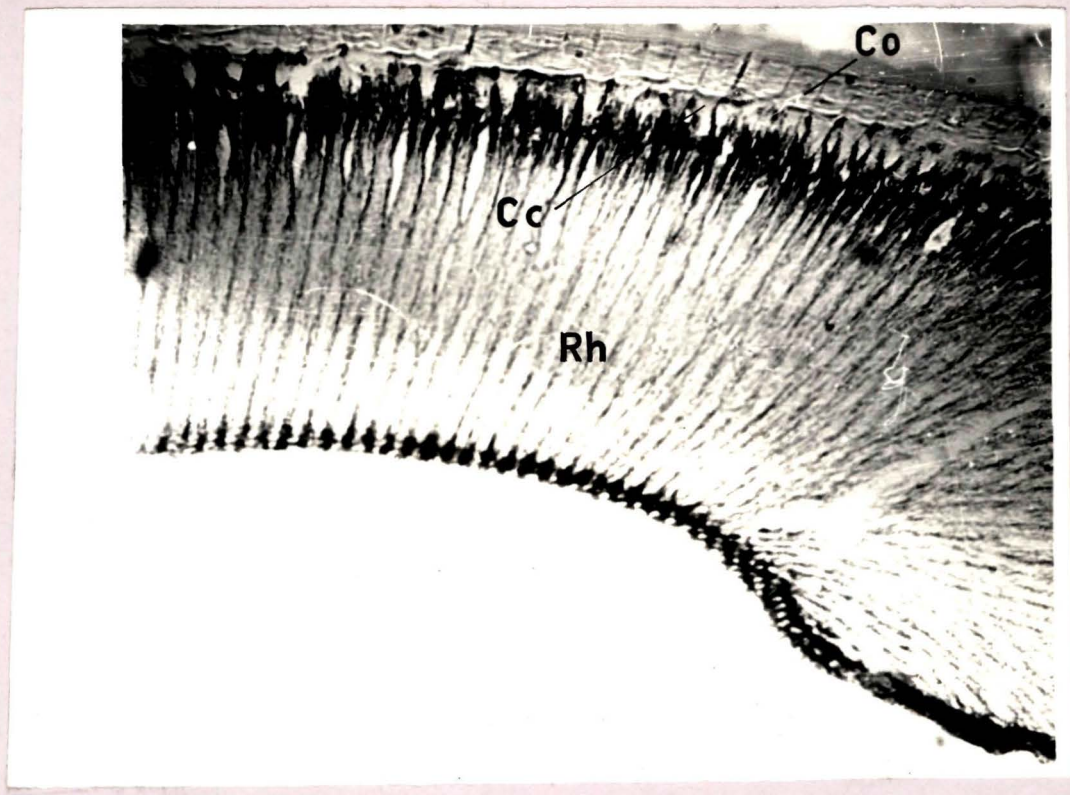

Co
Cc
Rh

Table :- 3 - Results of staining reactions and histochemical tests obtained with the lens-cuticle of the honey bee, Apis Cerana indica.

Sl. No.	Stains and tests	References	Epicutcle		Procuticle
			Outer	Inner	
1.	Mallory's tripple strain	Mallory (1938)	blue	red	Bluish
2.	Masson's trichrome test	Pantin (1948)	green	red	Green
3.	Heidenhain's haematoxylin	Lillie (1954)	blue	brown	light Brown
4.	Buiret test	Faeron (1946)	-	++	+
5.	Xanthoproteic test	Pearse (1961)	-	++	-
6.	Million's test	Bensley and Gersh (1933)	-	++	-
7.	Sudan black B	Baker (1956)	+++	++	-
8.	Nile blue	Cain (1954)	+	+	-

Key :- +++ intensely positive; + slightly positive;
- negative.

Table 4 : Results of staining reactions and histochemical tests for the detection of Acid mucopolysaccharides in the compound eye of honey-bee, Apis Cerana indica.

Sl No	Stains and tests	References	Regions		
			Corneal lens	Crystalline cone	Rhabdome
1.	Basic fuchsin	Stempien (1962)	Deep red	red	reddish brown
2.	Aldehyde fuchsin	Gomori (1950)	Purple (metachromatic)	Purple (metachromatic)	blue (orthochromatic)
3.	Toluidine blue	(Pearse, (1961)	Purple (metachromatic)	Purple (metachromatic)	blue (orthochromatic),
4.	PAS test	(Mc Manus and Caron, 1950; Hotchikiss, 1948)	+	+++	-
5.	Alcian blue	(Steedmann 1950)	+	+	-

Key : +++ intensely positive; + slightly positive ; - negative

tripple stain show that it comprises an outer epicuticle and an inner procuticle (Fig.4; Fig;5). The epicuticle consists of an outer pipid layer as evidenced from its positive reaction towards Sudan Black-B and Nile blue tests.

The inner portion of the epicuticle gives positive reactions to Xanthoproteic test, Millon's test, Bi-uret test, thus indicating that it contains proteins. In addition to this when the sections are stained with Sudan Black b or Nile blue reagents it gives some positive reactions(Table 5).

With Mallory's tripple stain, the pro-cuticle is feebly coloured red, corresponding to the pigmented layer of body-cuticle (Travis, 1965) and meso-cuticle of insects (Dennell and Malek, 1955).

When the sections of the lens-cuticle are stained with basic fuchsin, the pro-cuticle becomes red in colour, indicating the presence of sulfated mucopolysaccharides. The positive metachromatic reaction has been observed in entire width of the procuticle, when the sections are stained with Paraldehyde fuchsin stain. With this stain the peripheral portion of the crystalline cone also gives a strong metachromatic reaction. The central portion of the crystalline cone gives a blue colour reaction, i.e. the region is orthochromatic. The rhabdome region also gives an ortho-chromatic reaction(Table 6).

When the sections are stained with Toluidine blue, the pro-cuticle region gives a strong metachromatic reaction i.e. it becomes purple in colour. The peripheral portion of the crystalline

Table 5. Results of staining reactions and histochemical tests obtained with the lens-cuticle of the fresh-water prown, Macrobrachium birmancium.

Sl. No	Stains and tests	References	Outer epi-cuticle	Inner epicu-ticle	Procuticle
1.	Mallory's tripple stain	Mallory (1938)	deep blue	red	Light blue
2.	Masson's trichrome test.	Pantin (1948)	Deep green	red	Light green
3.	Heidenhain's haematoxylin	Lillie (1954)	Black	brown	brown
4.	Biuret test	Faeron (1946)	+	+++	+
5.	Xanthoproteic test	Pearse (1961)	+	+++	-
6.	Millon's test	Bensley and Gersh (1933)	+	+++	-
7.	Sudan black B	Baker (1956)	++	+	-
8.	Nile blue	Cain (1954)	++	+	-

Key : - +++ instensely positive; + slightly positive; - negative

EXPLANATION TO FIGURES

Fig. 4. T/S through compound eye of the fresh-water Prawn, Macrobrachium birmanicum 10 x 10., after staining with Mallory's Tripple stain.

Co , Cornea ,
Cc , Crystalline cone ,
Rh , Rhabdom.

Fig. 5. An enlarged view of a portion of the T/S through the eye of the fresh-water prawn , Macrobrachium birmanicum 10 x 40., after staining with Toluidine blue.

Co , Cornea (metachromatic)
Cc , Crystalline cone (metachromatic)
Rh , Rhabdom (orthochromatic)


Rh

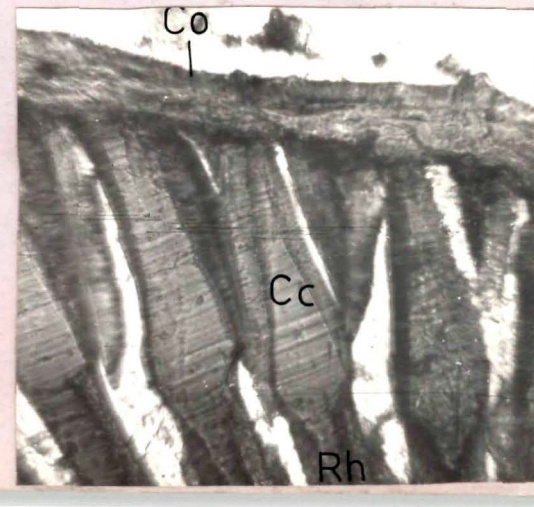

Co
Cc
Rh

Table 6 :- Results of staining reactions and histochemical tests for the detection of acid mucopolysaccharides in the compound eyes of the fresh-water Prawn, Macrobrachium birmanicum.

Sl.No.	Stains and tests	References	Corneal lens	Crystalline cone	Rhabdome
1.	Basic fuchsin	Stempien (1962)	Red	Red	brown
2.	Aldehyle fuchsin	Gomori (1950)	Deep purple	Light purple	blue
3.	Toluidine blue	Pearse (1961)	Dark purple	Purple	Light blue
4.	PAS test	(Mc Manus and Cason; 1950; Hotchi-kiss 1948)	+	+	-
5.	Alcian blue	(Steedmann, 1950)	+++	+	-

Key : +++ intensely positive ; + slightly positive ;
- negative.

cone also gives a strong metachromatic reaction similar to paraldehyde fuchsin stain. With this stain also, the central part of the crystalline cone gives orthochromatic reaction. In addition to this , the lower portion of the crystalline cone, which is in close association with the inner rhabdome region gives an intense metachromatic reaction, indicating the occurence of mucopolysaccharides in that region also. Some portion of the lens-cuticle and also the peripheral portion of the crystalline cone gives slight hypso-chromatic reaction to toluidine blue.(green-metachromasia) (Table 6).

Lens-cuticle of the marine Prawn, Metapinneus spp:

From the histological preparation of Metapinneus sp. after staining in Mallory's tripple stain, it has been observed that it consists of an outer smooth epicuticle and inner pro-cuticle which are elongated slightly in vertical directions forming cones (Fig. 6). The outer portion of the cones of Metapinneus shows affinity to acid fuchsin, Whereas the inner portion shows positive reactions to aniline blue, as the meso-cuticle and endocuticle respectively.

When the sections are stained with basic fuchsin, the major portion of the pro-cuticle becomes deep red in colour. The crystalline cone region also gives some positive reaction to the test.

Paraldehyde fuchsin stains the entire width of the pro-cuticle purple, indicating the occurence of acid mucopolysaccharides

EXPLANATION TO FIGURES

Fig. 6 T/S through eye of the marine prawn, *Metapinneus sp.* 10 x 10 . after staining with Mallory's Tripple stain.

Co , Cornea .
Cc , Crystalline cone ,
Rh , Rhabdom.

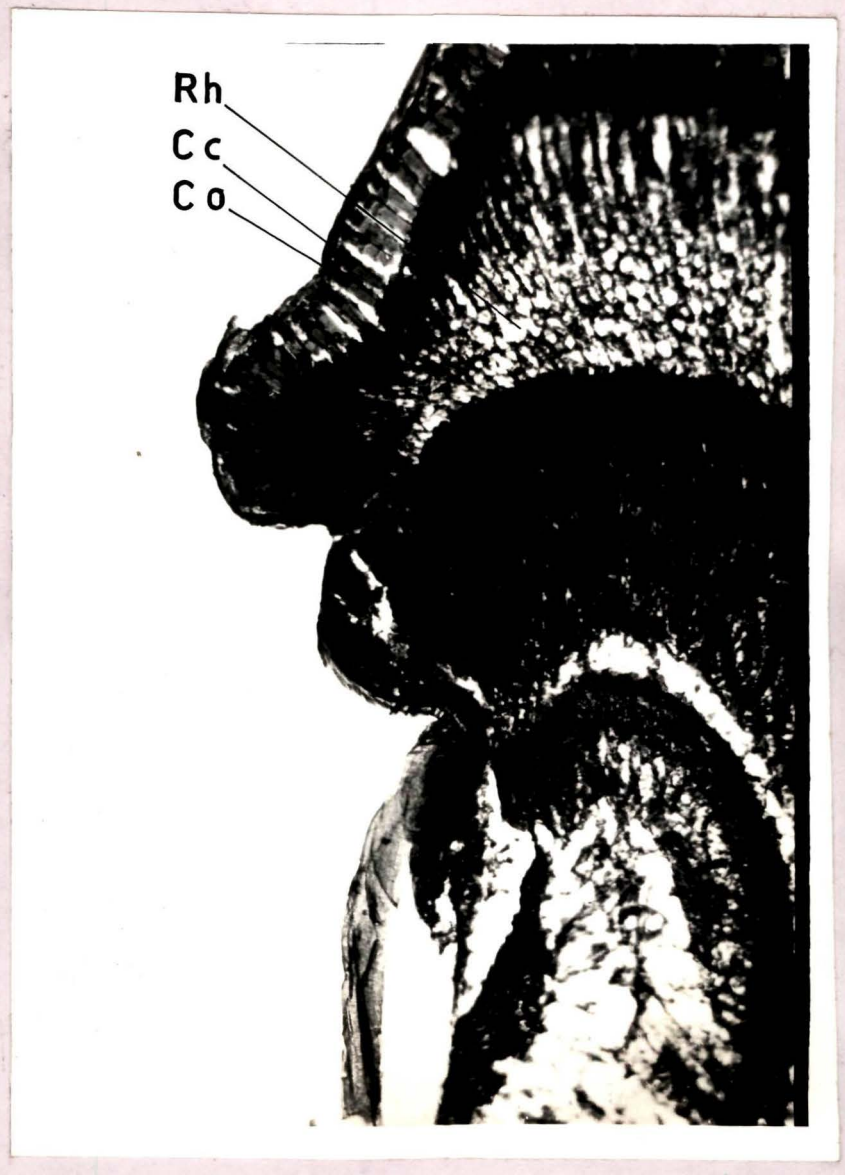

Rh
Cc
Co

Table: 7 - Results of staining reactions and histochemical tests obtained with the lens-cuticle of the marine Prawn, Metapinneus sp.

Sl. No.	Stains and tests	References	Outer epicuticle	Inner epicuticle	Procuticle
1.	Mallory's tripple stain	Mallory (1938)	deep blue	pink	blue
2.	Masson's trichrome test.	Pantin (1948)	deep green	red	green
3.	Heidenhain's haemaloxylin	Lillie (1954)	Black	light brown	brown
4.	Biuret test	Faeron (1946)	-	-	+++
5.	Xanthoproteic test	Pearse (1961)	-	+++	-
6.	Millon's test	Bensley and Gersh (1933)	-	+	-
7.	Sudan Black B	Baker (1956)	+++	-	-
8.	Nile blue	Cain (1954)	+	-	-

Key :- +++ intensely positive; + slightly positive ; - negative.

Table:8 Results of staining reactions and histochemical tests for the detection of Acid mucopolysaccharides in the compound eye of the marine prawn, Metapinneus sp.

Sl. No.	Stains and tests	References	Corneal lens	Crystalline cone	Rhabdome
1.	Basic fuchsin	Stampien (1962)	Deep red	red	brown
2.	Aldehyde-fuchsin	Gomori (1950)	Strongly metachromatic (purple)	Upper part (metachromatic) lower part:-Orthochromatic	Blue(orthochromatic)
3.	Toluidine blue	Pearse (1961)	purple (strongly metachromatic)	Upper: purple lower part: blue peripheral part:-green (hypsochromatic)	Blue(Orthochromatic)
4.	PAS test	(Mc Manus and caron, 1950; Hotchikiss, 1948)	+	+	-
5.	Alician blue	(Steedmann, 1950)	+	+	-

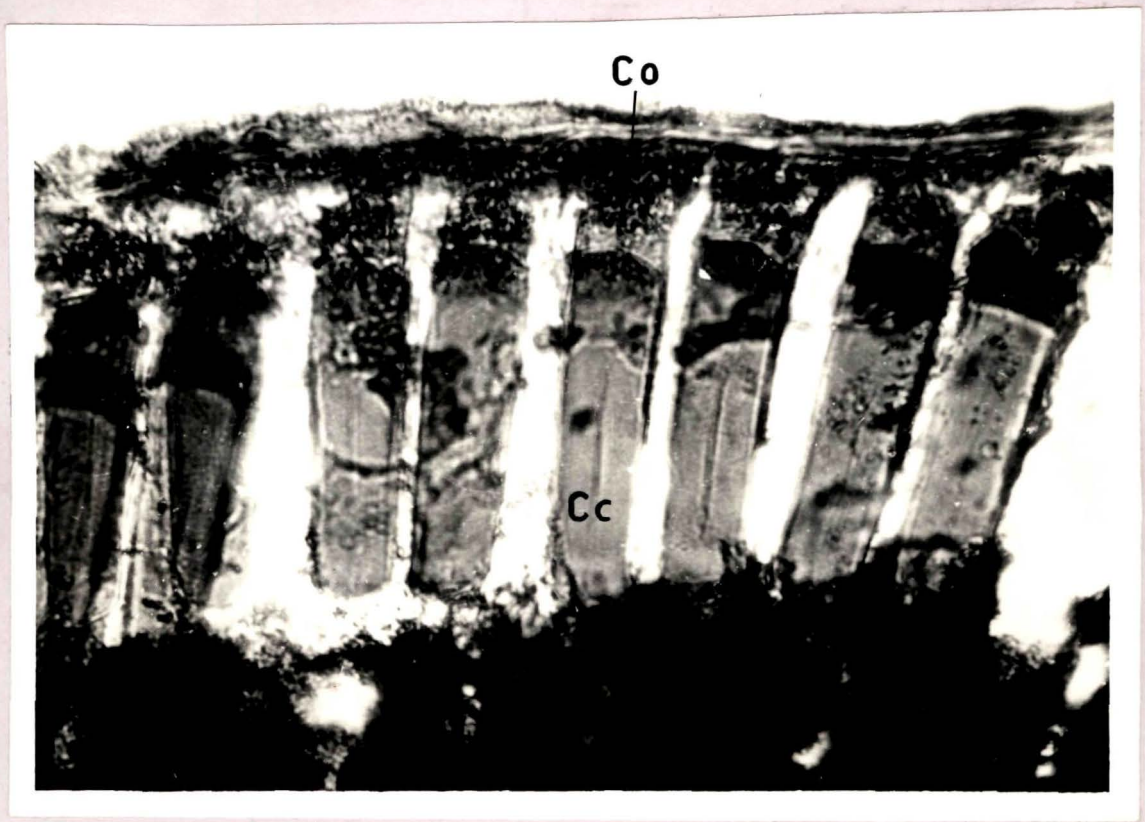
Key: +++ intensely positive; slightly positive; - negative.

EXPLANATION TO FIGURES

Fig. 7. An enlarged view of a portion of the T/S through the eye of the marine prawn, *Metapinneus* sp. 10 x 40..

Co , Cornea (metachromatoc)

Cc , Crystalline cone(metachromatic)


Co
Cc

in the region. The rhabdom region gives a blue colour reaction i.e. orthochromatic. The upper portion of the crystalline cone is metachromatic, whereas the lower part is orthochromatic (Table 7 and 8).

Staining of the histological preparations with Toluidine blue indicates that the pro-cuticle is very much metachromatic. The upper portion of the crystalline cone is also metachromatic, whereas the rhabdom region is orthochromatic. A careful onserving of the sections stained with Toluidine blue reveals that there is a slight hypsochromatic reaction in the peripheral portion of the crystalline cone(Fig.7).

Chromatography: Results of the chromatographi analysis of the acid mucopolysaccharides from the compound eye of Musca domestica and Macrobrachium birmanicum indicates the presence of three sugars. These are glucose, galactose and Xylose(Figures 8 and 9).

Due to paucity of materials, the amount of extracted mucopolysaccharides from the eye of honey-bee, Apis cerana indica and the marine prawn, Metapinneus spp. was so little that it has not been possible to make the acid hydrolysis and the chromatographic analysis.

Electrophoresis:- Comparison of electrophoretic movement pattern of the crude extract of mucopolysaccharides from Musca domestica, Apis Cerana indica, Macrobrachium birmanicum, Metapinneus spp. with several standard acid mucopolysaccharides in various buffer system shows that the main component of the mucopolysaccharide

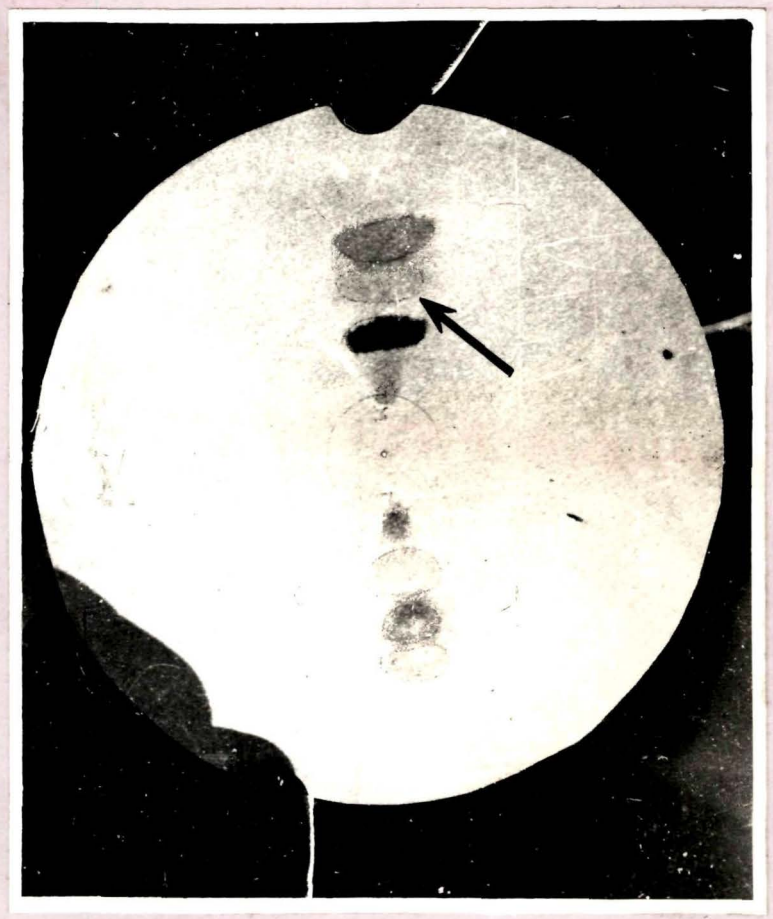
extracted from the compound eyes comprises of a sulfated mucopolysaccharide. It has been found that extracted mucopolysaccharides behave like chondroitin sulfate in regard to its Rf value in the electrophoretic fiel(Figures 10, 11, 12 & 13).

EXPLANATION TO FIGURES

Fig. 8. Circular paper chromatogram , showing the sugar components of the mucopolysaccharides extracted from the eye of the house-fly, *Musca domestica*.

Solvent: Butanol - Acetic acid - Water

(4 : 1 : 1, Vol/Vol).

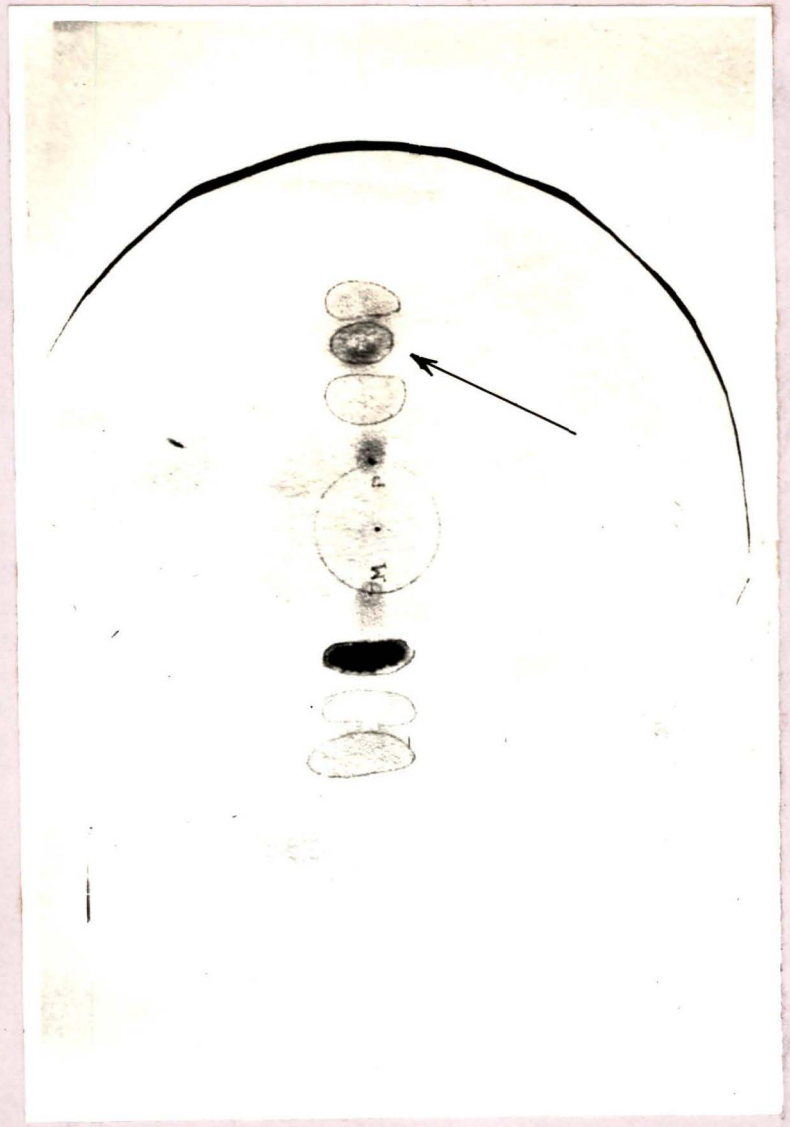


EXPLANATION TO FIGURES

Fig. 9. Circular paper chromatogram , showing the sugar components of the mucopolysaccharides extracted from the eye of the fresh-water prawn, *Macrobrachium birmanicum*

Solvent: Butanol - Acetic acid - Water

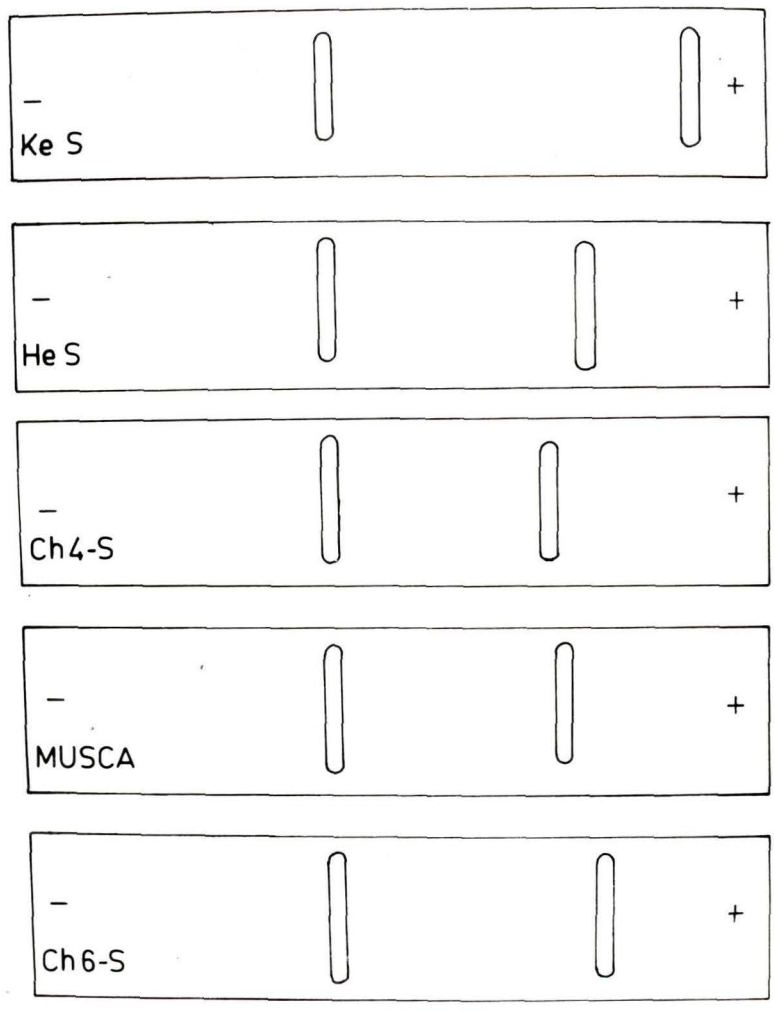
(4 : 1 : 1 , Vol/Vol).



EXPLANATION TO FIGURES

Fig.10. The electrophoretic movement pattern of the crude mucopolysaccharide extract from the compound eye of the house-fly, Musca domestica , as compared with the movement pattern of some standard mucopolysaccharides.

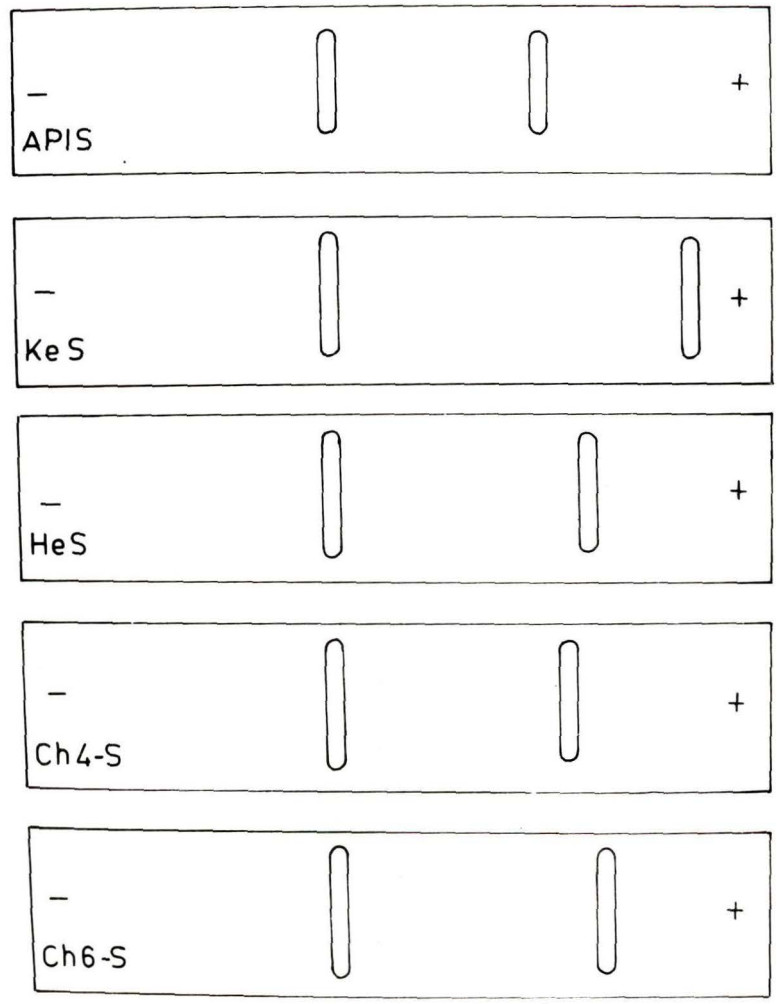
ch_4 - S , chondroitin - 4 sulfate
ch6 - S , chondroitin -6 - sulfate
KeS, Keratan Sulfate
HeS Heparan sulfate


–
Ke S
+
–
He S
+
–
Ch4-S
+
–
MUSCA
+
–
Ch6-S
+

EXPLANATION TO FIGURES

Fig. 11. The electrophoretic movement pattern of the crude mucopolysaccharide extract from the compound eye of the honey bee, Apis cerana indica, as compared with the movement pattern of some standard mucopolysaccharides.

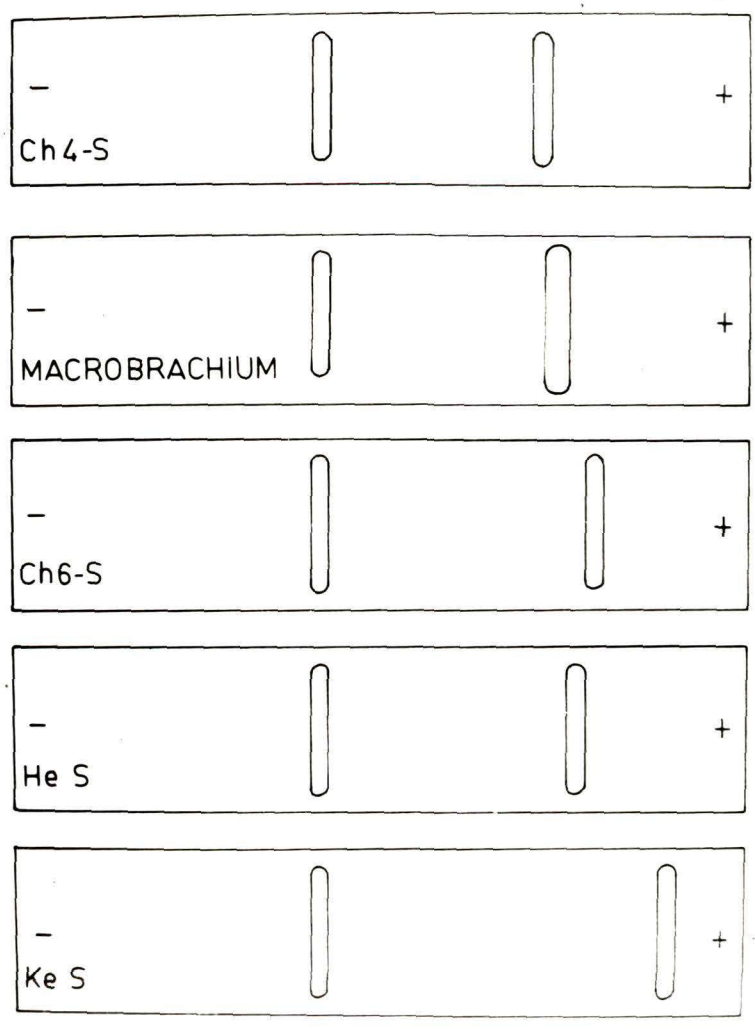
ch 4 - S, chondroitin 4 sulfate
ch 5 - S, chondroitin 6 sulfate
Kes, Keratan Sulfate
Hes, Heparan Sulfate.


–
APIS
+
–
KeS
+
–
HeS
+
–
Ch4-S
+
–
Ch6-S
+

EXPLANATION TO FIGURES

Fig. 12 . The electrophoretic movement pattern of the crude mucopolysaccharide extract from the compound eye of the fresh-water prawn, Macrobrachium birmanicum, as compared with the movement pattern of some standard mucopolysaccharides.

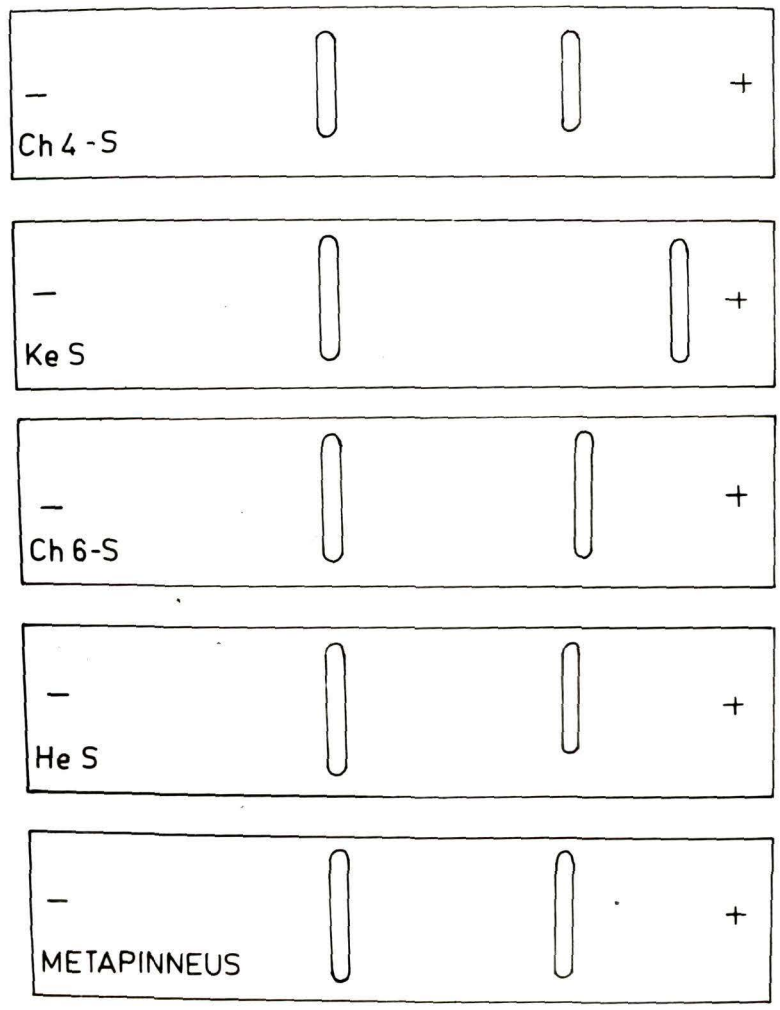
ch4 - S , chondroitin - 4 - sulfate
ch6 - S , chondroitin - 6 - sulfate
Kes , Keratan Sulfate,
Hes , Heparan sulfate.


–
Ch4-S
+
–
MACROBRACHIUM
+
–
Ch6-S
+
–
He S
+
–
Ke S
+

EXPLANATION TO FIGURES

Fig. 13. The electrophoretic movement pattern of the crude mucopolysaccharide extract from the compound eye of the marine prawn *Metapinneus sp.*, as compared with the movement pattern of some standard mucopolysaccharides.

Ch 4 -S , Chondroitin - 4 - sulfate
CH 6 -S , chondroitin - 6 - sulfate
Kes , Keratan Sulfate
Hes , Heparan Sulfate.


–
Ch 4 -S
+
–
Ke S
+
–
Ch 6-S
+
–
He S
+
–
METAPINNEUS
+

DISCUSSION

The foregoing observations reveal that the lens-cuticle and the crystalline cone of Musca domestica, Apis Cerana indica Macrobrachium birmanicum and Metapinnieus sp. contain mucopolysaccharides , more particularly some sulfated mucopolysaccharides.

The term " mucopolysaccharide " has originally been introduced by Meyer(1938) to describe substances having similar physical and chemical properties, isolated from the connective tissues. With increase in understanding of chemistry of these substances in recent years, many of these substances are now recognised as Glycoproteins, mucoproteins or mucopolysaccharides. However, the most recent trend is to name the substances as Glycosaminoglycans" ,which replaces the older terms " Mucopolysaccharides".

These polysaccharides are made of aminosugars, either 2 - amino-2-deoxy-D-glucose (D- glucosamine) and uronic acids, either D- glucuronic acid or L-iduronic acid, and may have N- acetyl, O - or N - sulfate groups.

The glycosaminoglycans are generally divided into the following classes:- (1) Hyaluronate (2) Chondroitin-4-sulfate (3) Chondroitin-6-sulfate (4) Dermatan sulfate(5) Keratan sulfate (6) Heparan sulfate and (7) Heparin.

Among these substances Keratan sulfate and heparin do not follow the definition of " Glycosamino-glycans" in the strict sense.

Keratan sulfate does not contain a uronic acid component and this is replaced by D-galactose. Heparin, on the other hand has similarities in its chemical structure to that of other glycosaminoglycans, but is is not usually present in the connective tissue.

Glycosaminoglycans or mucopolysaccharides have been detected in many vertebrate tissues and their biological significance has also been studied by different authors.

Mucopolysaccharides have been isolated from aorta of man (Barnes and Partridge, 1968; Berenson et.al. 1966), Cow (Radhakrisnamurthy and Berenson, 1973), pig (Wagh and Roberts,1972), horse, sheep(Robert, Robert and Robert, 1970), elephant (Mc Cullagh et.al. 1973) and chicken (Keeley et. al. 1969).

Skins of certain vertebrates such cattle (Bowes et al., 1958; Wolff et al., 1971), rat (Furth mayer and Timpl, 1970) and rabbit (Timpl et al.,1969, Borel et al., 1970) have also found rich in mucopolysaccharides.

Mucopolysaccharides have also been detected in cartilage of some vertebrates such as ox by Schubert and Hamerman (1968) Enomoto et al.,(1966), Janado and Dunstone(1972), Vittur et al. (1972 a.b.).

Among the various tissues of vertebrates, where mucopolysaccharides have been detected, the organic matrix of bone are reported to be the richest source(Herring, 1972). In addition to these, brain of some mammals e.g. rat are found to contain a rich

amount of some mucopolysaccharides (Di Benedetta et al.,1969; Margolis and Margolis, 1970).

Based on the occurence of mucopolysaccharides in these tissues, different functions have been suggested for these substances by various authors.

Rubin and Howard (1950) have observed acid mucopolysaccharides in association with calcification in bones and Cartilages. Mathews (1959) and Osawa (1971) have suggested that one of the characteristic properties of mucopolysaccharides is the selective association or binding with small inorganic cations, especially H^+ , Na^+ and Ca^{++} and also with Cationic groups of macromolecules. Farber and Schubert (1957) have found that in chondroitin- sulfate Ca^{++} is bound in a percentage greater than that of Na^+.

Urist et al.(1968) has also found a small preference for binding Ca^{++} over Na^+ in chondriotin sulfate. Mathews(1975) thus has rightly suggested that these substances act as a store for Ca^{++} in cartilage tissue and that is why have specific roles in tissue - calcification.

Mucopolysaccharides have also been reported to play some very essential roles in "Water-binding" and tissue osmotic pressure (Ogston and Wells 1972; Wells, 1973 b); Ogston (1970) has suggested that mucopolysaccharides play a role on tissue osmotic pressure, not only by influencing the water [illegible] balance but also by introducing excess swelling pressure, which is

balanced by an internal structural resistance.

Ogston and Wells (1972) and Wells (1973 b) has also suggested a role of mucopolysaccharides in maintaining mechanical flexibility and elasticity of tissues. Mucopolysaccharides may also have some functions in control of metabolism of cells, movement of metabolites on the basis of their rather specific chemical structure (Jeanloz,1970). Kobayashi and pedrini(1973) have suggested that mucopolysaccharides have a major role in structural organization of intracellutar matrix. According to them, mucopolysaccharides may be involved in electrostatic and steric interactions with other macromolecules of the matrix, such as collagen and elastin.

In spite of the fact , an extensive amount of works have been carried out on the mucopolysaccharides of various vertebrate tissues, the knowledge of histochemistry and biochemistry of invertebrate mucopolysaccharides is scanty. Hunt and collaborators (1970)have detected a glucan sulfate- peptide component from mucin from marine snail, Buccinum undatum . Mathews (1975) has detected a chondroitin- sulfate - like substance from cranial cartilage of L. opalescens.

Among the invertebrates, however some attentions have been paid in arthropods in the recent years to study their mucopolysaccharides. Some mucopolysaccharides, especially hyaluronic acid has been detected in various glands, e.g. salivary glands(Vadgama and Kamat 1971) and dermal glands (Baldwin and Salthouse, 1959). Its

occurance has also been detected in the connective tissue of brain, ganglia and imaginal disc of the house-fly, Musca domestica by Mustafa and Kamat (1970). Marshal (1966 a,b) has detected the occurence of mucopolysaccharides in the malpighian tubules and spittle of Cercopid larvae. In the locust, Locusta migratoria, Ashhurt and Costin (1971 a,b) have reported sulphated mucopolysaccharides in the connective tissue surrounding the ejaculatory duct and in the ganglia. Ashhurst and Richards(1964) have also detected mucopolysaccharides in the connective tissue, surrounding the nerve cord of pupa of the wax moth, Galleria mellonella.

Recently, some very interesting roles have been adduced to acid mucopolysaccharides, especially in the cuticle of arthropods. Meenakshi and Scheer (1959) and Sundara Rajulu(1969) have envisaged that acid mucopolysaccharide of the cuticle of Hemigrapsus nudus and Cingalobolous bunanioni respectively may haveimportant roles in the calcification of the cuticle. Krishnan (1965) has suggested that acid mucopolysaccharides may be associated with - s s- bonding of the cuticle in the scorpion, palamneus swammerdami.

The extreme flexibility of the intersegmental cuticles of the queen of Odontotermes Obesus has been considered to be due to the occurrence of acid mucopolysaccharides (Sannasi,1969). This assumption has been made on the basis of the fact that acid -

mucopolysaccharides are said to possess high water binding capacities (Ogston,1966 a; Laurent et al, 1969, and Katchalsky, 1964).

Now, the question is, what is the role of acid mucopolysaccharides that are present in the lens-cuticle and the crystalline cone of Musca domestica, Apis Cerana indica Macrobrachium birmanicum and Metapinnieus spp ?

Since the occurence of mucopolysaccharides is not a general feature of the arthropod cuticles and since it occurs in some special types of cuticle where it performs some special functions (Mecnakshi and Sheer,1959; Sundara Rajulu,1969; Krishnan, 1965; Sannasi, 1969), it is reasonable to presume that the specific occurrence of mucopolysaccharides in the lens-tuticle and the crystalline cone may have a bearing on the visual system of arthropods, examined in the present study.

In this context it is of interest to mention that mucopolysaccharides have been detected in several tissues of the eye of vertebrates and invertebrates by different authors, and its importance in visual phenomenon have been suggested.

Anseth (1961 a) and Moczar and Moczar(1973) have detected the occurence of mucopolysaccharides in the cornea of squids. It may be noted that squid cornea, unlike the analogous tissue in vertebrate eye is a direct continuation of the skin. Balazs et al (1959) have found that vitreous body of the eye of the squid also contains a high concentration of hexuronic acid and hexosamine, presumed to be present as hyaluronate.

Balazs (1965), have reviewed thoroughly the biochemistry of the cornea and other tissues of the eye. Mucopolysaccharides have been detected in the cornea of of chondrichthyes(Balazs, 1965), Osteichthyes (Anseth, 1961 a ;), reptilia e.g. turtte (Anseth, 1961a), aves, such as adult owl, turkey and chiken (Anseth, 1961 a) adult man, owl monkey, rabbit, sheep, cattle and Whale (Anseth, 1961 a).

The other ocular tissues, where mucopolysaccharides have been reported are vitreous (Balazs, 1965; Berman and Voaden, 1970), aqueous and ciliary body, interstitial martix surrounding the photoreceptor cells of Cattle matrix (Berman and Bach, 1968; Berman, 1969) and sclera of Ox.

With these reports in view, it is possible to assume a role of mucopolysaccharides in the lens-cuticle of arthropods. The lens-cuticle as already stated, besides playing a general deffensive role, performs a special optical function of conducting light rays to the inner rhabdomere. It is possible now, to presume that the transparency of the lens-cuticle, which is more than that of other types of cuticles(e.g. body-cuticle) may be affected by the occurrence of mucopolysaccharides. The basis of such presumption is that the transparency of the cornea of vertebrates has been reported to be effected by mucopolysaccharides (Anseth and Fransson, 1970). It is known that the bulk of cornea of vertebrate eye is the stroma, which functions as a supporting structure and is adapted for the transmission of a high percentage

of incident light of visible wave-lengths (Mawrice, 1969). Anseth and Fransson (1970) have found that during chick corneal development, the occurrence of a highly sulfated Keratan sulfate is associated with the rise in transparency of stroma. They have also suggested that stromal transperency is correlated with the presence of the normal proportions of Keratan sulfate and chondroitin 4 - sulfate.

A role of acid mucopolysaccharides can also be adduced in the compound eyes of arthropods studied, on the basis of the fact that acid-mucopolysaccharides can act as selective ion barriers (Jeanloz, 1970). It has often been suggested that the light stimulated potentials observed in photoreceiptor cells of invertebrates such as horse-shoe crab (Stieve, 1965), Cray-fish (Eguchi, 1965), squids (Haggins, 1965), barnacle (Brown et al, 1969) and honey-bee (Fulpius and Bauman, 1969) are a direct consequence of an increase in the permeability of the photoreceiptor cell membrane to sodium ions. This suggestion has been made from electro-physiological experiments.

Recently, Duncan et al. (1971) have measured the intracellular oationic concentrations in the isolated retina of squid and frog with and without stimulation of light and have unequivocally proved that there is an increase in membrane permeability to sodium ions during visual excitation. In the compound eye of Musca, Apis, Marcrobrachium and Metapinneus, as found in the present study, acid mucopolysaccarides are found not only in the lens-cuticle but also in the crystalline cone, which are close

connection with the inner rhabdomeres, and the rhabdomeres are the sites of chemical reactions the products of which may depolarize the membrane of the retinula cells and initiate impulse formation(Wigglesworth,1957). In view of the above reports, it is reasonable to presume that the acid mucopolysaccharides in the compound eyes may play an important role in visual excitation, when the light rays pass through the outer epicuticle to the inner endo-cuticular region (crystalline cone)- the sites of acid mucopolysaccharides.

In this regard, it is worth-mentioning that we have already detected acid mucopolysaccharides in the lens-cuticle and crystalline cone of some other insects such as Periplanata americana , Pierie brassicae, fresh-water bug, Belostoma sp. and nymphs of Aeshna sp.(Dey,1976) and in some crustaceans, such as Palaemon sp. and the horse-shoe crab, Limulus Polyphemus (Dey, Raghu Varman Michael, 1978).

The cosmopolitan occurrence of acid mucopolysaccharide in the lens-cuticle and crystalline cone of various arthropod groups further support our presumption.

Another interesting pointm which can be mentioned here is that Vitamin A has been shown to be involved in the synthesis of sulphated mucopolysaccarides. It is presumed that retinol, or an active form derived from retinol, or a retinoic acid releases an enzyme (or enzymes) necessary for the biosynthesis of mucopolysaccharides(Wolf and Varandani(1960) have reported that

deficiency of vitamin A in the tissue - culture of cartilage tissue blocks the synthesis of mucopolysaccharides.

In this context, it is of interest to mention here that vitamin A has been reported in the compound eye of many anthropods (Goldsmith, 1964, Wolken et al. 1960). Thus it will be of great significance to study whether there is any relationship between mucopolysachharides and vitamin A in the compound eyes.

Chapter - II

ASCORBIC ACID IN THE COMPOUND EYE.

INTRODUCTION

The visual ability of any animal is greatly influenced by the generation of energy in their photoreceptor organs. The generation of energy in the biological system is chiefly due to the oxidative process, which is carried out by two mechanisms (i) the addition of oxygen to a substance and (ii) removal of hydrogen from the substance. Goldsmith(1924) while studying the energy generation in the photoreceptor of vertebrates has found that the oxidative process of the eye, especially the lens is dependent mainly on the second method of oxidation, i.e.removal of hydrogen. For such a method of oxidation, it is known that some reducing substances should essentially be present in the photoreceptor organ. Glick et al (1936); Bellows and Rosner(1936) Rosner et al (1938); Henkes (1946) have reported the occurrences of two reducing substances, namely glutathione and ascorbic acid in the lens of some vertebrates.

Pirie (1946) has reported the presence of ascorbic acid in the cornea of ox at a concentration of approximately 1.8 u moles per gm. wet weight of the tissue. Heath (1962) has reported ascorbic acid in the lens of rat, dog, guinea-pig, rabbit, sheep frog, pig, man, cow and horse. Ascorbic acid has also been reported in the aqueous humour of rabbit (Muller and Buschke, 1934; Podesta and Baucke, 1938), ox (Muller and Buschke, 1934; Podesta and Bucke 1938; Vladesco and Stefanesco, 1939; Johnson,

1936), man (Muller and Buschke 1934; Purcell et al. 1954), monkey (Kinsey and Jackson, 1949) Guineapig (Muller and Buschke, 1934; Podesta and Baucke, 1938; Johnson, 1936), Sheep (Vladesco and Stefanesco, 1939; Podesta and Bauchke, 1938; Vladesco and Stefanesco, 1939) Cat (Langham, 1950), Rat (Muller and Buschke, 1934) and Frog (Muller and Buschke, 1934).

The occurrence of ascorbic acid has been reported in the vitreous body (Balazs, 1961) and also in the retina(Heath, 1962; Heath et al., 1962b ; Heath and Fiddick, 1964, 1965a,b; Fiddick and Heath, 1966) of several vertebrates.

Detection of high levels of ascorbic acid in various parts of vertebrates photoreceptors have led to the attempts to study the importance of this substance in the visual function of animals by a number of authours (Heath and Fiddick,1964; 1965a; b, ; Pirie 1964; Kinoshita 1964; Henkes 1946).

In an attempt to study the importance of ascorbic acid in the visual system, Rawal and Rao,(1977) have made an estimation of the substance in the lens of a number of vertebrates such as, fishes (Ophiocephalus; Saccobranchus fossils; Barbus pinnuratus); amphibian (Rana hexadactyla; Bufo metanostictus); birds (Columba livia) and mammals (Rattus norvegicus; Cavia porcellus, Taphozous longimanus). The interesting observation made by them is that, fishes which swim on the surface or on the upper strata of water, e.g. Ophiocephalus and Barbus have a small amount of ascorbic acid in the lens. In contrast to this , they have observed that

in fishes which live in bottom or deeper water, e.g. cat fishes, the amount of ascorbic acid in the lens is significantly higher. In the similar way, they have observed that in amphibians also, the frogs which live in well-lighted area contain a small amount of ascorbic acid in the lens, whereas the members living in shaded orless-lighted area have comparatively higher amount of ascorbic acid in the lens. Among the mammals, the nocturnal bats have comparatively higher amount of ascorbic acid in the lens. In higher forms, ascorbic acid shows degradation except in birds, where it is lower than amphibians and fishes and higher than reptiles and birds.

A gradual decrease in the concentration of ascorbic acid, however, has been observed in the lens as the animals become old. In spite of the wide variations in the concentration of this vitamin between species, in aging lens of almost all the species investigated so far, the level has been found to be greatly decreased. As for example, the rat and cow differ in lens-size, life-span and average ascorbic acid level but both of them show similar drops in ascorbic acid concentration in the ageing lens. (Kuck Jr., 1961).

All these reports suggest that ascorbic acid may play some very important roles in the visual system of animals. But a review of literature on the subject reveals that the study has been confined mainly to the vertebrate eyes. No attempt has been made so far in the photoreceptor of invertebrates, especially of the arthropods.

Ascorbic acid has been reported in various tissues of several species of arthropods (Giroud and Rakoto-Ratsimamanga, 1936; Haydak and vivino, 1943; Metcalf, 1943; Smyth, Bingley and Hill, 1944; Swamy and Sreenivasaya, 1942; Woolman, Giroud and Ratsimamanga, 1937), although it has not been reported as an essential dietary constituent in most of the species. Wollmann et al (1937) have reported that the cockroach, Blatella can synthesize ascorbic acid. Wollmann, Giroud and Ratsimamanga (1937) have reared Blatella on a diet free from ascorbic acid for 15 years. But it has been observed that, in spite of rearing of these insects on ascorbic acid- free diet, they contain 10-15 mg/g of the tissue, the amount which is found in newly caught specimens. Joly (1940) has reported the occurrence of ascorbic acid in the blood of the queen termite, Bellicositermes natalensis(Hav). A considerable quantity of ascorbic acid has also been reported in the honey bee Apis mellifera by Haydak and Vivino (1943). Giroud and Rakoto-Ratsimamanga (1936) have reported that the muscles of Dytiscus contain a very high amount of ascorbic acid; about three times higher than that of vertebrate muscles. In the malpighian tubules of the cockroach, Periplanata americana, the amount of ascorbic acid is reported to be 0.6 - 10 mg / g of the tissue.

Day (1949) has detected ascorbic acid in various tissues of a number of arthropods, such as the silverfish, Ctenolepisma longicaudata Esch; the cockroach, Blattela germanica (L) workers of the termite, Nasutitermes exitiosus (Hill), larvae

and adult of the mealworm Tenebrio molitor L.; adubts of the flour beetle, Tribolium confusum Duval; larvae, pupae and adults of the blowfly, Lucilia cuprina wied; larvae and adults of potato moth, Gnorimoschema Operculella Zeller, larvae of the clothes moth, Tineola visellilla Hummel, and worker of the honey bee, Apis mellifica L. by means of histochemical tests. He has detected ascorbic acid in the dermal tissues, blood, circulatory system, alimentary canal, exee excretary system, storage tissue, muscle tissue, respiratory tissue, nervous tissue, glandular tissue, organs of intermediary metabolism, and reproductive system of those arthropods.

But inspite of all these , it seems that no attempt has been made on the compound eyes of insects and other arthropods.

With these in view, histochemical and biochemical studies have been performed on the compound eyes of the housefly, Musca domestica; the honey bee, Apis Cerana indica; the fresh-water prawn, Macrobrachium birmanicum and the marine prawn, Metapinneus sp. to determine the ascorbic acid status in the compound eyes.

Materials Methods

Histochemistry : The histochemical tests employed for the detection of ascorbic acid is that followed by Bacchus (1950). The eyes are separated from the head very carefully and immersed in the dark for thirty minutes in 5% silver nitrate containing two drops of acetic acid per milliliter at 56°C. The tissues are washed thoroughly in several changes of distilled water for thirty minutes and treated in 5% Sodium thiosulphate for thirty minutes and again washed in distilled water and transfered to 70% alcohol. Dehydration and infiltration are performed in subdued light. The materials are sectioned mounted on slides and counterstained in cosin.

Colorimetric detection: The colorimetric method for the estimation of ascorbic acid followed in the present investigation is the modified method of Chinoy et al (1976). It is known that ascorbic acid in the biological materials occurs not only in free form (AA), but also it occurs in the bound form, or ascorbigen (ASG). It is also known that ascorbic acid is continuously acted upon by a number of oxidizing enzymes. These include a special peroxidase which catalyses the formation of its free radical - (FR) - monodehydroascorbic acid(MDHA) (Gurevich), 1963). Further, chinoy (1967, 1969) has reported that in living system endogenous as well as exogenously added ascorbic acid forms a complex with macromolecules like proteins and nucleic acids. Isherwood and Mapson (1962) have suggested that in a tissue, the actual concentration of ascorbic acid

represents the excess formed in synthesis over that used in metabolism. Thus, according to them any study regarding the concentration of ascorbic acid in free form alone may lead to an incorrect inference. Keeping all these facts in view, ascorbic acid turn-over in the compound eyes has been studied by simultaneous determination of (i) free form of ascorbic (AA), (ii) bound form of ascorbic acid(ASG), (iii) enzymatic utilization of ascorbic acid(AAU), and (iv) complexing of ascorbic acid with marcromolecules (AA_MM).

Reagents :

(1) Metaphosphoric acid (HPO_3)

(i) 3 % W / V solution (0.275 M) .

(ii) 15 % W/ V solution (1.375 M).

These solutions have been kept in the refrigerator (3^oC).

(2) Buffer Solutions.

(i) Buffer solution A (0·5 M) : 10·55g. of Citric acid are dissolved in 1N NaOH and the volume is made to 100 ml (PH 4·8).

(ii) Buffer solution B (1·5 M) :31·65gm of Citric acid are dissolved in 3N NaOH to a volume of 100 ml (PH 4·8).

(3) Buffered HPO_3 .

The volumes of 3% HPO_3 have been mixed with one volume of buffer solution A. The PH of this solution is 3·6. Buffered HPO_3 is freshly prepared every time.

(4) Standard ascorbic acid solution.

10 mg. of ascorbic acid is dissolved in glass-distilled water, which is previously boiled, cooled and saturated with CO_2. The volume of the solution is made upto 100 ml and stored in an amber - coloured bottle at 3-5°C. Ascorbic acid solution is always freshly prepared.

(5) Standardised dye solution.

10 mg of 2,6- dichlorophenol- indophenol (BDH) is dissolved in de-ionised water at 80°C. It is cooled and the volume is made upto 200 ml.

Standard Curve : Ascorbic acid solutions of concentrations ranging from 10 µg to 90 µg/ ml are prepared from a stock solution by dilutions with cold CO_2- saturated glass distilled water to the requisite concentration.

1 ml aliquot of each AA solution is mixed with 1 ml of buffered HPO_3 (pH 3·6) solution (pH does not change by the addition of ascorbic acid since the buffering capacity is high) and 5 ml of standardised dye solution and the reading on the scale of photoelectric colorimeter is recorded using a green filter (i.e. 485-550 nm). Colorimetric readings are taken for each AA solutions and a standard graph is prepared by plotting these values.

Extraction of the materials

The eyes are separated from the head very carefully so that the adhering tissues are removed from them, as far as possible.

Weighed sample of the material is placed in a mortar, covered with 1-2 ml of cold CO_2 saturated distilled water

as well as a pinch of purified silica sand and quickly homogenised. The contents are transferred to a test tube. The mortar and pestle are rinsed two to three times with 1 - 2 ml of cold CO_2 - DW, transferred to the test- tube and the volume of homogenate is made upto 12 ml with CO_2 - saturated distilled water.

The homogenate is divided into three parts:

(i) 4 ml for the estimation of AA.

(ii) 4 ml for the estimation of ASG; and

(iii) 4 ml for the estimation of AAU and MM complex.

Determination of ascorbic acid :- 4 ml of cooled buffered HPO_3 is added to 4 ml of the original homogenate and after thorough shaking a 2 ml aliquot of the mixture is diluted with 5 ml of distilled water and the pointer on the Colorimetric scale is adjusted at '0' for the turbidity factor. 5 ml of standard dye solution are added to another 2 ml aliquot of the same mixture and the colorimetric reading is noted.

Calculation : The concentration of ascorbic acid in 2 ml of the original extract is calculated as follows. As each 2 ml aliquot contains 1 ml of the original homogenate the concentration of ascorbic acid / gm fresh weight of the material is :-

$$A = \frac{a \cdot V}{W} \times 1000$$

Where, A = AA content of the sample in µg/ g. fresh weight.

a = AA in µg/ ml of the original homogenate.

V = Total volume of the original homogenate.

and W = Weight of the sample taken for analysis (in mg).

Determination of ascorbigen (ASG)

2 ml of 15% HPO_3 is added to 4 ml of the original homogenate and the mixture is kept in a water-both at 75°C for 15 minutes for hydrolysing ascorbigen. After cooling, the system is buffered at pH 3·6 by adding 2 ml of the Buffer solution B thus increasing the volume of the mixture to 8·0 ml and the colorimetric reading is recorded as in free ascorbic acid.

Concentration of ascorbic acid in 2 ml of the buffered hydrolysed extract is determined from the standard graph. The value of free ascorbic acid in 1 ml of the homogenate, which has been determined previously, is subtracted from it to obtain the ascorbic acid equivalent of ascorbigen in 1 ml of the homogenate.

Ascorbigen content per gm. fresh weight is calculated as follows:

$$B = \frac{V\ (\ b-a\ )}{W} \times 100$$

where, B = Ascorbic acid equivalent of ascorbigen in ug per gm. fresh weight of the sample.

b = Ascorbic acid (µg) in 2 ml of buffered - hydrolyzed extract.

a = Ascorbic acid (µg) per ml. of the original homogenate.

V = Total Volume of the original homogenate.

W = Weight of the ssmple taken for analysis (in mg).

Ascorbic acid utilization (AAU) and ascorbic acid - macromolecule complexing (AA - MM complex):

4 ml of ascorbic acid solution (100 µg/ ml) are added to 4 ml of the original homogenate. The mixture is incubated at 30 ± 2°C with thorough shaking after every 10 minutes. After incubation, aliquots of 3 ml are taken out separately for the anslysis of AAU- MM complex respectively.

Determination of AAU : 3 ml of buffered HPO_3 are added to 3 ml of the incubated solution, and ascorbic acid content is determined. The ascorbic acid content of 2 ml aliquot of the buffered incubated solution (i.e.0·5 ml of the original homogenate) is determined from the standard graph. The value of ascorbic acid (in µg) thus obtained is multiplied by 2 to obtain the value of ascorbic acid per 1 ml of the original homogenate left unutilized after incubation.

The calculation of AAu per gm. fresh weight is as follows:

$$C\ 1 = \frac{V(a + 100) - 2C}{W} \times 1000$$

where, C 1 = Abscorbic acid in µg utilized per gm. fresh weight during a given period of incubation.

C = Amount of ascorbic acid in µg left over in 2 ml of the buffered - incubated solution (i.e. 0·5 ml of the original homogenate.

a = Ascorbic acid in µg/ ml of the original homogenate.

V = Total Volume of the original homogenate.

W = Weight of the sample taken for analysis(in mg).

Determination of AA-MM complex :

At the end of the incubation period 2 ml of 15 % HPO_3 are added to 4 ml aliquot and the mixture is hydrolyzed. After cooling, the mixture is buffered at pH 3·6 by adding 2 ml of buffer solution - B bringing the total volume to 8 ml and the complexing of ascorbic acid is determined as in ascorbigen . The ascorbic acid content in the aliquot is determined from the standard graph and is multiplied by 2. Substracting the value of ascorbic acid left over in 1 ml of the original homogenate before hydrolysis from the above value gives the amount of ascorbic acid released by hydrolysis of the AA_MM complex

(in /ug/ ml). The amount of ascorbic acid complexing with macromolecules per gram.fresh weight has been calculated as follows:-

$$D_1 = \frac{2V\ (d-c)}{W} \times 1000$$

Where, D_1 = /ug of ascorbic acid released from the complex per g. fresh wt of the material.

d = ug of ascorbic acid in 2 ml of the incubated hydrolyzed buffered solution (i.e. in 0·5 ml of the original homogenate)

V = Total volume of the original homogenate.

C = Amount of ascorbic acid in /ug left over in 2 ml of the buffered-incubated solution (i.e. 0·5 ml of the original homogenate).

W = Weight of the sample taken for analysis.

Observations and Discussion

The principle of the histochemical tests for the detection of ascorbic acid in biological system lies on the fact that silver nitrate reduces ascorbic acid in tissue sections and produces a characterstic pattern of black granules scattered in the region, where ascorbic acid is present. The efficiency of the method, however, has been criticised by Barnett and Fisher (1943), which has mainly been answered by Bourne(1944).

Barnett and Bourne (1941), on the basis of the available evidences have stated that in vertebrates it is "justifiable to assume that the reactions observed are unlikely to be due to reducing substances other than ascorbic acid".

Day (1948) has stated that this suggestion is very much applicable to insects also. But one of the difficulties is that melanin granules are also known to reduce silver nitrate in acid solution. However it has been found that proper controls readily distinguish them from the ascorbic acid - positive granules(Day,1948).

The histo-chemical tests employed for the detection of ascorbic acid in the present investigation have failed to indicate ascorbic acid- positive granules in the

in the corneal lens, which is a modified cuticle. This is in agreement with the suggestion of Day(1948), who has reported that cuticle of most of the insects and other arthropods usually does not contain any substance which reduces silver nitrate. However, he has reported that the cuticle of the larva of the potato-moth, Gnorimoschema contains some very small ascorbic acid-positive granules. Similarly, the larva of the mealworm, Tenebrio molitor is reported to show a peculiar distribution of larger granules in a few regions of the cuticle. These granules are present in abundance near the hypodermes and they are arranged in rows and at right angles to the hypodermis. Because of this unusual appearence of the granules, it is not easy to comment boldly on the occurrence of ascorbic acid in the cuticular regions of arthropods. However, Day (1948) has suggested that the characteristic pattern of ascorbic acid positive granules in the hypodermal cells of several insects, are very similar to those reported in other tissue. In Locusta, these ascorbic acid-granules are very much conspicuous in cells located at muscle - cuticle attachments. Hypodermal cells which, however, are not attached to muscles do not show any such ascorbic acid- positive granules (Day 1948). Day (1948) has also reported that the hypodermal cells of the larvae of the blowfly, Lucilia cuprina wied, potato moth, Gnorimoschema Operculella Zeller, mealworm, Tenebrio molitor L., workers of the termite,

Nasutitermes exitious (Hill), and the silver fish, Ctenolepisma longicaudata Esch. contain ascorbic acid- positive granules but their appearence is less striking than in other tissue.

In the present investigation, although ascorbic acid could not be detected in the corneal lens by histochemical tests, careful observations of a large number of sections using suitable controls reveals the presence of ascorbic acid positive granules in the rhabdom regions of Musca domestica, Apis cerana indica, Macrobrachium birmanicum and Metapinneus sp.(Fig.14). To confirm the results obtained by histochemical tests, chemical estimation of ascorbic acid from the compound eye has been performed. The ascorbic acid turn over (Free form of ascorbic acid or AA, bound form of ascorbic acid or ASG, enzymic utilization of ascorbic acid or AAU and association of ascorbic acid with macromolecules or AA-MM complex) in the compound eye of Musca, Apis Macrobrachium and Metapinneus are presented in Tables 9, 10, 11, and 12 respectively.

The major source of error in studying the ascorbic acid concentration in biological system is the auto-oxidation of ascorbic acid. This auto-oxidation has been prevented by a fairly easy method as described by Chinoy et al(1976), in which glass-distilled water used for extraction as well as for preparing ascorbic acid solution is saturated with CO_2. Chinoy et al (1975) while studying the auto oxidation of ascorbic acid

EXPLANATION TO FIGURES

Fig.14. Diagramatic representation of the distribution of ascorbic acid positive granules in the compound eye.

c, cornea ,

Co, Crystalline cone

pp, primary pigment cell

rc, retinula cell

r , rhabdom

bm, basement membrane

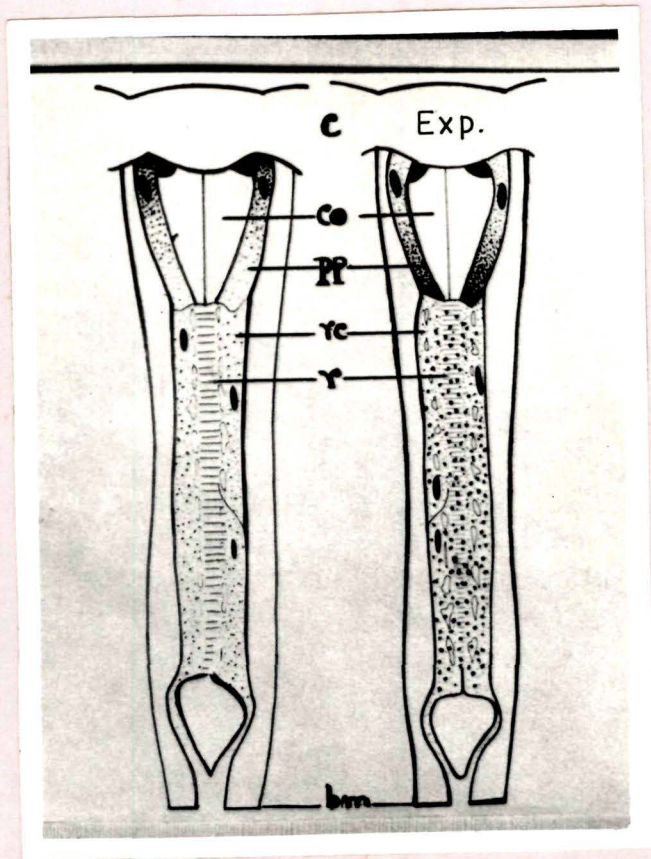

C
Exp.
Co
Pp
Yc
Y
bm

Table 9. Ascorbic acid turn over (AA or Free form of ascorbic acid; ASG or bound form of ascorbic acid; AAU or enzymic utilization of ascorbic acid and AA-MM or association of ascorbic acid with macromolecules) in the compound eyes of the house-fly, Musca demestica.

Different forms of ascorbic acid	Concentration (mg / gm)
1. Free form (AA)	0.41 ± .026
2. Bound form of ascorbic acid (ASG)	0.44 ± .218
3. Enzymic utilization of ascorbic acid (AAU)	2.1 ± .125
4. Association of ascorbic acid with macromolecules (AA-MM complex)	0.21 ± .017

Table 10. Ascorbic acid turn over (free form, AA; bound form of ascorbic acid, ASG; Enzymic utilization of ascorbic acid, AAU; Association of ascorbic acid with macromolecules AA - MM) in the compound eyes of the honey bee, Apis Cerana indica.

Ascorbic acid	Concentration (mg/g)
1. Free form of ascorbic acid (AA)	0.60 ± 0.026
2. Bound form of ascorbic acid (ASG)	2.1 ± 0.125
3. Enzymic utilization of ascorbic acid (AAU)	2.3 ± 0.127
4. Association of ascorbic acid with macromolecules (AA - MM complex)	2.1 ± 0.122

in relation to time have found that auto-oxidation of ascorbic acid is rapid in glass-distilled water kept at 30°C. When glass-distilled water cooled to 3-5°C after boiling, is used, it has been observed that the rate of auto-oxidation is slowed down, which is noted upto 180 minutes. When the glass-distilled water is saturated with CO_2, it has been observed that auto-oxidation is completely checked at both 30°C and 3- 5°C.

Another probability of error in the estimation of ascorbic acid is due to the instability of the dye, 2,6-dichlorophenol indophenol. It is known that the dye, 2,6-dichlorophenol indophenol solution is very much unstable at low PH, which is characteristic of indophenol dyes. At low PH, these dyes decompose to give quninones and aminophenols. (Karrer,1950).

Chinoy et al (1976) have reported that unbuffered HPO_3 decolourises the dye rapidly and the rate of decolourisation is proportional to the concentration of HPO_3 present in the system. In our study the dye has been effectively stabilized by buffering it with Citric-NaOH buffer at a PH 3.6.

Another difficulty in estimation of ascorbic acid turn over, is that, during the determination of ascorbigen

Table 11. Ascorbic acid turn over (free form of ascorbic acid, AA; bound form of ascorbic acid, ASG; enzymic utilization of ascorbic acid, AAU; and association of ascorbic acid with macro-molecules, AA - MM complex) in the compound eyes of the fresh water prawn , Macrobrachium birmanicum.

1. Free form of ascorbic acid (AA)	0.75	±	0.214
2. Bound form of ascorbic acid (ASG)	.22	±	0.025
3. Enzymic utilization of ascorbic acid (AAU)	3.12	±	0.061
4. Association of ascorbic acid with macro-molecules (AA - MM complex)	. 63	±	0.024

Table 12. Ascorbic acid turnover (free form of ascorbic acid , AA; bound form of ascorbic acid, ASG; enzymic utilization of ascorbic acid; AAU and association of ascorbic acid with macromolecules, AA-MM complex) in the compound eyes of the marine prawn, Metapinneus sp.

ascorbic acid	Concentration (mg / gm)
1. Free form of ascorbic acid (AA)	.9 ± 0.032
2. Bound form of ascorbic acid (ASG)	.24 ± 0.021
3. Enzymic utilization of ascorbic acid (AAU)	12.16 ± 3.611
4. Association of ascorbic acid with macro-molecules (AA - MM)	.24 ± 0.024

and AA-MM complex, hydrolysis with metaphosphoric acid is carried out at 75°C for 15 minutes. At such a high temperature, there is always a possibility of loss of ascorbic acid due to heating. This loss is effectively checked in the present investigation by using 15% metaphosphoric acid in the system.

Chinoy et al (1976) while studying the effect of metaphosphoric acid on the protection of ascorbic acid during hydrolysis have suggested that 15% or higher concentration of metaphosphoric acid (HPO_3) effectively checks the loss of ascorbic acid during heating at 80°C upto 15 minutes. When HPO_3 is used to check the loss of ascorbic acid the system is buffered to p^H 3.6 before the addition of the dye to it. While using 15% metaphosphoric acid, buffer solution B (Ref. Materials and Methods) effectively brings the PH range to 3.6 when added in 1:1 ratio.

Finally, the interference of substances other than ascorbic acid is checked by carrying out the determinations in strong acid solution. Thus it can be inferred that the dye reducing property observed in the eye-homogenate of Musca domestica, Apis Cerana indica, Macrobrochium birmancium and Metapinneus sp. is very likely to be due to the presence of ascorbic acid in the compound eyes.

Now, the question is, what can be the significance of occurrence of ascorbic acid in the compound eyes of these arthropods ?

As already stated, studies on the occurrence and and significance of ascorbic acid in the visual system has been confined mainly to the photoreceptor of vertebrate. Thus in any attempt to study the significance of ascorbic acid in vision, one should depend at first on the investigations made on the vertebrate eye.

Kinoshita (1964) , Henkes (1946) and Rawal and Rao(1977) have suggested that ascorbic acid may play some very important role in maintaining the transparency of lens in the eye of some vertebrates. The basis for such a suggestion is that the concentration of ascorbic acid in the lens is reported to be relatively higher in those animals where their lens needs more energy. Kinoshita (1964) and Henkes (1946) have reported that "Cataract", the most common lens-disease of human being is caused due to some disturbances in the metabolic process of the lens and they have suggested that one of the reasons for the formation of " Cataract" may be the depletion of ascorbic acid and glutathione . In this context, it is of interestto mention that Kinoshita(1964) has reviewed some of the biochemical

reactions involving glutathione and ascorbic acid, which may be relevant to lens metabolism . It is known that the main source of energy in the crystalline lens is the pentose phosphate pathways and it is suggested that the respiratory link between ascorbic acid and glutathione provides $NADP^{+}$ AV available for the enzyme of the pentose phosphate pathways (Pirie, 1964).

One of the reasons for assuming some very important functions to ascorbic acid in vision lies on the fact that eye has relatively more capacity to retain ascorbic acid than other tissues, when the dietary source of the Vitamin is cut off. Hughes et al (1971) have reported that when guineapigs are deprived of dietary sources of the vitamin, the brain and the eye lens show retention of a substantial amount of it while the other organs show almost complete depletion.

In their experiment, Hughes et al (1971) have maintained guineapigs on an ascorbic acid-free diet for 14 days. After 14 days, when they have estimated ascorbic acid from organs of the guineapig, it has been found that the concentration of the vitamin has fallen to 1% of the initial in the spleen, to 4% in the adrenal glands and less than 1% of the initial concentration in the aorta. In contrast

to this, the corresponding values for brain and eye lens have been found to be 24% and 28% respectively. Hughes, Hurley and Jones (1970) have suggested that the differences that has been observed on the concentration of ascorbic acid between eye-lens and other organs may be due to the fact that the specific mechanisms that ensure retention or uptake of ascorbic acid may operate more efficiently in the eye than in other organs.

Hughes et al (1971) have suggested that the function of ascorbic acid may be very important to the eye lens and that may account for the maintenance of the level of the vitamin in the eye as long as possible. Even after the fall of the concentration the necessary functions are maintained even longer by an increased turnover rate. This may explain the experiment of Baker (1946) , in which it has been reported that a rabbit lens in vitro looses all detectable ascorbic acid after 10 days but transparency of the lens is maintained even after 21 days out of culture. Besides the lens, aquous humour of theeye of many species retains a high concentration of ascorbic acid by a mechanism of active transport from blood to eye (Kinsey,1947; Barany and Langham,1955). But in spite of all these reports, the reason why this high concentration of ascorbic acid should exist in the eye remains unclear.

Recently a very important functions of ascorbic acid has been suggested by Buck and Zadunaisky (1975) in the corneal epithelium and other ocular tissues of frog and rabbits on the basis of the fact that ascorbic acid stimulates ion transport through inhibition of 3′ : 5′ - cyclic - AMP phosphodiesterase.

In their experiments, Buck and Zadunaisky (1975) have observed that ascorbic acid exerts an inhibitory action on the 3′ : 5′ - cyclic AMP phosphodiesterase(EC 3.1.4.17 (activity of sub-cellular homogenates of corneal epithelium. They have found that the addition of ascorbic acid produces an increase in the short-circuit current of the isolated corneas of frog and rabbit. They have also reported that in frog, addition of ascorbic acid at a concentration of 10 mM in the reaction mixture cause a 37% inhibition of phosphodiesterase activity in the corneal epithelium of frog. To find out whether this phosphodiesterase inhibition is due to change in PH of the reaction medium, they have determined the p^H of the reaction mixture at the start and end of the incubation period; they have found it to be essentially similar. This inhibitory action of ascorbic acid is found not only in the frog cornea, but it has also been reported in the corneal epithellium of rabbit and toad (Buck and Zadunaisky, 1975)

By inhibiting 3′ : 5′ - cyclic phosphodiesterase activity, ascorbic acid produces an increase in cyclic AMP content of corneal epithelium and other tissues. (Buck and Zadunaisky 1975; Lewins, 1973). This increase in cyclic AMP content in the corneal epithelium possibly stimulates active ion transport across the cornea.

All these reports give some ideas regarding the way in which ascorbic acid may play some roles in the photoreceptor of vertebrates.

Now, the compound eyes of arthropods as already mentioned (Ref. General Introduction) are some what different from the vertebrates eye, as far as the structure is concerned. But in spite of these differences, it seems that chemically, and also functionally both the arthropod compound eyes and vertebrate eyes are more or less similar. As for example, it has been reported that, the arthropod compound eyes use vitamin A and a retinene-complex for the visual pigment chemistry as is done by vertebrate. In addition to this, it has been reported that the rhabdomere microtubules of arthropods are very similar to that of the vertebrate retinal rod outer segment sacs (Wolken, 1968).

Thus , it is reasonable to presume that the functions which have been suggested for ascorbic acid in the photoreceptors of vertebrates, may similarly be applicable to the compound eyes of arthropods also.

In the present investigation, while studying the utilization of ascorbic acid it has been observed that the part of ascorbic acid of the aliquot incubated for AAU forms some complexes, presumably with macro-molecules instead of getting oxidised. The complexing, however, can be recovered after hydrolysis with metaphosphoric acid. This complexing ability of ascorbic acid, most probably is responsible for the formation of bound form of ascorbic acid (Ascorbigen). Such complexing may lead to the formation of charge transfer complexes which has also been reported to occur in biological system very frequently, and they are known to take active part in the process of energy transfer (Szent Gyorgyi, 1960).

Szent Gyorgyi (1960) has suggested for the first time the importance of these charge transfer complexes in biological system. Since this report, complexing of ascorbic acid with macromolecules has been reported from time to time (Chinoy, 1967, 1969; Chinoy et al ., 1971; 1973; 1974). Bonner (1957) has reported that ascorbic acid is acted upon by a number of enzymes which oxidise ascorbic acid directly

or indirectly and over and above this oxidation, it forms complexes with macromolecules, such as protein and nucleic acid.

Another interesting point which should be considered here is that the rhabdomeres of the compound eye of arthropods are known to contain polyphenolic substances (Suzuku,(1962) In the course of histochemical studies, Suzuku (1962) has observed that in the housefly, Musca vicina(Mackquart) periodic acid- schiff (PAS) positive substances are distributed in the rhabdomeres of the dark- adapted insects. He has also found that P AS reaction of rhabdomere is always positive both in a dark-adapted eyes treated with saliva for one hour in an incubator and in a light-adapted eye. In addition to this he has observed that Bauer's reaction is very weak in the rhabdomeres of the insects. He has reported that rhabdomeres are positive to Millon's reaction, Potassium iodate, Potassium bichromate and ammoniacal silver, but it is negative for the detections of - amino radical and indole radical. Positive Millon's reaction indicates the presence of tryptophan, tyrosine and other phenolic substances, but since the rhabdomeres are negative to both the=tests for - amino and indole radicals, Suzuku (1962) has suggested that rhabdomeres do not contain - amino acids such as tryptophan and tryrosine, rather it contains some polyphenolic derivatives.

In this context, it is of interest to mention that ascorbic acid may play some important roles in synthesis of polyphenolic substances (Dennell, 1947; Dadd, 1960).

Dennel (1947) has suggested that ascorbic acid may be implicated in melanin formation in larvae of Sarcophaga falculata. Recent studies on the process of sclerolization and melanization reveal that ascorbic acid in the blood plays an important role as an inhibitory factor in these process. In silkworms the amounts of ascorbic acid present in each larval stage undergo cyclic flucturations and are minimal during the moulting period (Gamao, 1941). Its concentration in the haemolymph of grass-fed Schistocerca larvae varies characteristically during the course of the fourth and fifth instars. Immediately after ecdysis, when the insect is still soft, the titer of the blood is Zero, thereafter it steadily increases at a high level from mid-instar until the following moult, after which it is again found to be Zero (Dadd, 1960).

In view of the above reports, it is reasnable to presume that the ascorbic acid in the compound eyes of Musca domestica, Apis Cerana indica, Macrobrachium birmanicum and Metapinneus sp. may have some important roles in synthesis of polyphenolic substances in the rhabdome.

In addition to this, ascorbic acid may also play some very important functions in vision of the above-mentioned arthropods in the same way as has been suggested in the case of vertebrates (Kinoshita, 1964; Rawal and Rao 1977).

Chapter - III

FLUORESCENT SUBSTANCES OF THE COMPOUND EYE

Section 1. RESILIN

Section 2. PTERIDINES.

Section - I.

R E S I L I N

INTRODUCTION

The lens-cuticle of the compound eye of arthropods can be regarded as ' transitional ' cuticle (Neville, 1975). The term has originally been introduced by Anderson and Weis- Fogh (1964)to describe that type of cuticle, which is intermediate in physical and chemical properties between solid and rubber - like cuticles. Weis-Fogh (1960) has described the distinction between the solid cuticle and rubber - like cuticle, when he discovered the latter for the first time. Both the solid cuticle and rubber - like cuticle contain chitin but they differ in their protein component. Solid cuticle is generally present in the sclerities and its supermolecular organisation is much more precise than that of arthrodial membrane or the cuticle between sclerites.

The characteristic feature of a rubber-like cuticle is the presence of an elastomer protein, resilin, which has been discovered by Weis - Fogh (1960).This type of cuticle is generally identified by a combination of techniques. The structure with resilin stains sapphire with Methylene blue or toluidene blue-light green combination. When it is irradiated with ultra-violet light , it autofluoresces with a bright blue colour in the range of

420- μ. The fluorescence increases in presence of alkaline conditions. Upon deformation, it shows marked strain - birefringence, which disappears immediately after the strain is removed. The most important property of a rubber-like cuticle is its extensive and reversible elasticity. The rubber like cuticle stores and releases elastic energy with very high efficiency and thus it functions in assisting the muscles. This property is correlated with the mode of cross - linking of the cuticle.

The transitional cuticle is distinguished from both the solid cuticle and rubber-like cuticle by the presence of certain properties which are intermidiate between the two. This type of cuticle contains chitin and characteristic proteins of solid cuticle, and at the same time, it also contains cross - links typical of resilin in the rubber - like cuticle (Neville, 1975).

As already stated, the rubber-like cuticle is distinguished by the presence of the protein, resilin. Resilin is a colourless, structural elastic protein, the name of which is derived from the latin word " resilire " which means to jump back. Weis-Fogh (1960) was the first to discover this protein in certain elastic hinges and tendons in the cuticle of locusts and dragonflies. The protein is colourless and is very elastic (Weis-Gough 1960)

and posses characteristically di - and tri-tyrosines, which are fluorescent (Bailey and Weis - Fogh, 1961). Because of its occurrence in certain cases in pure isotropic form, the protein can be analysed both physically and chemically without further purification.

Resilin, in many respects resembles the vertebrate structural protein, elastin and thus it may well be called " arthropod elastin " (Anderson and Weis-Fogh, 1964). But inspite of the fact that resilin resembles elastin in many points, it also differs from elastin in many important ways.

Thus, both resilin and elastin posses high content of glycyl, alanyl and prolyl and in this respect they are very much similar to each other. The most significant difference between the two, however, is that resilin has a high proportion of residues with polar side chains whereas elastin has an unusually small amount. But in any case, both of them are elastic and rubber-like and have very similar physical properties. LLoyd and Garrod(1946) and Kendrew (1954) have suggested that the rubber - like properties of elastin is maintained by the scarcity of polar groups. But since the polar groups are abundant in resilin, their absence in elastin may not be very important

in maintaining the rubber- like properties, Purified elastin is faint yellow in colour and they show fluorescence in ultra violet light. Regarding the nature of the fluorescent components of elstin, there is a great amount of disagreement among the authorities. But it is certain that they act as cross-links between the peptide chains. The nature of the fluorescent substances isolated from resilin is however very much different from those isolated from elastin but the function of both types are essentially the same.

Resilin is present in certain patches of cuticles of insects and some crustaceans (e.g. crayfish). It has been detected in the elastic ligaments in flight system of Aeshna (Weis-Fogh, 1960), Schistocerca (Weis-Fogh, 1960) Calliphora (Andersen and Weis-Fogh, 1964), Panorpa(Neville and Rothschild, 1967) and Bombus (Andersen and Weis-Fogh, 1964); Remnants of flight-system in wingless jumping insects Xenopsylla (Bennet clark and Lucey, 1967; Nevile and Rothschild, 1967); Clypeo-labral spring of Schistocerca (Andersen and Weis- Fogh, 1964); Abdominal tergites of Schistocerca (Andersen and Weis-Fogh, 1964); and Oryctes (Andersen and Weis-Fogh, 1964) sucking pump in mouthparts of Rhodnius (Bannet-Clark, 1963 a), Glossina (Rice,1970) Mesosternal plate for jumping in click beetles, Melanotus (Sannasi, 1969); base of bristles of Apis (Thuom, 1964);

tarsel and aroliar pads, in Schistocerca (Andersen, 1971 a) spermathecal duct wall of Aedes (Clements and Potter, 1967) Periplanata (Gupta and smith, 1969); Spiracle opening mechanism in Schistocerca (Miller, 1960); leg-hinge of Astacus (Andersen and Weis-Fogh, 1964); in the regions between abdominal segments of Panulirus (Crustacea) (Neville and Luke, 1971b); Anal leg joints of Scolopendra (Sundara Rajulu, 1971); sound organs of pyralidae (Lepidopteral) and Cacama (Homoptera: Cicadidae) (Scott, 1970) jaw cuticle of Eoperipatus (Krishnan, 1970). Although these are some of the very important examples of the occurrence of resilin in arthropods, the protein is likely to be present in many more situations in arthropods, as suggested by Andersen and Weis-Fogh (1964) and Nevile (1975).

Miller (1960) has observed that in the locust, the cuticle of the pleura of the pterothorax, dorsal to the second spiracle contains resilin and he has suggested that this rubber-like property of the cuticle may be partly responsible for the wide-openning reaction of the valve during flight. Thurm (1963) has reported that the base of each hair in the sensory hair fields of bees stains with methylene blue like resilin, and the structure is highly elastic. Since resilin does not flow and also it does not suffer any permanent deformation this report of Thurm(1963)

is of considerable significance, and Andersen and Weis-Fogh(1964) have suggested that the material like resilin would be an absolute frame of reference for a mechanical sence organs.

From all these reports it is clear that resilin acts as a elastic material from which the epidermal cells construct mechanical springs and thus it plays a very important role in storage and release of mechanical energy. Weis-Fogh (1960) has suggested that it is metabolically inert but Jensen and Weis-Fogh (1962) have observed that after deposition simple chemical modification may take place and thereby mechanical properties may slightly be altered. But this is yet to be proved directly. As compared with other structural proteins, resilin is remarkably stable, no major changes take place. In fact, its physical properties are very much unchangeable. Elasticity and insolubity are the two most important properties of resilin. It is insoluble in water at a temperature below 140^{o}C and also in all other solvents (Weis-Fogh, 1960; Andersen and Weis-Fogh 1964). Another very important property of resilin is that it swells considerably in effective protein solvents such as phenol, formamide, formic acid etc. It also swells in concentrated solution of Lithium thiocyanate, and cupric ethylene diamine.

While reviewing the functions of resilin in arthropod

cuticles, Andersen and Weis-Fogh (1964) have suggested that resilin or related proteins may play an optical role in the transparent cuticles over the eyes and ocelli, but there is not much information regarding the study of this protein in the lens-cuticle of compound eyes of arthropods excepting the report of Sannasi(1970).

Sannasi (1970) has detected for the first time the occurrence of the structural elastic protein, resilin in the lens-cuticle of the firefly, Photinus Pyralis by staining reactions, swelling properties and also by detecting the UV fluorescence with a maximal intensity at 420 m/μ .

Considering all these, certain histochemical and biochemical studies have been performed in the compound eyes of Musca, Apis, Macrobachium and Metapinneus in relation to the structural elastic protein resilin.

MATERIALS AND METHODS

The materials used in the study comprise the lens-cuticle of house-fly Musca domestica; the honey-bee Apis cerana indica ; fresh- water prawn, Macrobrachium birmanicum ; and the marine prawn, Metapinneus sp.

Histochemistry : For histochemical study, the material has been fixed in 5% neutral formaldehyde for 5 hours. The material is then washed in several changes of buffer at 5°C for 30 minutes. After that, the material is transferred directly to 70% aquous ethanol, dehydrated and cleared and embedded in paraffin and the sections are taken at 8/u thick. The sections have been stained with stains customarily used in arthropod cuticular study such as Mallory's tripple. Masons trichrome and Heidenhains iron haematoxylin.

The histochemical tests for the detection of resilin have been employed according to the method described by Andersen and Weis-Fogh (1964) as follows:

The living arthropod has been killed by immersion in hot, buffered water for a few minutes(pH 6.7, 95-100°C). After separating the eyes from the head region, the lens-cuticle is freed from the adjoining tissues by means of a thin

strong jet of tap-water. The rinsed cuticle is placed for 24_ 28 h at room temperature in dilute phosphate buffer (M/20,pH about 7) to which 5 mg toluiding blue and 5 mg light green per 100 ml. buffer is added. A crystal of thymol is added to prevent microbial growth.

The histological preparations of the lens-cuticle has also been similarly treated with light-green-toluiding blue combination and are examined in light microscope.

Swelling properties: Andersen and Weis-Fogh(1964) have reported that one of the characteristic features of the protein resilin is that it swells strongly in water and in water containing media and also in many anhydrous solvents. The degree of swelling depends on pH. Weis-Fogh(1960) has suggested that the liquids solvate the peptide chains and break the secondary bonds and thus the chains become flexible. and free of each other. Thus, it is thought that investigations of swelling properties of the lens-cuticle will also be of interest in this regard. The solvents used for studying the swelling properties are formic acid, acetic acid, Phenol, formamide, acetamide, dichloroacetic acid trichloroacetic acid and glycerol. Some solvents such as formic acid and formamide have been used at room temperature. In other solvents such as glycerol heating to 60 - 70°C has been performed. The solvents such as trichloroacetic acid, acetamide

and phenol have been used at few degrees above their melting points.

Analysis of amino acids : For analysis of the amino acids of the lens-cuticle, the lens cuticle has been separated from the adhering tissues carefully, the cuticle is analysed without involving extraction procedures as has been done by Hackman and Goldberg(1971). This is prefered as Andersen and Barrett(1971) have suggested that extraction may involve considerable change in organic components. The lens-cuticle has been kept in fresh aquous ethanol, and adhering tissues are removed by scraping with a blunt scapel. The cuticle is then dried in vaccum desiccator over calcium chloride, is powdered and the cuticular material is collected by centrifugation. The residue is hydrolysed in 6N hydrochloric acid at a temperature of 105°C for 1.2 to 18 hours. The hydrolysate is evaporated to dryness over a boiling water bath and the dry residue is dissolved in double distilled water. The test material, is then spotted in Whatman No.1 filter paper with the help of glass-capillary.

One dimensional, descending chromatography with subsequent use of two solvents in the same direction is carried out as suggested by Andersen(1963). The first solvent is iso-propanol-concentrated amonia -water (8:1:1 V/V). A small beaker with 3% amonia has been placed at the bottom

of the chromatography for 30-60 min.before starting the chromatogram, otherwise,the fluorescent compounds might migrate to some extent. When the solvent has reached the lower edge of the paper, it has been removed, dried and rechromatographed in the same direction in n-butanol-acetic acid-water (4:1:1: V/V) for 16-20 hours. The fluorescent amino-acids are easily located by examining the chromatogram in UV light.

Because of the fact that the light microscope with ultra-Violet attachment is not available in our department, we could not examine the autofluorescence of the lens cuticle of the arthropods, we have chosen for our study.

OBSERVATIONS

Lens-cuticle of the house-fly, Musca Domestica:

When the histological preparation of the lens-cuticle of Musca domestica is stained in a mixture of 5 mg. toluidine blue and 5 mg. light green in 100 ml. buffer solution (M/20) for 40 hours and examined after 6 hrs washing in pure buffer solutions, the following observations has been made:

At PH 4.6, the epicuticle is faint green blue and the procuticle shows blue colour. At PH 6.1 the epicuticle is faint-sky-blue and the procuticle is blue. At PH 7.1, the epicuticle is sky-blue and the pro-cuticle is sapphire in colour.(Table 13).

Swelling properties: When the lens-cuticle of the house fly, Musca domestica has been subjected to the treatment of certain solvents, which are generally used for the study of swelling of resilin, it has been observed that in solvents like formic acid and formamide, the lens-cuticle swells considerably within few minutes, even at room temperature. When glycerol is used, the swelling does not take place so quickly and the process is much slower as compared to formamide or formic acid. The lens-cuticle also swells in tri-chloro-acetic acid, acetamide, phenol, Dichlorocetic acid etc. to considerable extent.(Table 13).

Amino-acids: Chromatographic analysis of the lens-cuticle for amino acids, shows that besides the usual amino acid constituents of the insect cuticle , two fluorescent amino-acids (di-and tri- tyrosine) are present in the chromatogram. They are easily located by their blue fluorescence in UV light and the RF values at 0.05 and 0.18 respectively. The fluorescence has increased when the chromatogram has been exposed to ammonia vapour, whereas vapour from hydrochloric acid has quenched the fluorescence almost completely.

Table 13. Staining and histochemical reactions shown by the different layers of the lens-cuticle of the house fly, Musca domestica.

Stains and tests	References	Epicuticle	Procuticle
Mallory's tripple stain	Mallory, 1938	red	blue
Toluidine-blue-light green Ph 4.6	Andersen and Weis-Fogh, 1964	green-blue (faint)	blue
Toluidine-blue-Light green, PH 6.1	Andersen and Weis-Fogh, 1964	Sky-blue (faint)	blue
Toluidine blue-Light green, PH 7.1.	Andersen and Weis-Fogh, 1964	Sky-blue	Sapphire
Formic acid	Andersen and Weis-Fogh, 1964	swells	swells
Acetic acid	Andersen and Weis-Fogh, 1964	swells	swells
Phenol	Andersen and Weis-Fogh, 1964	swells	swells
Formamide	Andersen and Weis-Fogh, 1964	swells	swells
Acetamide	Andersen and Weis-Fogh, 1964	swells	swells
Dichloroacetic acid	Andersen and Weis-Fogh, 1964	swells	swells
Trichloroacetic acid	Andersen and Weis-Fogh, 1964	swells	swells
Glycerol	Andersen and Weis-Fogh, 1964	swells	swells

Lens-cuticle of the honey bee, Apis cerana indica:

The transverse section through the eye of the honey-bee Apis also shows almost the same type of colour reactions when the preparation is stained with toluidine-blue-light green stain from PH 4-7. At PH 4.6, the epicuticle is faint blue green in colour and the procuticle is greenish blue; at PH 6.1, both the epicuticle and pro-cuticle are faint green-blue; at PH 7.1 the epicuticle is sky-blue and the procuticle is sapphire.

The lense-cuticle swells in formic acid, acetic acid, Phenol, formamide, acetamic, dichlo-acetic acid, trichloro-acetic acid and glycerol.

When the chromatogram, run for aminoacid anglysis, has been examined with a UV-lamp, two blue-fluorescent spots has been detected. On exposure of the chromatogram to amonia vapour, the fluorescence of these spots have been increased, whereas hydrochloric acid vapour has quenched the fluorence of the spots. From the Rf values of the two fluorescent spots(0.05 and 0.18 respectively) they have been identified as di-and tri-tryosine.

Lens-cuticle of the fresh-water Prawn, Macrobrachium birmanicum: When the transverse sections through the eye of Macrobrachium birmanicum are stained with light-green-Toluidine blue combination, it is observed that at Ph 4.6

the epi- and pro-cuticle is sky blue in colour, at PH 6.1 the epicuticle is blue and pro-cuticle sapphire, at PH 7.1 only the procuticle is sapphire.

The lens-cuticle of Macrobrachium also has exhibited considerable swelling on all the solvents tested, e.g. formic acid, acetic acid, Phenol, formamide, acetamide, dichloro-acetic acid, trichloroacetic and Glycerol. (Table 14).

The fluorescent amino acids, di and tri-tyrosine have also been identified in the chromatogram by using UV light.

Table 14. Staining and histochemical reactions shown by the lens-cuticle of the different layers of the honey-bee, Apis cerana indica

Stains and tests	References	Epi-cuticle	Pro-cuticle
Mallory's tripple stain	Mallory, 1938	red	red
Toluidine blue-Light green PH 4.6	Andersen and Weis-Fogh, 1964	Blue-green (faint)	Greenish blue
Toluidine blue-Light green PH 6.1	Andersen and Weis-Fogh, 1964	Green-blue	Green-blue
Toluidine blue-Light green, PH 7.1	Andersen and Weis-Fogh, 1964	Sky-blue	Sapphire
Formic acid	Andersen and Weis-Fogh, 1964	swells	Swells
Acetic acid	Andersen and Weis-Fogh, 1964	swells	Swells
Phenol	Andersen and Weis-Fogh, 1964	swells	Swells
Formamide	Andersen and Weis-Fogh, 1964	swells	Swells
Acetamide	Andersen and Weis-Fogh, 1964	swells	Swells
Dichloro-acetic acid	Andersen and Weis-Fogh, 1964	swells	Swells
Trichloro-acetic acid	Andersen and Weis-Fogh, 1964	swells	Swells
Glycerol	Andersen and Weis-Fogh, 1964	swells	Swells

Table 15. Staining and histochemical reactions shown by the different layers of the lens-cuticle of the fresh-water prawn, Macrobrachium birmanicum.

Stains and tests	Reference	Epi-cuticle	Pro-cuticle
Mallory's tripple stain	Mallory, 1938	red	light blue
Toluidine-blue-light green, PH 4.6	Andersen and Weis-Fogh, 1964	sky-blue	Sky blue
Toluidine-blue-light green, PH 6.1	Andersen and Weis-Fogh, 1964	Blue	Sapphire
Toluidine-blue-light green, PH 7.1	Andersen and Weis-Fogh, 1964	Sapphire	Sapphire
Formic acid	Andersen and Weis-Fogh, 1964	swells	Swells
Acetic acid	Andersen and Weis-Fogh, 1964	swells	Swells
Phenol	Andersen and Weis-Fogh, 1964	swells	Swells
Formamide	Andersen and Weis-Fogh, 1964	swells	Swells
Acetamide	Andersen and Weis-Fogh, 1964	swells	Swells
Dichloro-acetic acid	Andersen and Weis-Fogh, 1964	swells	Swells
Trichloro-acetic acid	Andersen and Weis-Fogh, 1964	swells	Swells
Glycerol	Andersen and Weis-Fogh, 1964	swells	Swells

Lense cuticle of the marine Prawn, Metapinneus sp:-

When the transverse section of the eye of the marine prawn, Metapinneus sp. has been stained with light-green toluidine blue stain it is found that PH 4.6, the epicuticle stains greenish blue and the pro-cuticle, sky-blue. At PH 6.1. the epicuticle is blue in colour and the procuticle sapphire. At PH 7.1 only the pro-cuticle has given sapphire-colour reaction.

Like the lens-cuticle of other arthropods studied, the lens-cuticle of Metapinneus also shows characteristic swelling properties in solvents like formic acid, acetic acid, Phenol, formamide, acetamide, dichloroacetic acid, trichloroacetic acid and Glycerol (Table 16).

The fluorescent amino acids(Di-and Iri-tyrosine) have also been detected in the chromatogram by their characteristic blue fluoresence and their Rf values of 0.05 and 0.18 respectively. When the chromatogram has been exposed to ammonia vapour, the fluorescence has increased and the hydrochloric acid vapour has quenched the fluorescence, almost completely.

Table 16. Staining and histochemical reactions shown by the different layers of the lens-cuticle of the marine prawn, Metapinneus spp.

Stains and tests	References	Epicuticle	Pro-cuticle
Mallory's tripple stain	Mallory, 1938	Pink	blue
Toluidine blue-light green, PH 4.6	Andersen and Weis-Fogh, 1964	greenish blue	Sky-blue
Toluidine blue-light green, PH 6.1	Andersen and Weis-Fogh, 1964	blue	Sapphire
Toluidine blue-light green, PH 7.1	Andersen and Weis-Fogh, 1964	Sapphire	Sapphire
Formic acid	Andersen and Weis-Fogh, 1964	swells	Swells
Acetic acid	Andersen and Weis-Fogh, 1964	swells	Swells
Phenol	Andersen and Weish-Fogh, 1964	swells	Swells
Formamide	Andersen and Fogh, 1964	swells	Swells
Acetamide	Andersen and Weis-Fog, 1964	Swells	Swells
Dichloro-acetic acid.	Andersen and Weis-Fog, 1964	Swells	Swells
Trichloro-acetic acid.	Andersen and Weis-Fog, 1964	Swells	Swells
Glycerol	Andersen and Weis-Fog, 1964	Swells	Swells

DISCUSSION

Recent studies on arthropod cuticle show that the properties and chemical nature of the body-cuticle varies from region to region of the animal and also during development which involves a series of moults.

Andersen (1976) has reviewed the cuticular enzymes and sclerotization in insects and has mentioned three important types of cuticular sclerotization, namely
(i) Quinone- tanning where the tanning of the cuticle is due to O - Quinone formed from diphenols.
(ii) β - sclerotization, which is an alternative pathway of sclerotization to the formation of Co-valent links between the β - position of N - acetyl dopamine and amino or phenolic groups in the cuticular proteins, and
(iii) di- and tri- tyrosine which is a special type of cross-linking mechanism in the cuticular protein, resilin.

Resilin occurs in certain specialized patches of insect cuticle (Weis-Fogh, 1960; Andersen and Weis-Fogh, 1964) and it is charecterised by having long-range rubber-like elasticity. From the mechanical analysis of the elasticity, Weis-Fogh (1961 a,b) concluded that the protein is composed of flexible randomly coiled chains, which are linked together by stable cross-links. The

existence of cross-links has later been confirmed by their isolation and chemical charecterization, and eventually they have been identified as di - and tri tyrosine (Andersen, 1964, 1966).

Subsequently Locke (1969) has demonstrated that even the actual process of hardening in other non-elastic regions of the cuticle of Calpodes ethlius (Stoll) involves the cross-linking by di- and tri tyrosine, which has been presumed to be catalysed by peroxidases (Coles, 1966). Krishnan (1970) has also reported that the jaw-cuticle of the onychophoran Eoperipatus is also hardened by cross-links involving di- and tri tyrosine. In addition, resilin has been reported in some non-elastic structures such as the egg - envelopes of dragonflies, Sympetrum infuscatum and Sympetrum frequens (Kawaski, et. al, 1974) and silk fibroin (Raven, et. al, 1971).

In the present study also we have observed the presence of resilin in the lens-cuticle of Musca domestica; Apis cerana indica; Macrobrachium birmanicum and Metapinneus sp., which are non-elastic but transparent. Now, the question arises as to what is the special role of resilin, if any, in the cuticular region which forms lens in these arthropods. From the fact that lens-cuticle which is transparent is also mechanically resistant and since resilin has also

been reported to occur in some non-elastic regions (Kawaski, et al, 1974 ; Raven et.al., 1971), it is reasonable to presume that the hardening of the cuticle in the photoreceptors forming " Lens" may involve cross-linking by di- and tri- tyrosine, which are charecteristically autofluorescent. However, the " hyaline" transparency of the lens-cuticle may not only be due to the occurrence of resilin but also due to other factors such as absence of phenolically tanned proteine and/or melanin pigments which normally owe amber or black colour to the cuticle. Besides, acid mucopolysaccharides which behave as gums of plants having the property of conversion from sol to gel and from gel to sol , may also impart hyaline transparency to the lens-cuticle (Refer Chap. 1).

Lastly, the characteristic amino acids, di- and tri-tyrosine of resilin are uniquely autofluorescent (420 m μ).

In view of the above reports it is presumable that the lens cuticle which is patched or coated with resilin which has the property of autofluorescence, may aid in absorbing and filtering the suitable light rays of the required range in the spectrum and thereby allow the harmless light rays into the interior of the compound eyes to be utilized for subsequent photo-activation and perception of objects by the arthropods.

Section - 2

PTERIDINES.

Pteridines constitute a group of compounds having the molecular structure of pyrimido 4, 5 - b pyrazine, They have a common structure of either 2 - amino - 4 hydroxy - Pteridines or 2, 4 - dihydroxypteridine and the are customarily called pterin or lumazine respectively. The name pteridine comes from the greek word " pteros ", which means feather or wings. (Wieland and Schopf, 1925; Schopf and Becker, 1936; Wieland and purrmann, 1940; Schopf and Reichert 1941). The compounds are so named because of their discovery for the first time in the wings of butterflies by Hopkins(1889).

The molecular structure of pteridines has been revealed by Purrman (1940) for the first time, who has found it to be pyrimido 4, 5 - b pyrazine (Purrman, 1940 a, 1940 b, 1941, 1943). Purrmann(1940) has reported that the structure of pterines resembles that of purines, imidazo 4,5 - c pyrimidine. Albert (1957) and Cresswell et al,(1965) have shown chemical similarities between purines and pteridines. Weygand and Waldschmidt (1955); Ziegler-Gunder, Simon and Wacker (1956); Aaronson and Rodriguez (1958); Brenner- Holzach and Leuthardt(1959, 1961; Vieira and shaw (1961) and Mc Nutt (1964) have shown that metabolically the pteridine molecules are closely related to

purines. A number of workers have shown that most of the pteridines that occur in nature are synthesized from purine necleot(s)ides, (Weygand et al, 1959; 1961, 1964; Jaenicke, 1961; Stuart and Wood 1962, Baugh and Shaw, 1963; Kidder and Dewey, 1968; Sugiura and M.Goto,1968)

On the basis of these findings, some authors have suggested a close relationship between pteridines and purines with regard to the fundamental synthetic pathway and general chemical properties such as enzyme susceptibility, chromatographic behavious and solubility(Buchanan, Sonne and Delluva,1948; Alber, 1954; Ziegler-Gundler,1956,a,b,c; Aaronson and Rodguez, 1958; Buchanan and Hartman,1959; Moat and Friedman, 1960; Brenner-Holzach and Leuthardt,1961; Weygand et al, 1961, Stackhouse,1966).

Since the discovery of the chemical structure of some naturally occuring pteridines such as leucopterin, xanthopterin and iso-xanthopterin(Purrmann, 1940 a, 1940b, 1941), neumerous derivatives of pteridines have been reported from the biological system by different authors. Pteridines have been reported to occur either as conjugated or unconjugated, monomer and polymer, oxidized or reduced, phosphorylated or as glycoside.

Pteridines and their derivatives have been reported to perform a variety of functions in the biological system.

They have been detected in the eyes of some arthropods, especially certain insects such as Drosophila, Calliphora, Ephestia etc. (Gregg and Smucker, 1964; Viscontini, and stierlin, 1961,1962, 1963) and are reported to perform the function of screening pigments. But in spite of a substantial amount of work on pteridines from the compound eyes, it seems that the eyes of certain insects and also some crustaceans have not been examined for pteridines.

With this view a study has been performed on the compound eyes of Musca, Apis, Macrobrachium and with regards to the occurrence of pteridines, and their possible functions have been discussed.

MATERIALS AND METHODS

Solubility tests: The principle of this test is based on the fact that different pigments show specifity in their solubility in various solvent systems. In alkaline aqueous solutions, e.g. dilute(1% Vol/Vol) ammonia solution, pteridines are easily soluble, but in organic solvents such as acetone, absolute ethanol, ether. petroleum ether, benzene, toluene, Carbon tetrachloride, they are not readily soluble. (Matsumoto etal,1971).

For solubility tests, the eyes are fixed in a chloroform-ethanol mixture (2:1 Vol/Vol). for more than two hours. Routine histological preparations are made, using two changes of absolute ethanol and benzene, and by paraffin as the embedding medium. Relatively better tissue fixation has been obtained with the use of a fixative composed of 2 parts of formaldehyde or glutaraldehyde; 7 parts 90% ethanol and 1 part acetic acid. In this fixation, the dehydration has been started from 95% ethanol and has been followed by ordinary techniques of histology. The specimens thus prepared are examined after treatment with carbontetrachloride, benzene, chloroform, ethyl ether, or petroleum ether for more than two hours at room temperature.

Chromatography: For the separation and identification of pteridines from the compound eyes, paper chromatography has been carried out. Paper chromatography is the most

commonly used technique for studying pterdines, because of the simplicity and also because it yields excellent separation (Matsumoto et al,1971). Chromatography has been carried out with Whatman No.1 filter paper, in dark or in dim light, because it is known that light may cause the decomposition of sepiapterin, biopterin, and most of 6-substituted pteridines into 6- carboxy pterine and other unidentified pteridines(Matsumoto et al,1971).
For chromatographic analysis the tissue sample has been applied to the chromatogram either directly or after suitable extraction using the following methods:

(1) Squash technique: This technique has been suggested by Hadron, and Mitchell (1951). After careful dissection, the tissue has been directly squashed on a chromatographic paper by firmly pressing with a clean microscope slide. The squashed materials are then dried with an electric dryer. The chromatogram is run with ethanol: n- butanol: conc. ammonia: Water(10:3: 2: 5 vol./vol.).

(2) Quick extraction: Matsumoto et al (1971) have suggested that chromatographic separation of pteridine could be achieved from the extract itsel. For this purpose, the eyes are separated from the head and they are then cut into small pieces. Approximately 10 mg. of the sample has been treated in a small glass vial with a few drops of ab. alcohol: 1% ammonia mixture(7: 3 Vol/Vol). The sample is then warmed in

a water bath at a temperature of 70°C. The extract thus prepared is then applied to filter paper with the aid of glass capillary. Chromatogram is run using n-butanol: acetic acid: Water(4:1:1), vol./vol.).

(3) Extraction with Ethanol : (Awapara's method): The extraction procedure is a slight modification of Awapara's method(Awapara,1948). The material is homogenized in a volume of absolute ethanol 20 times that of the fresh weight of the tissue in a glass homogenizer. The homogenate are diluted with redistilled water to give a 70% ethanol solution. The ethanol solution is heated in an 80°C water bath for 20 minutes and after adjusting its volume by the addition of absolute ethanol, it is centrifuged 3000 rev/min for 5 minutes to remove proteins. The resulting clear supernatant is mixed with chloroform 3 times its volume. By centrifusing at 3000 rev/min for 20 minutes, the aqueous layer containing the fluorescent substances has been separated from the ethanol-chloroform mixture. The extract thus obtained is then concentrated in a vacuo to about 0.5 ml and then chromatographed.

Extraction with trichloracetic acid(TCA): In this extraction procedure, the materials have been homogenized in 10% (W/V) TCA and is extracted three times with a volume equal to the fresh weight of the tissue, in a water bath for 20 minutes at 70°C. TCA is later removed by washing the supernatant

with one-half of the volume of ethyl ether, for 6 times. This has been done because TCA is an unfabourable contaminant for paper chromatography. The ether layer is sepated by centrifugation and is discarded at each washing. The resulting aqueous extracts are then reduced to a volume of about 0.5 ml to 1 ml in vavuo. The remaining ether in the extracts is removed by treating them in a water bath at a temperature of 55°C.

Extraction with phenol(Crammer's method): The materials are heated in a boiling water-bath for 5 minutes, for denaturing They are then homogenized in a volume of distilled water 20 times that of the fresh weight of the tissue. The homogenate is incubated in a water-bath at a temperature of 80°C for 20 minutes. After that, the homogenate is deproteinized by adding half its weight of ammonia sulfate and then centrifuged. After centrifugation, the supernatant is mixed with phenol equal to one-tenth of its volumw and then it is again centrifuged. The procedure is repeated three times. Ethyl ether in a volume three times that of phenol solution, and distilled water about one-tenth the volume of ether are added to the phenol layer, and then centrifuged. The aquous layer thus separated is then evaporated to about 0.5 ml in vacuo(Crammer,1948).

Extraction by hydrochloric acid-hydrolysis: The materials are homogenized with 0.1 N Hydrochloric acid in a volume 20 times that of the fresh weight of the tissue. The homogenate is then hydrolysed for 24 hours at 60°C. The resulting solution is neutralized with ammonia, and is then centrifuged. After centrifugation, the supernatant is concentrated in a desiccator and is used for analysis by paper chromatography.

One-and Two-dimensional chromatography and re-chromatography:

The extracts are applied to the filter paper in the form of a spot for one- and two-dimensional chromatography. For rechromatography, the extracts are applied in the form of a narrow streak. Every paper chromatogram has been subjected at first to one-dimensional separation. Matsumoto et al (1971) have reported that some pteridines overlap one another in one-dimensional chromatography. Because of this they have suggested the importance of two-dimensional separation. Both the ascending and descending techniques have been tried in the one-dimensional chromatography. The ascending technique is known to be better for separating pteridines in general, whereas descending method is advantageous for separating pteridines of low Rf values (Matsmoto et al, 1971).

In the present investigation, the ascending technique is repeated two times. The chromatogram is dried in the dark

after each development. The combination for rechromatography is one run with n- prapanol: 1% ammonia (2:1 V/V) and a second with iso-propanol: 2% (W/V) ammonium acetate(2:1 V/V),

In two-dimensional chromatography, pteridines first separated in one direction are subjected to further separation through the second development made at angle prependicular to the first.

The position of pteridines are determined by examining the chromatogram with UV lamp.

OBSERVATIONS AND DISCUSSION

The chromatograms after examining with UV light shows that the eye of house-fly, Musca domestica contains pterin, biopterin, and isosepiapterin(Fig.15).

The pteridines from the compound eye of the honey bee, Apis cerana indica have been tentatively identified as 6-carboxypteridine, isosepiapterin and biopterin(Fig.16). In the compound eye of the fresh-water prawn, Macrobrachium birmanicum, the pteridines which have been identified are xanthopterin and xanthine (Fig.17). The pteridines from the compound eye of the marine prawn, Metapinneus sp. have also been identified tentatively as xanthopterine and xanthine (Fig.18).

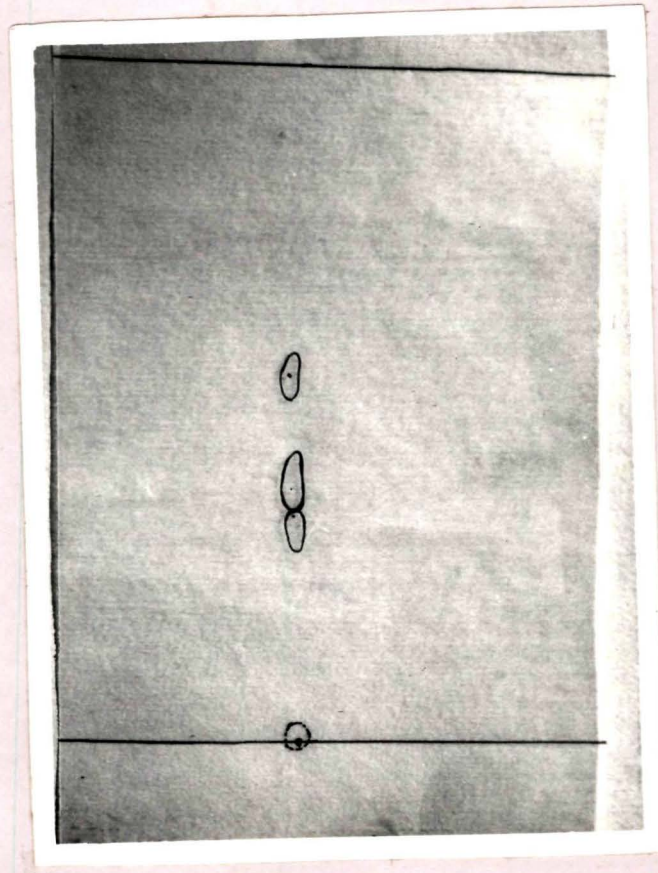
It is known that pteridine and its derivatives perform a variety of functions in the biological system. Farber et al (1948)1 Handschumacher and Welch(1960) have reported that conjugated pteridines functions as principal therapeutics for acute leukemy. Some conjugated pteridines are also known to function metabolically as coenzyme in the enzymatic synthesis of inosinic acid, serine, methionine, thymidylate etc. (Buchanan, Larrabee, Rosenthal and Canthou,1964; Huennekens and Scrimgeour,1964; Blakley,1969). It is also known that some unconjugated pteridines take part in biosynthesis of riboflavin(Masuda,1955,1956 a, 1956b,Masuda,Kishi and Asai,

EXPLANATION TO FIGURES

Fig. 15. Trace of the chromatogram showing the pteridine components of the eye of the house-fly, *Musca domestica*

Solvent: n-butanol : acetic acid: water

(4 : 1 : 1)

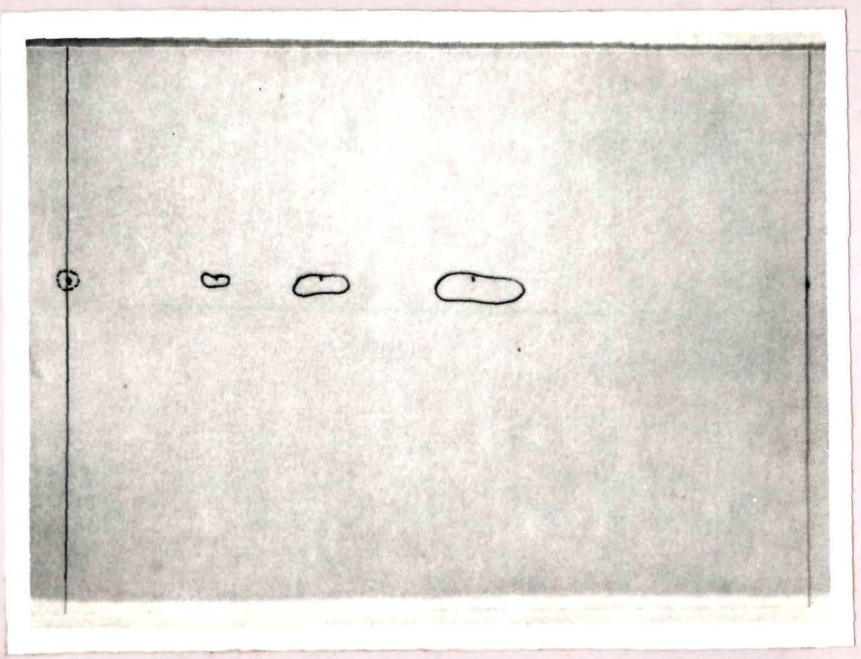


EXPLANATION TO FIGURES

Fig.16. Trace of the chromatogram showing the pteridine components of the eye of the honey bee, *Apis cerana indica*.

Solvent : n-butabol : acetic acid: water

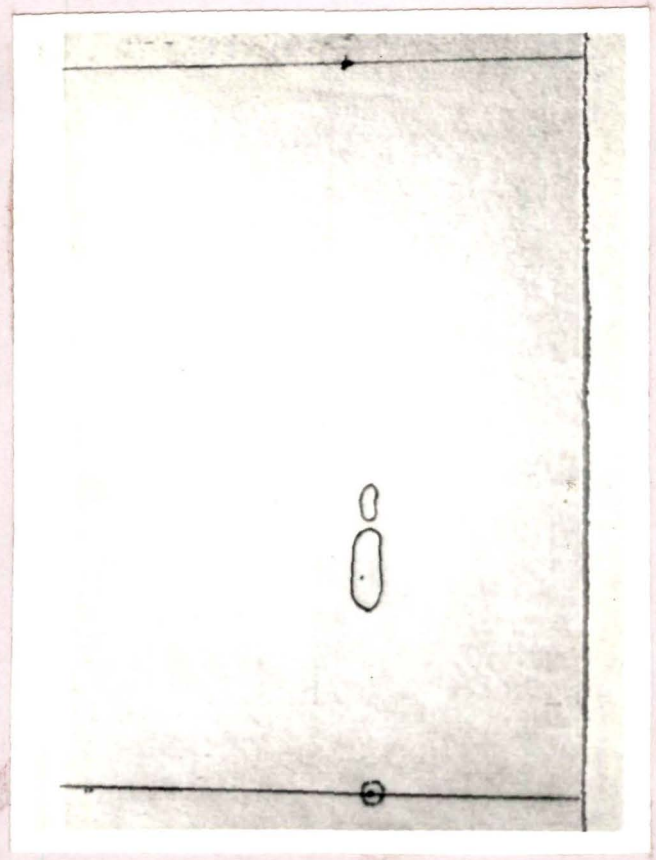
(4 : 1 : 1)



EXPLANATION TO FIGURES

Fig. 17. Trace of the chromatogram showing the pteridine components of the eye of the fresh-water prawn, Macrobrachium birmanicum.

Solvent: n-butanol: acetic acid : water
(4 : 1 : 1)

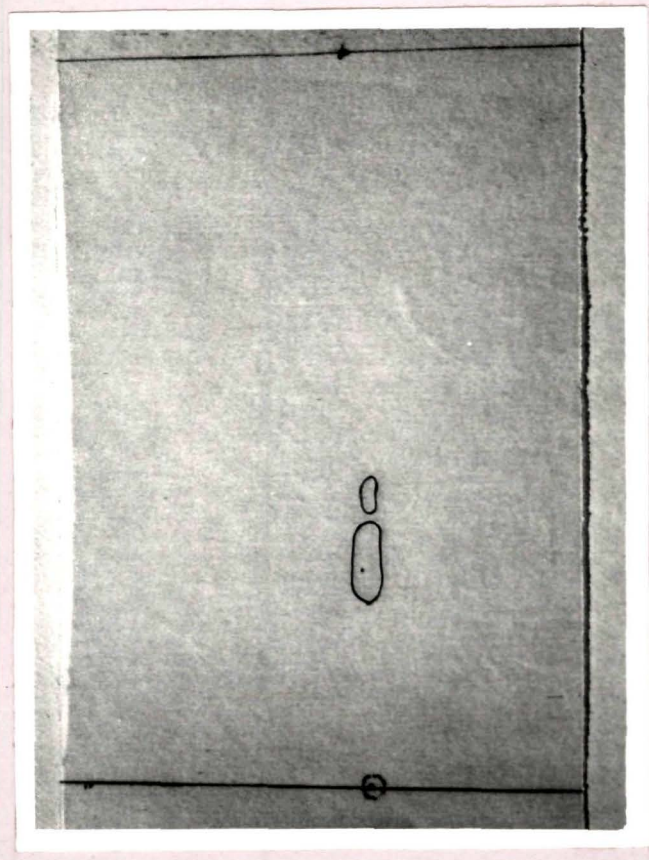


EXPLANATION TO FIGURES

Fig. 18. Trace of the chromatogram showing the pteridine components of the eye of the marine prawn,

Metapinneus sp.

Solvent: n-butanol: acetic acid: water.
(4 : 1 : 1)



1957,1959, Masuda, Kishi, Asai and Kuwada,1959; Forrest and McNutt, 1958; Kuwada, Masuda, Kishi and Asai 1958; Maley and Plaut,1959; Plaut and Maley 1959; Brown 1960; Creswell and Wood, 1960; Davol and Evans 1960; Plaut 1960 1961a,b,1963,1964; Goodwin and Horton 1961; Winestock and Plaut 1961; Rowan and Wood 1963; Winestock, Aogaichi and Plaut 1963; Kuwada and Hori 1964& McNutt 1964; Hemmerich, Heizmann, Mengel and Pfleiderer, 1969).

A role for certain reduced pteridines such as sepiapterin, dihydrobiopterin, and tetrahydro folic acid in the hydroxylation of phenylalanine to tyrosine has been reported by some authors(Kaufman, 1957, 1958 a,1958b,1959, 1961,1062,1963,1964,1967, 1969; Kaufman and Levenberg 1959; Matsubara, Katoh, Akino and Kaufman,1966). Pteridine derivatives have also been shown to be involved in oxidation of glyceryl ethers (Tietz et al,1963) and steroids (Hagerman 1964).

Ptertidines are also reparted to be associated with synthesis of lipid(Kidder and Dewey,1958), and nucleic acid (Forrest,1969(and also in sexual differentiation of insects (Butenandt and Rembold,1958; Schmidt and Viscontini,1962 Schmidt,1969) . Of all these functions cited however, the most significant role played by pteridines is that they act as pigment in the biological system. In this context, it can be pointed out that, in vetebrates pteridines are synthesized

in developing pigment cells and they accummulate specifically in brightly coloured pigment cells (Hama and Obika, 1960; Obika 1963; Hama et al 1960; Matsumoto, 1965 a, Richards and Bagnara, 1967).

During the last 30 years, pteridines have been reported in a variety of animals from lepidopteran insects to higher vertebrates and it has now become evident that these occur as red, yellow or orange pigments. Pteridines have been detected in both the eyes of certain insects such as - Drosophila (Ephrussi and Herold, 1944; Heymann Chan and Clancy, 1950; Hadorn and Mitchell, 1951; Nolte, 1952; Forrest and Mitchell, 1954 a, 1954 b; Rasmussen, 1954; Viscontini et al; 1955; Viscontini Hadorn and Karrer, 1957; Viscotini, Loesser and Karrer 1958; Viscontini and Mohlmann, 1959 a, 1959 b, Forrest et al; 1961 b, Taira, 1961 a; Rembold, 1961; Hadorn, 1962; M.Goto et al, 1964; Gregg and Smucker, 1966;). Calliphora (Ziegler, 1961 c), Ephestia, (Hadorn and Kuhn, 1953; Kuhn and Engelhaaf, 1959; Viscontini and stierlin, 1961, 1962, 1963) and some butterflies (Hopkins 1889, 1895; Schopf and Becker, 1933; Becker and Schopf 1936; Tarttar, 1940; Purrmann, 1940 a, 1940 b, 1941, 1943; Purrmann and Maas, 1944 a, Purrmann and Eulitz, 1948 pfleiderer, 1962, 1963; Pfleiderer and Rukwied, 1961; Umenachi, 1962, Descimon 1965,

Usually those tissues which are red or orange in colour contain drosopterin whereas the tissues which are yellow in appearence are rich in sepiapterin. The distinguishing feature of pteridines is their fluorescence under UV irradiation. Depending upon the concentration and PH of the medium, the fluorescence ranges from violet to red (Rauen and Stamm, 1952; Uyeda and Rabinowitz, 1963). This characteristic fluorescence of most of the pterdine derivatives has long been used for the detection of these compounds.

The significance of occurrence of pteridines in the compound eye has been studied by a number of authors and it has been suggested that they act as screening pigments (Goldsmith, 1958 a, b ; Langer and Thorell, 1966).

Hoffmann and Langer (1961), while studying the spectral sensitivity of the eye of Calliphora erythrocephala have observed that the 'White- apricot' mutant eye, which contains pteridines has a U.V.- sensitivity maximum same as the ' Chalky' mutant, which lacks pteridines. They have further observed that in ' white-apricot' mutant the U.V.- maximum is less than in the 'chalky' eye mutant in relation to the visible maximum. Thus, in the 'white-apricot' mutant

the screening pigments, pteridines function to decrease the U.V. sensitivity relative to visible sensitivity.

All these reports suggest that pteridines play some very important roles in the visual system of arthropods. But as already mentioned, pteridines have not been ~~sutid~~ studied in the compound eyes of all groups of arthropods, and thus there is a need for further studies on the pteridine components of the compound eye of various groups of arthropods.

Chapter - IV

ADENOSINE TRIPHOSPHATASES (ATPase) OF THE COMPOUND EYE.

INTRODUCTION

Adenosine triphosphatase (ATPase) system frequently been shown to be intimately associated with the process of photoreception (Langer, 1964, 1974; Weber and Schorrath, 1971; Drujan and Ali 1972; Kuhn and Dreyer 1972, Bownds, et al, 1972) and there are strong indications that ATPase has an important role in the process of phosphorylation.

To understand the mechanism by which one quantum of light reacting with a single molecule of the visual pigment causes nervous stimulation, three main hypothesis have been put forward. They are :

I The Enzyme hypothesis

II The solid - State hypothesis, and

III The ionic hypothesis.

According to the enzyme hypothesis, the visual pigment rhodopsin is a pro-enzyme. Upon absorption of light, this is converted to an active enzyme, which catalizes the electrochemical process leading to visual excitation.

Mc Connell and Scarpelli (1963) have claimed that the visual pigment rhodopsin is an ATPase, which is activiated by light. However, later on, Bonting and Bangham(1966) have found that the experimental evidence for this claim is faulty

and neither the (Na^{+} - K^{+} - Mg^{+2}) - activated ATPase nor the Mg^{+2} - activated ATPase can be identical with the visual pigment.

The solid - state hypothesis suggests that after absorption of light by the photoreceptor cell, a solid - state process, such as energy transfer by resonance or photoconduction may take place .However, Hagins and Jannings (1960) have found that in frog rods, there is no such energy transfer by resonance or photoconduction.

The ionic hypothesis says that there is a movement of ions across the photoreceptor membrane following visual excitation just as it occurs in a nerve after stimulation. Adams and Hagins (1960) have found that in the photoreceptor cells of the squids there is a local inflow of current within 7 μm from the site of light absorption.

This receptor current consists of an influx of Na^{+} ions due to a local light-induced increase in Na^{+}-permeability as well as an outward flux of potassium ions.

In this context, it is worth - mentioning that the light - stimulated potentials observed in photoreceptor cells of Limulus (stieve 1965), Crayfish(Eguchi,1965), barnacle

(Brown, et al, 1969) and the honey bee (Fulpius and Bauman, 1969) are a direct consequence of an increase in permeability of the photoreceptor cell membrane to sodium ions.

Sekoguti (1960) and Bonting et al. (1963) have detected the presence of ($Na^+ - K^+$) activated ATPase in the rod outer sigments and thus the ionic hypothesis has further been supported, because this enzyme system has been implicated in the repolarization process of nerve, muscle and electric organ (Bonting, et al, 1963)

Thus according to the ionic hypothesis, maintainence of ionic gradients across the photoreceptor membrane is important for visual excitation, and this ionic gradient is maintained by the enzyme, adenosine triphosphatase.

The available data show that the studies of this enzyme in the photoreceptor organ is confined mainly to the vetebrate eye. Sekoguti (1960) has detected the high activity of this enzyme in cattle retina and outer segment. He has found that addition of potassium to sodium-containing assay medium has stimulated the ATPase activity. Bonting et al (1963) have observed a high activity of this enzyme in several ocular tissues such as retina, cilliary body, Iris-sphincter, choroid, sclera,Cornea, lens, and vitreous of cat and man. De Pont and Bonting(1971) have detected high activity of this enzyme in retina of the cuttle fish, Sepia officinalis.

In spite of a considerable amount of work on the ATPase system from the photoreceptor organs of various animals, there is very little information on the Mg^{2+} - (Na^{+} - K^{+}) - ATPase activity of the photoreceptor of arthropods.

The compound eye of arthropods has been extensively studied in the past years by different groups of authors from electrophysiological and biochemical point of view. Langer (1960 a; 1960 b, 1962, 1963) has studied the chemical composition of the eye of the blowfly, Calliphora erythrocephala and its changes upon illumination. He has observed that after illumination, there is an enhancement of metabolic activity of the eye, and he has suggested that it provides the sensory cells with necessary energy for converting the visual stimulus to nervous excitation. This mechanism appears to involve cation exchange process, which seems to be through the enzyme (Na^{+} - K^{+}) - ATPase.

The first report on the studies of the ATPase system from the compound eye of arthropods has been made by Weber, et al. (1971, 1972). They have studied the enzymology of the compound eye of the white - eyed mutant of Calliphora erythrocephala by means of histochemical tests. The study demonstrated the existence of two types

ATPase activity - one distributed in the cytoplasm of the sensory cells and in the rhabdomes and another located within pigment and semper cells.

Later on Rivera (1974) has made a biochemical study on the ATPase system of the compound eye of Calliphora erythrocephala and compared the data with the histochemical observations of Weber et al. (1971, 1972). This seems to be the only biochemical report on the ATPase system from the compound eye of arthropods.

Keeping this in view, an attempt has been made to a study the ATPase system from the compound eye of the house - fly , Musca domestica, the honeybee, Apis cerana indica and the fresh-water prawn, Macrobrachium birmanicum.

The ATPase system of the eye- homogenate of Macrobrachium birmanicum has been subjected to a detail study. The effect of substituting Mg^{2+} by other divalent cations, especially calcium has been investigated. The effect of increasing concentration of Na^{+} and K^{+} on the ATPase activity has also been done. In addition to this, relation between the enzyme and the co-substrates and the effect of bicarbonate on the ATPase activity has been carried out.

MATERIALS AND METHODS

Tissue Preparation :

Immediately after decapitation, the eyes are dissected out by a clean razor. The eyes are then frozen over dry ice. The eye preparation contains cornea, crystalline cones, semper cells, sensory cells and pigment cells.

Eyes are homogenized in ice- cold 0.25 M sucrose.

Protein determination :

Protein has been determined by the method of Lowry et al (1951) as follows :

0.1 ml of 0.5 N NaOH solution, containing 10% $Na_2 CO_3$, 0.1% K_2- tartrate, and 0.05 % Cuso have been added to 0.1 ml extract ; after 15 minutes 0.5 ml of a 4% aquosus solution (V/V) of Folin- Cio Calteus Phenol reagent is added. The mixture is heated in a water-bath at a temperature of 50°C for 10 minutes, cooled under tap-water and the colour read at 700 nm.

Bovine serum albumine in a sucrose solution is used as standard.

Enzyme assay :

Incubation medium : The complete medium or Medium I contains 5 mM ATP; 5mM $Mgcl_2$; 100 mM Nacl ; 15 mM Kcl; 50 mM Tris-Hcl,

PH 7.4, in a final volume of 0.1 ml. as suggested by Rivera(1974). This medium gives the total or $(Na^{+} - K^{+} - Mg^{++})$- activated ATPase activity.

Medium II : This medium contains 5mM ATP; 5 mM $Mgcl_2$, 100 mM Nacl; 10^{-3} M ouabain and 50 mM Tris - Hcl. This medium gives the Mg^{2+} - ATPase activity.

Activity in medium I minus activity in medium II gives the ouabain - sensitive $(Na^{+}-K^{+})$ - ATPase activity.

Incubation has been carried out at 37°C. After 20 minutes the reaction is stopped by the addition of 17% trichloroacetic acid.

The reaction mixture is centrifuged and ig inorganic phosphate is determined in the supernatant after the method of Fiske and Subba Row(1925) as follows:

Reagents :

(1) 5 N H_2SO_4

(2) 2.5 % Ammonium molybdate.

(3) Reducing reagent (Fiske- Subba Row-reagent).25gm of the reducing reagent is added in 10 ml of water.

Standard solution :

1.3613 g of analytically pure KH_2PO_4 is dissolved in 1000 ml. of water. A few drops of chloroform are added, and the solution is stored in the refrigerator. For use it is diluted 1: 10, so that 1 ml. corresponds to 1 micromole of Phosphorous.

Procedure :

One milliliter of sulfuric acid is added, followed by 1 ml. of ammonium molybdate. After mixing, 0.1 ml of reducing solution is added. The volume is made upto 10 ml. After mixing again, the absorbancy at 660 m u is measured after 10 minutes in Beckman Spectrophotometer.

Determination of Properties of the ATPase system:

Mg^{2+} activation curve : Mg^{2+} activation curve is obtained by varying the magnesium concentration from 0-12 mM in the incubation medium.

Effect of Calcium : The effect of calcium on the ATPase activity of the eye-homogenate is tested by adding graded amount of calcium (0-15 mM) to incubation medium without magnesium.

Sodium activation curve : The Na^{+} - activation curve is obtained by adding graded amounts of NaCl (12 different steps of varying concentrations between 50 and 150 m M) in the total incubation medium.

Potassium-activation curve: Potassium activation curve has been obtained by adding graded amounts (0- 30 m M) of KCl.

Effect of bicarbonate: Effect of bicarbonate on the ATPase system of the compound eye is studied by substituting Nacl by $NaHCO_3$ in the incubation medium.

Relative ATPase activity in various substrate media : In order to test the relative ATPase activity in incubation media with different co-substrates, the following media has been used.

Medium III : In this medium KCl has been ommited from the total incubation medium.

Medium IV : In this medium both NaCl and KCl are ommited.

Medium V : This medium contains Mg^{2+} , K^{+} and ouabain. NaCl is ommited.

Medium VI : From the total incubation medium both NaCl and KCl are ommited. 10^{-3}M ouabain has been added to the incubation medium.

Effect of cations on the ATPase activity :

Several di-valent cations have been tested by substituting $Mgcl_2$ by their chlorides in both the incubation media I and II. The cations used are calcium, cobatt, manganese, copper, nickel and barium.

Effect of temperature :

For the determination of the effect of temperature on enzymes activity, extracts and media are preincubated at each temperature for 10 minutes.

Effect of pH :

The effect of pH has been studied by preparing the media with Tris- histidine buffers (final concentration of

each compound ; 50 mM) in a pH range of 6.0 to 9.1.

The pH of the resulting medium has been measured, and this value is used in plotting the assay results.

RESULTS

Table 17. ATPase activities in the homogenate of the compound eye of house-fly Musca domestica, honey-bee, Apis Cerana indica, fresh-water Prawn, Macrobrachium birmanicum in umoles Pi/mg Protein/ hr, given as means with standard errors.

Samples	Mg^{2+} - ATPase	$(Na^{+} - K^{+})$ - ATPase
House-fly, Musca domestica	1.85 ± 0.59	1.69 ± 0.40
Homey-bee, Apis Cerana indica	1.91 ± 0.60	1.71 ± 0.37
Fresh-water Prawn, Macrobrachium birmanicum.	2.88 ± 0.59	1.93 ± 0.35

Properties of ATPase system from the eye-homogenate of the fresh water prawn, M. birmanicum:

Effect of Mg^{2} :-

The effect of increasing concentration of Mg^{2+} on the total ATPase activity is shown in Fig. 19.

Effect of Calcium :

Figure 20 shows the activation Curve of Calcium for the eye-homogenate. The Mg^{2+} - ATPase activity is found to be depressed when calcium is used instead of magnesium. The $(Na^{+} - K^{+})$- ATPase activity is almost completely inhibited by calcium.

Effect of Sodium and Potassium:

Effect of increasing concentration of Na^{+} and K^{+} on the total ATPase activity is shown in the figures 21 and 22 respectively.

Effect of bicarbonate:

The effect of substituting gradually Nacl by $NaHCO_3$ in the total ATPase activity is shown in Figure 23. It has been found that bicarbonate enhance the total ATPase activity.

Relative ATPase activity in various substrate media:

Table 18 and Fig. 24 shows that the effect of different co-substrate in the ATPase activity.

Effect of Cations on the ATPase activity: The effect of substituting magnesium by other di-valent cations upon the ATPase activity in the eyes is shown in Table 19 and Figs.25 and 26.

Table 18. Relative ATPase activities of eye homogenate of the fresh water prawn, Macrobrachium birmanicum in various substrate media. Activity are expressed as the means of 8 experiments with ± S.E.

Medium	ATPase activity %
I. Mg^{2+}, Na^{+}, K^{+}	100
II. Mg^{2+}, Na^{+}, ouabin	56.9 ± 6.8
III. Mg^{2+}, Na^{+}	61.4 ± 7.1
IV. Mg^{2+}	57.5 ± 7.1
V. Mg^{2+}, K^{+} ouabain	56.3 ± 6.1
VI. Mg^{2+}, ouabain	54.2 ± 5.9

Table 19. Effect of substituting Mg^{2+} by other divalent cations upon the ATPase activity of eyes of M.birmanicum in the standard incubation media ATPase activity in media with Mg^{2+} was set at 100 . Values are means of 6 experiments with standard error.

CATIONS	Mg^{2+} - ATPase (%)	$(Na^{+} - K^{+})$ - ATPase (%)
Magnesium	100	100
Manganese	100± 9.2	62.5±9.1
Nickel	25.3 ± 4.1	11.2 ± 3.1
Copper	12.4 ± 2.8	18.3 ± 4.1
Cobapt	94.2 ± 9.1	88.4 ± 9.5
Barium	6.9 ± 1.8	6.2 ± 2.2

It is found that the activity is enhanced only when Mn^{2+} is used instead of Mg^{+2} . Other cations tested, on the other hand had inhibitory effect on the ATPase activity. Both the ATPase remains allmost at the same level with cobalt.

Effect of temperature :

Figure 27 shows the effect of temperature on the enzymes activities. Maximal rates are obtained at 40°C.

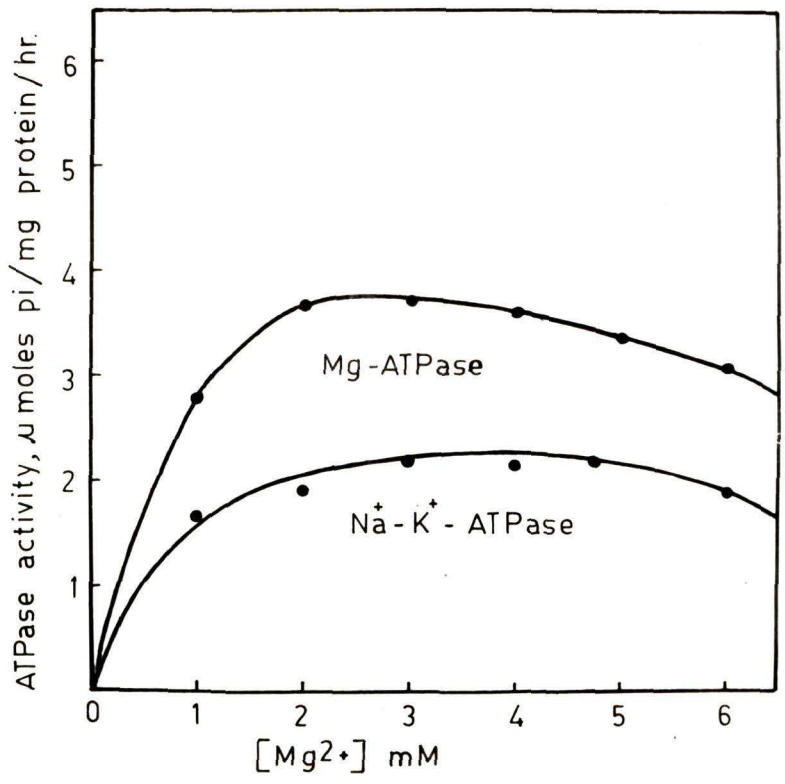
Effect of pH :

Effect of p H on (Na^+ - K^+) - ATPase and Mg^{2+} - ATPase is shown in figure 28. At pH 8.6, Mg^{2+} - ATPase activity is optimal. The optimal activity of (Na^+ - K^+)- ATPase is observed at the pH 7.2.

EXPLANATION TO FIGURES

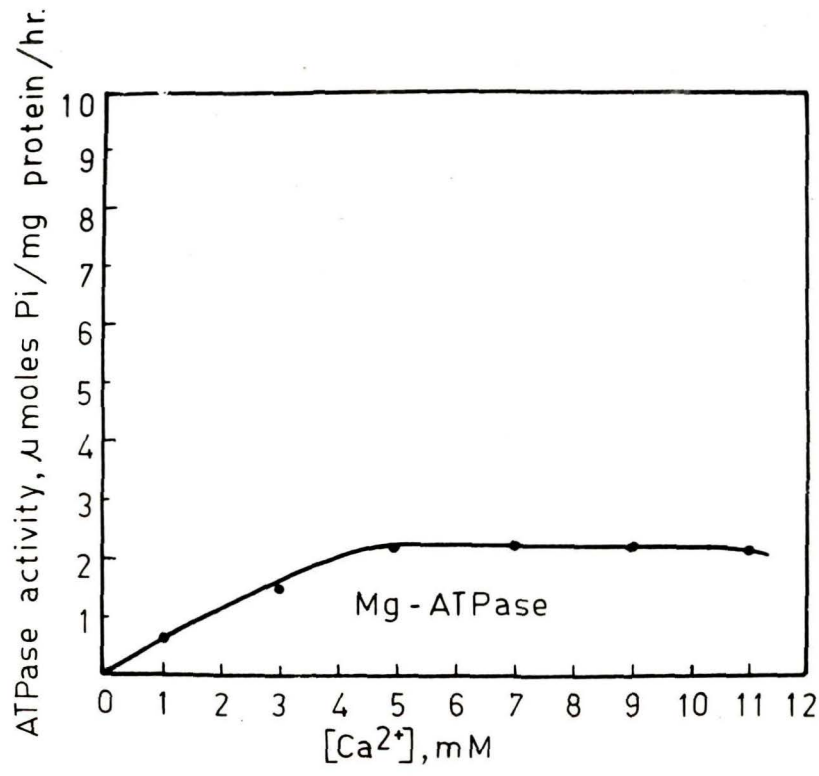
Fig. 19. The effect of increasing concentration of Magnesium on the total ATPase activity in the eye-homogenate of the fresh-water Prawn, Macrobrachium birmanicum.

Each point is the mean of 10 determinations.


ATPase activity, μ moles pi/mg protein/hr.
6
5
4
3
2
1
0
1
2
3
4
5
6
[Mg2+] mM
Mg-ATPase
Na+ - K+ - ATPase

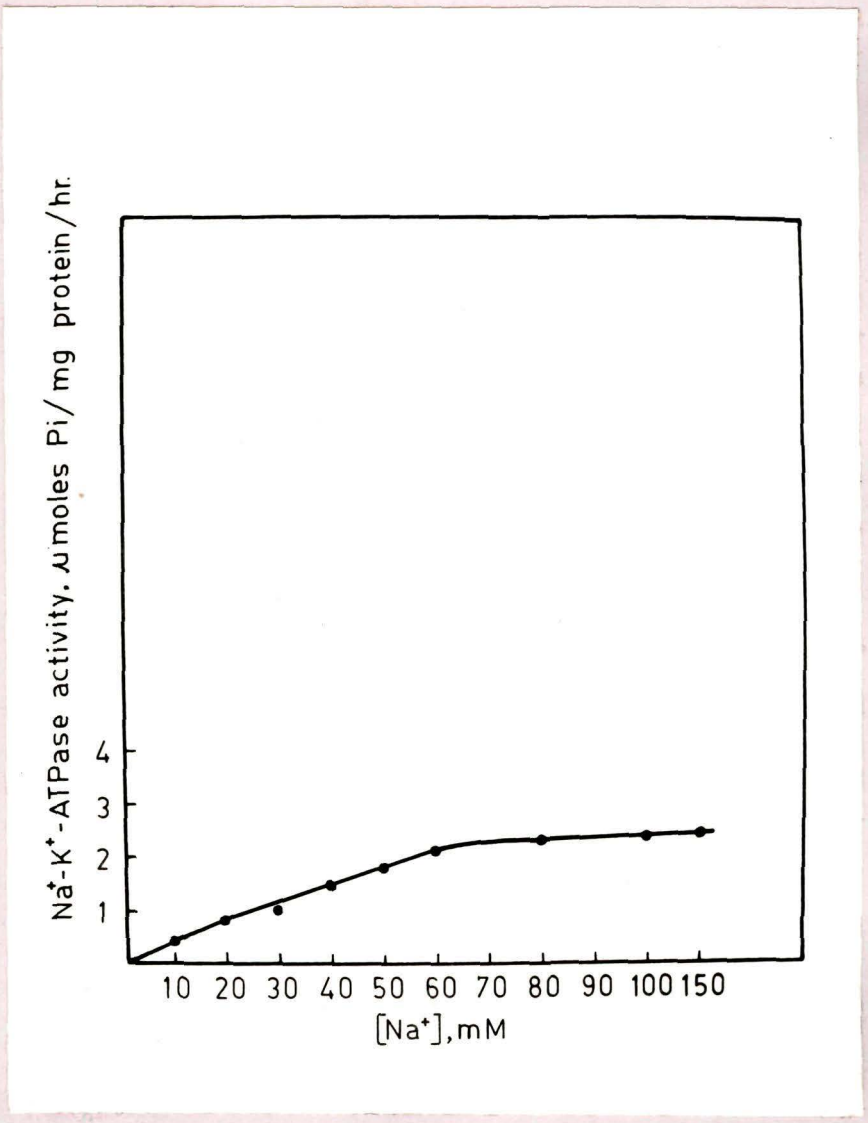
EXPLANATION TO FIGURES

Fig. 20. Relationship between reaction velocity of ATPase activities in eye-homogenates of the fresh-water Prawn, Macrobrachium birmanicum and the Ca^{2+} concentration, used instead of Mg^{2+} in incubation media. Each point is the mean of 10 determinations.


ATPase activity, μ moles Pi/mg protein/hr.
10
9
8
7
6
5
4
3
2
1
Mg - ATPase
0 1 2 3 4 5 6 7 8 9 10 11 12
[Ca2+], mM

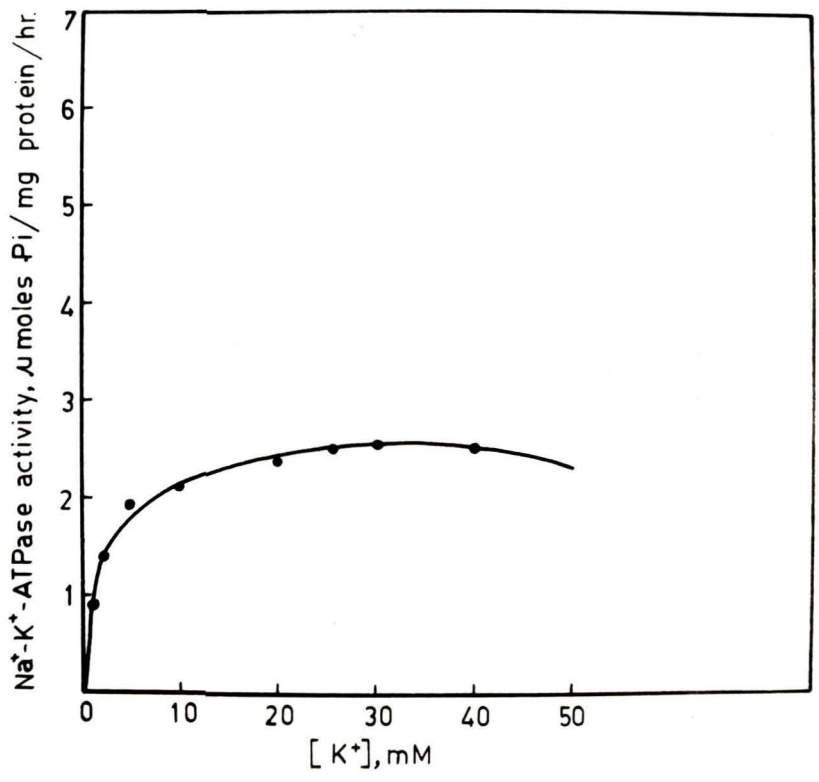
EXPLANATION TO FIGURES

Fig. 21. Effect of the Na^+ concentration upon [redacted] ATPase activity in eye homogenates of Macrobrachium birmanicum. Each point is the mean of 6 determinations ± S.E.


Na+-K+-ATPase activity. μmoles Pi/mg protein/hr.
4
3
2
1
10 20 30 40 50 60 70 80 90 100 150
[Na+], mM

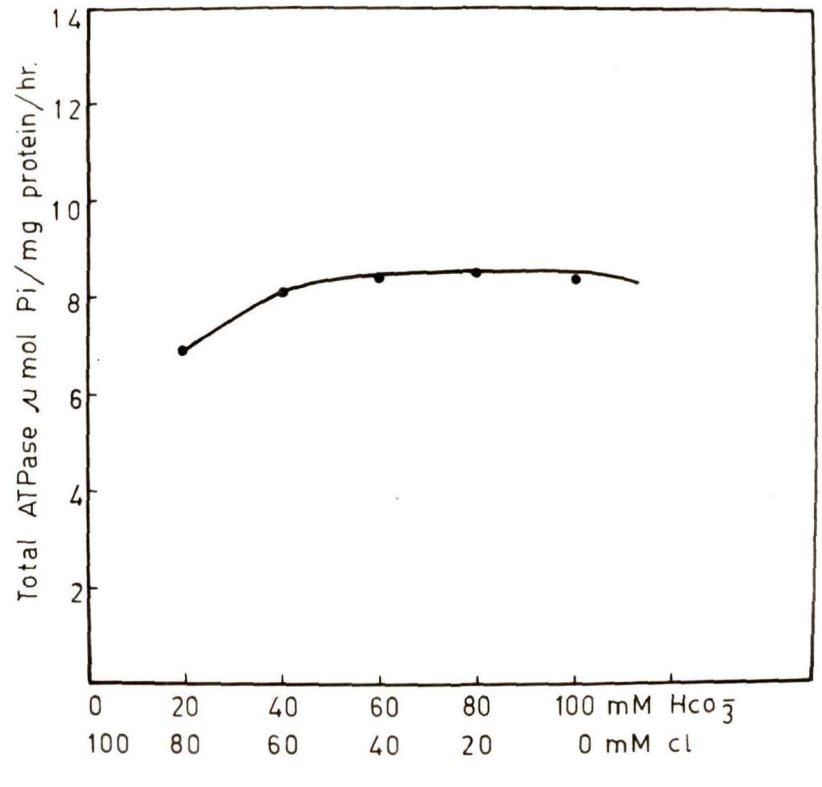
EXPLANATION TO FIGURES

Fig. 22. Effect of the K^+ concentration upon the total ATPase activity in eye-homogenates of *Macrobrachium birmanicum*. Each point is the mean of 6 determinations ± S.E.


Na⁺-K⁺-ATPase activity, μmoles Pi/mg protein/hr.
7
6
5
4
3
2
1
0
10
20
30
40
50
[K⁺], mM

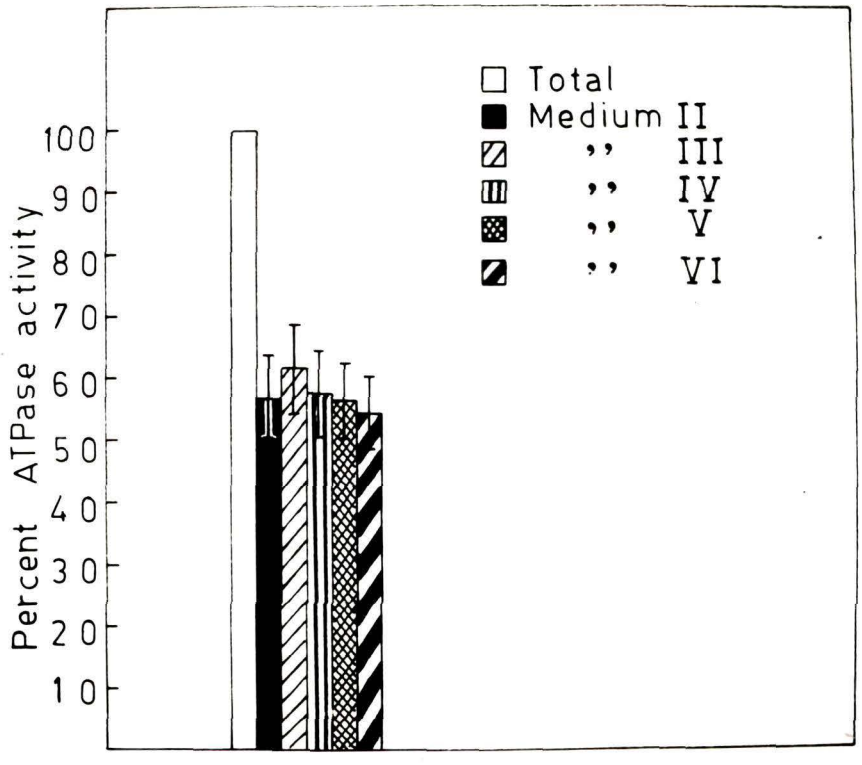
EXPLANATION TO FIGURES

Fig. 23. Effect of substituting chloride for increasing concentrationf of bicarbonate upon ATPase activities of eye homogenates of *Macrobrachium birmanicum*. Each point is the mean of 10 determination.


Total ATPase μ mol Pi/mg protein/hr.
14
12
10
8
6
4
2
0
20
40
60
80
100 mM Hco3
100
80
60
40
20
0 mM cl

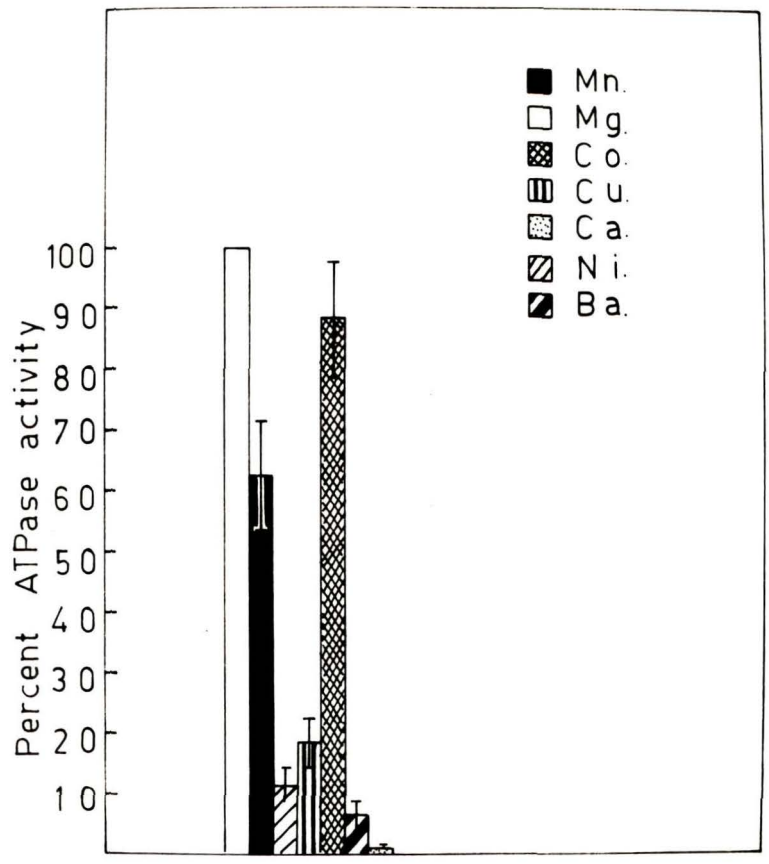
EXPLANATION TO FIGURES

Fig. 24. Effect of different co-substrates on the ATPase activity of eye-homogenate of the fresh-water prawn, *Macrobrachium birmanicum*.


Total
Medium II
,, III
,, IV
,, V
,, VI
Percent ATPase activity
100
90
80
70
60
50
40
30
20
10

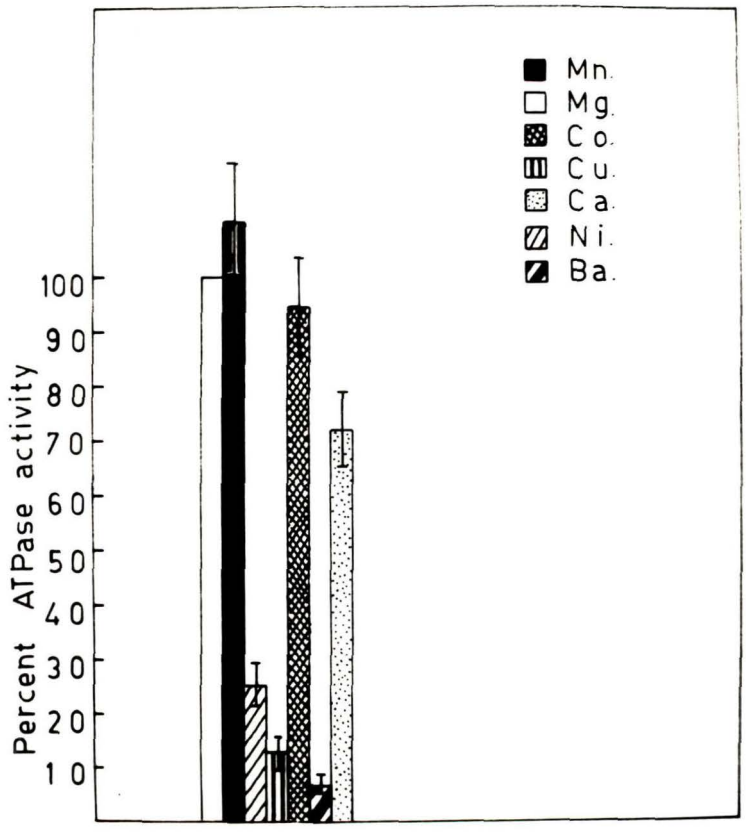
EXPLANATION TO FIGURES

Fig. 25. The effect of substituting magnesium by other di-valent cations upon the (Na^+ —— K^+). ATPase activity in the eye-homogenate of the fresh-water prawn, Macrobrachium birmanicum.


Mn.
Mg.
Co.
Cu.
Ca.
Ni.
Ba.
Percent ATPase activity
100
90
80
70
60
50
40
30
20
10

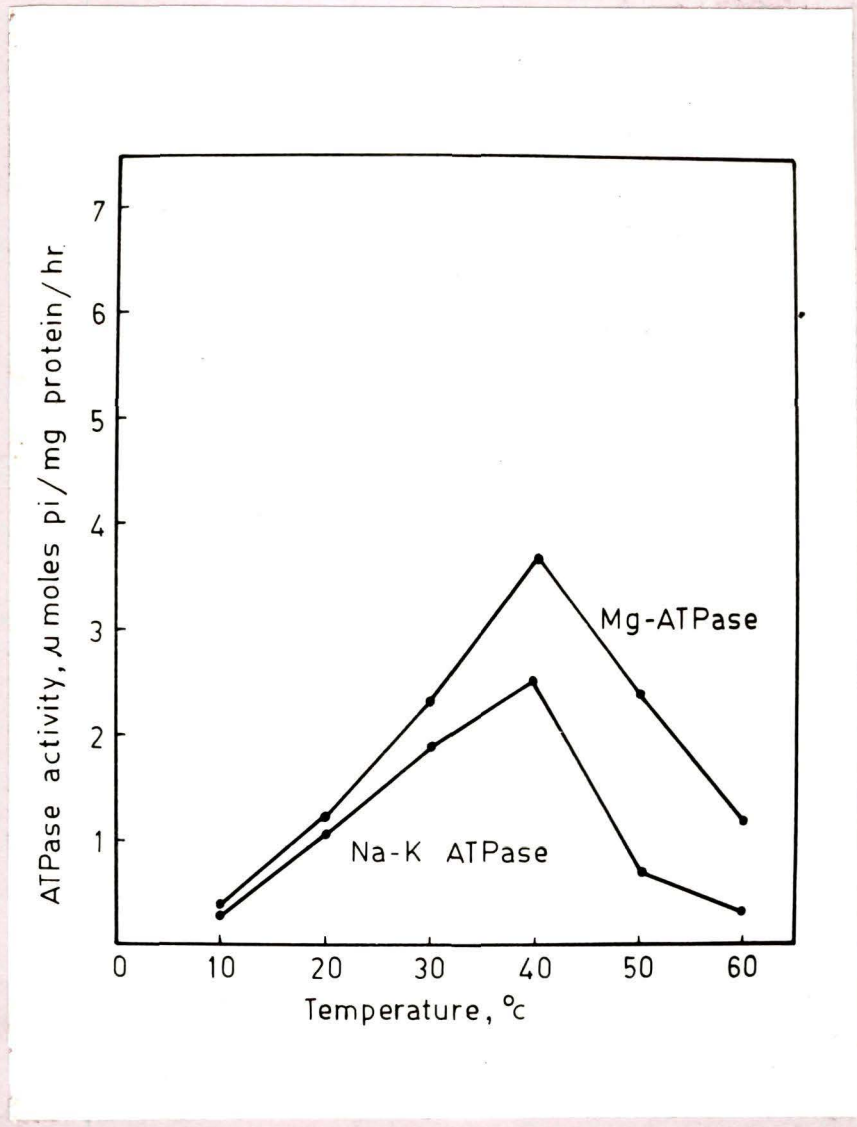
EXPLANATION OF FIGURES

Fig. 26. The effect of substituting magnesium by other divalent cations upon the Mg^{2+}— ATPase activity in the eye-homogenate of the fresh-water prawn , Macrobrachium birmanicum.


Mn.
Mg.
Co.
Cu.
Ca.
Ni.
Ba.
Percent ATPase activity
100
90
80
70
60
50
40
30
20
10

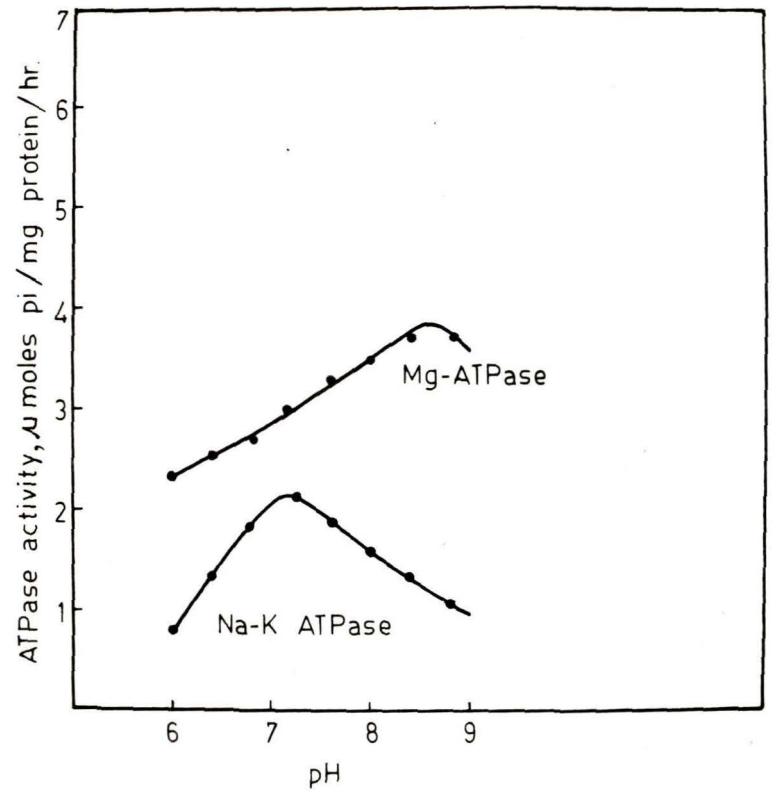
EXPLANATION TO FIGURES

Fig. 27. Effect of temperature on ATPase activities of eye homogenates of the fresh water prawn, *Macrobrachium birmanicum*. Incubation media and extracts were pre incubated at each temperature for 10 minutes. Each point is the mean of 6 determinations ± S.E.


ATPase activity, μ moles pi / mg protein / hr.
7
6
5
4
3
2
1
0
10
20
30
40
50
60
Temperature, °c
Mg-ATPase
Na-K ATPase

EXPLANATION TO FIGURES

Fig. 28. The pH profile of ATPase activities of eye homogenates of the fresh-water prawn, Macrobrachium birmanicum. Each point is the mean of 6 determinations ± S.E.


ATPase activity, μ moles pi / mg protein / hr.
7
6
5
4
3
2
1
Mg-ATPase
Na-K ATPase
6
7
8
9
pH

DISCUSSION

The results show that there are two distinct types of ATPase activity in the homogenate of the compound eye of the house-fly, Musca domestica, honey bee, Apis Cerana indica and the fresh-water prawn, Macrobrachium birmanicum .

Among the two types of ATPase activity, one is Mg^{2+} - activated and the other (Na^+- K^+)- stimulated.

Sodium- Potassium - activated adenosine triphosphatase (Na^+- K^+- ATPase) is considered to play a major role in cation transport across cell membranes (Skou, 1965; Katz and Epstein 1968). It is presumed that this enzyme is identical to the "sodium-potassium pump". The reason for such presumption is that the hydrolysis of ATP catalyzed by the Na^+-K^+ - ATPase is translated into the movement of sodium and potassium against their electrochemical gradients(Skou 1972).

The enzyme, sodium- potassium-activated adenosine triphosphatase (Na^+ - K^+ - ATPase) occurs in nearly all-cell-membranes over which a gradient for sodium and potassium exisits (Bonting 1970). In the Kidney tissues, Na^+- K^+-ATPase is present in the highest concentration in outer medulla (Handler, Torretti and Epstein 1971). The activity has been detected more particularly in the thick ascending limbs of Henle's loops, mainly at the peritubular cell- membrane in

accordance with its Postulated roles in active cation transport (Schmidt and Dubach 1969). In the photoreceptor cells of various vertebrates, Bonting et al (1964 a) have reported that a substantial part of the (Na^+- K^+)- ATPase activity is localized in rod outer segments. Frank and Goldsmith (1965) have reported similar findings for isolated pig outer segments. The high activity of this enzyme in the rod outer segments led Bonting and Banghan (1967) to formulate their " Cation Channel" hypothesis for the visual mechanism. In view of the high activity of the enzyme, Bonting and Bangham (1967) have assumed of that the enzyme is located not only in the plasma membrane of the outer segments but also in the rod sac-membranes. Earlier, Scarpelli and Graig(1963) established histochemically, the presence of ATPase activity in the rod sac membrane.

In view of the above statements, it is reasonable to presume that in the compound eye of arthropods ATPase activity may be localized in the rhabdomes.

In the literature, there is only one biochemical report on the ATPase system of the compound eye of Calliphora erythrocephala (Rivera 1974). The results of the present study has been compared with the data of Rivera(1974).

The properties of Mg^{2+} - ATPase and the (Na^+- K^+)- ATPase of Macrobrachium birmanicum are in good agreement with

Weber and Schorrah (1971, 1972) while studying the enzyme histochemistry of the compound eye of the blow-fly, Calliphora erythrocephala, have found that the ATPase of the pigment cells and semper cells is stimulated by manganese and calcium, while the enzymes of sensory cells and rhabdomes are inhibited by both the cations. But by histochemical study, it has not been possible to determine the effect quantitatively.

In the present study, the effect of several cations have been tested on both the $(Na^{+} - K^{+})$- ATPase and Mg^{2+}-ATPase activity. It has been found that manganese stimulates the Mg^{2+}- ATPase activity and depresses the $(Na^{+} - K^{+})$ dependent activity. The total activity is only slightly enhanced.

The effect of bicarbonate on the ATPase activity of the eye-homogenate of M.Birmanicum is of considerable significance.

It is found that the enhancement of tal ATPase activity produced by bicarbonate is due to stimulation of Mg^{2}- ATPase activity. The $(Na^{+} - K^{+})$- ATPase activity is gradually inhibited by bi-carbonate ions. Since the pH in the incubation medium is held constant, the increased ATPase activity observed is due to change in bicarbonate concentration and is independent of pH.

The similar type of Mg^{2}- ATPase activity by bicarbonate is reported in many other tissues. For example, Hastings et al (1963) have found that in rat liver mitochondria, Mg^{2}- ATPase activity is enhanced by bicarbonate ions. Racker (1963) has reported a similar stimulation in the mitochordrial ATPase in the heart.

The stimulation produced by bicarbonate on the ATPase activity raises the possibility of its role in ion-transport. It is well-established that sodium and potassium ions move against the electrochemical gradients through the energy produced by breakdown of ATP by the ouabain-sensitive $(Na^{+}- K^{+})$ ATPase (SKou, 1965). Whether the transport of bicarbonate and chloride ions also take place by means of similar ion-dependent ATPase is not known clearly and it is very much controversial.

However, Kasbekar and Durbin (1965), Kinne- Saffran and Kinne (1974), Riley (1977) have detected HCO_3-dependent ATPase in several tissues, where active HCO_3 fluxes have been established.

Winker and Riley(1977) have demostrated the existence of a bi-carbonate-stimulated ATPase activity in the retina of rat. They have also established the importance of bicarbonate in retinal function.

It has been observed that the metabolic activity of an isolated mammalian retina is markedly affected by bicarbonate

ions (Cohen, and Noell, 1960; Riley 1964; Winkler, Simson and Benner 1977).

Riley (1965) and Riley and Voaden (1970) have found that a great amount of decrease takes place in the glycolytic and respiratory rate of rat and rabbit retinas, when phosphate buffer is substituted for bicarbonate. Winkler, Simson and Benner (1977) have found a similar change in the electroretinogram potentials when bicarbonate is ommited, and phosphate or Tris is used instead of it to maintain a constant pH.

These findings suggest that the HCO_3^- ATPase detected by winker and Riley(1977) is of great significance in retinal function. Our present observations on the stimulation produced by bi-carbonate ions on the ATPase activity of the homogenate of the compound eye of M. birmanicum suggests that there may be a bi-carbonate activated ATPase in the rhabdome of compound eyes. However, the sub-cellular localization of such bicarbonate stimulated ATPase is yet to be done.

Chapter - V

PIGMENT MIGRATION

INTRODUCTION

A characteristic feature of the compound eyes of arthropods is the migration of screening pigments during changes in condition of illumination.

In Grustacea, the three sets of retinal pigments, distal retinal pigments, Proximal retinal pigments and reflecting pigments change their position in relation to light or dark-adaptation. Distal and Proximal retinal pigments are black and they consist of Ommatin, which is related to melanin. The reflecting pigments on the other hand, are white in colour.

During light adaptation, both the distal and the proximal retinal pigments undergo dispersion, and thus they form a sort of screen which isolates one ommaditium from the next. The white reflecting pigments are withdrawn through the basement membrane, and correspondingly their reflecting power is diminished.

In the dark-adapted condition, a reverse phenomenon takes place. The distal and proximal pigments are concentrated, and the white reflecting pigments migrate above the level of the basement membrane.

However, the pattern of migration of these retinal pigments does not occur in the same manner in all groups, and it seems that the extent of the movement and also the number of retinal pigments showing such movements may be a species- specific phenomenon (Nagabhusanam and Sarojini 1970).

In Insecta, the migratory pigments are confined to the pigments of iris cells, which are also known as screening pigments or accessory pigments. The most important and widely distributed groups of accessory pigments are the Ommochromes which are formed from the amino acid tryptophan through the intermidiate Kynurenine and hydroxy-kynurenine(Butenandt et al,1954).Different kinds of Ommochromes are now recognised, among which Ommatin, Ommin, and Ommidin are the most important.

There are three different types of ommatin, of which Xanthomatin is the only one found in the eyes. It it the principal pigment of Calliphora and related flies.

Ommins are the most widely distributed among the ommochrome pigments of the compound eyes of insects. It differs from ommatin by having higher molecular weight, and by showing less tendency to auto-oxidation(Wiglesworth,1957).

Ommidin , which is related to Ommin is found in the eyes of Orthoptera. These accessory pigment granules change their position in relation to light or dark-adaptation. However, the anatomical changes during light or dark-adaptation in insect compound eye do not show the same pattern in all forms.

In apposition type of compound eyes, the light-dark adaptation is associated with radial movement of screening pigments. In the dark - adapted state, the sacks of the endoplasmic reticulum known as Palisade surround the rhabdom. Major portion of the retinula cells upto one-third of its width from the rhabdom is occupied by the elongate, large vesicles of the palisade. The pigment granules occur between the vesicles of the palisade as well as in the peripheral cell-cytoplasm (Kirscfèld and Franceschini, 1969).

During light-adapted condition, the palisade is reduced in extent. Accessory pigment granules of the retinula cells move towards the rhabdomeres. In Musca, accessory pigments approach the rhabdomeres closely during the light- adapted condition, but not palisade has been clearly demonstrated (Kirschfeld and Franceschini, 1969).

In Lepidopteran compound eyes and also in the Fire-flies, light-dark adaptation is associated with the longitudinal movement of pigment granules in the retinula and pigment cells. Most of the animals in this group are nocturnal and are not active in the presence of bright light.

In some compound eyes, eg. in the beetle Dytiscus, light-dark adaptation, causes the movement of somes of the retinula cell bodies (Walcott 1969).

In some acone type of compound eye, the most notable feature during light or dark-adaptation is the extensive changes in the shape of the cone cells and also the movement of cone-cells, crystalline tract and the rhabdom of the retinula cells. A typical example of this case is the compound eye of the hemiptera, Notonecta (Walcot,1974)

Despite the fact that the studies on pigment migration in the compound eyes of arthropods started long back, as early as in 1889 by Exner, the mechanism by which pigment granules change their position during light-dark adaptation is not clearly understood even now.

In crustacea, however, it is known that the movement of the distal retinal pigments are controlled by some hormones present in the eye- stalk (Bennit 1932).

Welsh (1939) has demonstrated in the eye- stalk of the crayfishes Procambrus clarki and Orconectes limosus a factor which could bring the distal retinal pigments to light-adapted position. A similar type of light-adapting hormone has been reported in the eye-stalk of Palaemonetes by Kleinholz (1936). Brown, Hines and Fingerman(1952) have found a distal retinal pigment light-adapting hormone in the supra-oesophageal ganglia of Palaemonetes. The presence of this hormone in the eye-stalks and supra oesophangeal ganglia of dwarf crayfish, Combarellus shufeldti has been demonstrated by Fingerman (1957).

Similarly, a proximal retinal pigment dark-adapting hormone has been proposed in the eye- stalks of Palaemonetes and Orconectes by Fingerman, Nagabhusanam and Phillipot (1962).

In insects, the mechanism which controls the migration of the screening pigments is very poorly understood. Most of the works in the area have been performed in the eyes of lepidopteran insects.

Day (1941) has found that accessory pigments of moths move to the light adapted position when the animal is subjected to cold, narcosis, CO_2 or sometime general injury . Stavenga (1977) has reported that the circadian

movement of distal pigments in the praying mantis and Katydids are influenced by temperature. He has found that day-adapted state can be established at night by cooling.

These findings suggest that the compound eyes possess a greater metabolic capacity in order to move the pigments to the dark adapted position than for movement in opposite direction. It is suggested that some energy requiring processes are operative during the distal migration of the screening pigment and light has an inhibitory effect on it. (Goldsmith and Bernard 1974). Veron (1973) has found that in the dragonflies, Austrolestes annulosus and Ischnura heterosticta, pigment migration in the eyes is associated with pronounced colour changes which superficially resemble those of their epidermal chromatophores. When the pigment is concentrated around the base of the crystalline cone, a dense layer of tyndall blue bodies produce bright " blue-phase" colours. During dark-phase, distal migration of the pigment occurs, which disrupts the Tyndall effect and the eyes turn grey - brown in colour. By performing many experiments, Veron (1973) has proved that at least in Austrolestes annulosus and Ishnura heterosticta the eye pigment

migration and the migration of the epidermal chromatophores are under similar environmental and physiological control. He has found that changes in temperature can influence pigment migration of the eye, as well as the epidermal chromatophores.

Keeping these in view, an attempt has been made to study certain factors especially the temperature in relation to pigment migration in the compound eyes of the house- fly, Musca domestica and the honey - bee, Apis cerana indica. A preliminery study on the effect of light and darkness on neurosecretion of these insects has also been performed in view of the suggestions by some authors regarding the influence of neurosecretion in pigment migration (Veron, 1973).

In addition to this, the effect of cyclic- AMP, 5 - hydroxytryptamine and colchicine has been studied on the migration of pigment granules in Musca and Apis.

However, it has not been possible to perform any experiment on the pigment migration in the eye of the fresh water prawn, Macrobrachium birmanicum and the marine prawn, Metapinneus sp. because of the practical difficulties in maintaining them in the laboratory.

MATERIALS AND METHODS

Studies on light or dark adaptation: To examine the position of the pigment granules in light or dark- adapted condition, two groups of experimental insects, each containing about 20 insects have been collected. One group has been kept in normal Sun light for about 5 hrs. After the required period of light- adaptation, the insects are kelled and then the eyes are fixed in " Susa" fixative. Routine histological preparations are made by paraffin methods and sections are cut at about 8 μ thick.

The other group of insects has been adapted to complete darkness by keeping them in the photographic " Dark room" for the same period as the light- adaptation experiment. After the experiment, the eyes are similarly fixed in " Susa " fixative and section are cut at 8 ^{u} thick.

Effect of temperature:

Veron (1973) has reported that temperature effects the migration of screening pigments of the compound eye as well as the epidermal chromatophores. In view of the above report, the effect of temperature on pigment migration in the eyes of the house-fly, Musca domestica and the honey bee, Apis cerana indica have been tested.

Experiment No.1. Effect of high temperature(28 - 30°C) on light adapted insects:

A group of experimental insects are light adapted by exposing them to normal sun-light. They are then divided into two groups. One group is kept as control and the other is given a high temperature - treatment by placing them in glass jars submerged upto their neck in a water- batch, maintained at a temperature of about 28 - 30 °C.

EXPERIMENT NO.2

Effect of low temperature on Dark-adapted insects

Experimental insects are kept in glass jars, which are painted with black paint to prevent the entry of light. The jars are then placed in a water-bath, the temperature of which is maintained at about 15°C .

After the required period of temperature treatment, the insects are fixed in " Susa " fixative, routine histological preparations are made and sections are taken at about 8/μ thick.

The sections are then compared with the control ones.

Effect of light and darkness on Neurosecretion.

To study the effect of light or darkness on the

neurosecretory system of Musca and Apis the insects are given a light or dark treatment for about 5 hrs.

After the required period of light or dark - adaptation the insects are killed immediately and the heads are fixed in alcoholic Bouin's fixative.

For the histological preparation of the neurosecretory cells, a number of fixatives such as Zenker- formol, Helly's fluid, Regaud's fixative, and 8% formalin are tried. However, the best results are obtained when the tissues are fixed in alcoholic Bouin's fixative. The complete head of the experimental insects are kept in Bouin's fixative for 24 hrs. Routine histological preparations are made and sections are prepared at 10 μ thickness. To detect the neurosecretory cells the sections are stained in Gomori's aldehyde - fuchsin (AF), modified by Cameron and Steele (1959), as follows -

(1) The paraffin sections, after deparaffinization, are brought through ethanol series down to distilled water.

(2) The sections are then oxidized in oxidant (3g $KMnO_4$ in 100 ml water containing 0'30 ml concentrated H_2SO_4) for 2 to 3 minutes.

(3) Oxidants are then blotted off with stips of filter paper. The sections are bleached with 4% Sodium bisulfite. The solution is renewed once or twice until it it becomes perfectly white.

(4) The sections are then washed in distilled water for 5 to 10 minutes.

(5) The sections are transferred through 30% to 70% alcohol.

(6) Staining is done in paraldehyde fuchsin stain(Basic fuchsin 1g; conc.Hcl 1 ml; paraldehyde 2 ml; 60% alcohol 100 ml) for 2 to 10 minutes.

(7) Excess stain is quickly blotted off with a filter paper.

(8) Stain is differentiated in 95% alcohol until no more superfluous stain is given off.

(9) The sections are dehydrated, cleared and mounted in DPX.

Effect of cyclic AMP(Calbiochem),5 HT, and colchicine

To study whether there is any effect of any drug on the migration of screening pigments in the compound eyes of Musca and Apis, cyclic AMP, 5- HT and colchicine have been applied to both the dark and light-adapted insects.

Cyclic AMP is used in the concentration of 10^{-6}M and the concentration of 5-HT is 10^{-8}M. These concentrations have been used successfully by other authors in other tissues (Berridge 1971; Hood 1973).

Both the substances are dissoved in Insect Ringer solution and are applied topically.

Effect of colchicine: Colchicine has been tried only in the dark-adapted insects to examine whether it can bring the screening pigments to light adapted position as has been suggested by Miller and Cawthon (1974).

The insects have been dark- adapted and colchicine has been applied topically in the dark-adapted condition itself. Colchicine was dissolved in insect Ringer solution and the concentration used was 10^{-2}M

OBSERVATIONS

The position of the pigments during light and dark adaptation in house fly, and honey bee are shown in Figs. 29, 30 and 31, 32 respectively.

Effect of temperature:

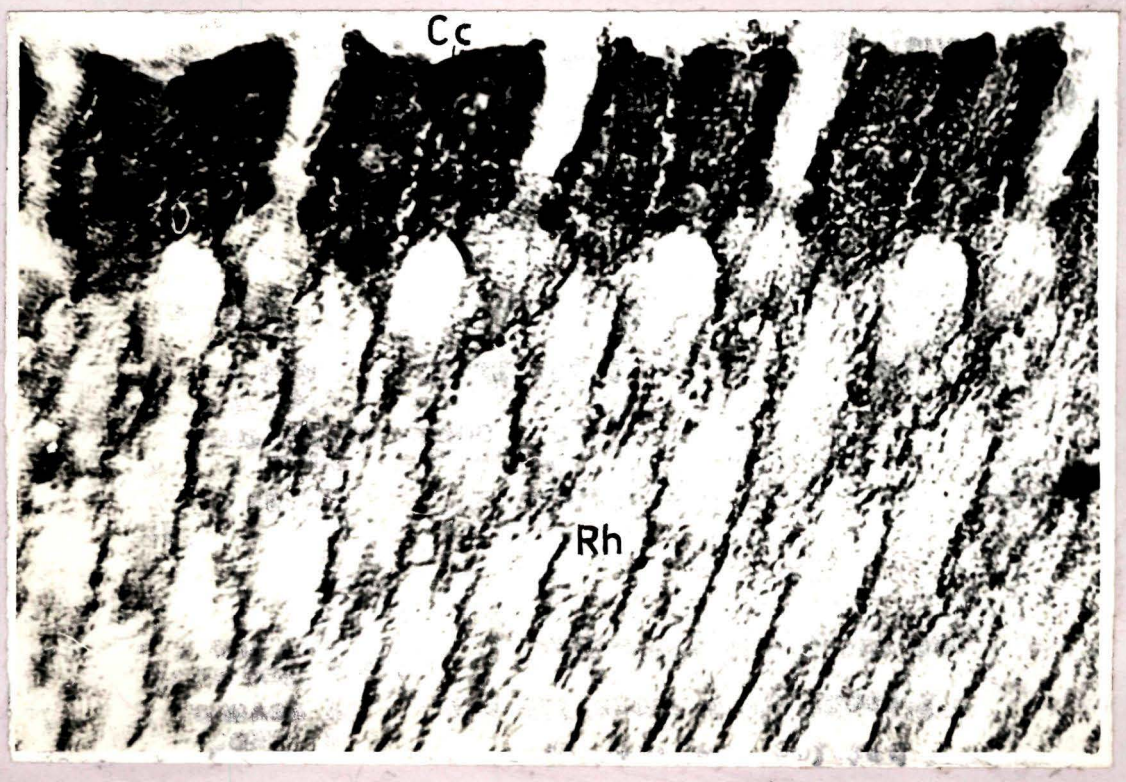
It has been observed that the pigment migration in the eyes of both Musca and Apis are very much affected by temperature.

When the light -adapted insects have been exposed to high temperature, it is found that after about 5 hrs. of such treatment, the section of the eyes appear more or less like dark - adapted ones, in relation to the position of the migratory pigment granules.

EXPLANATION TO FIGURES

Fig. 29. T/S through the eye of light-adapted house-fly, Musca domestica showing the radial movement of pigment granules. 10 x 40

Cc , Crystalline cone,
Rh , Rhabdom.

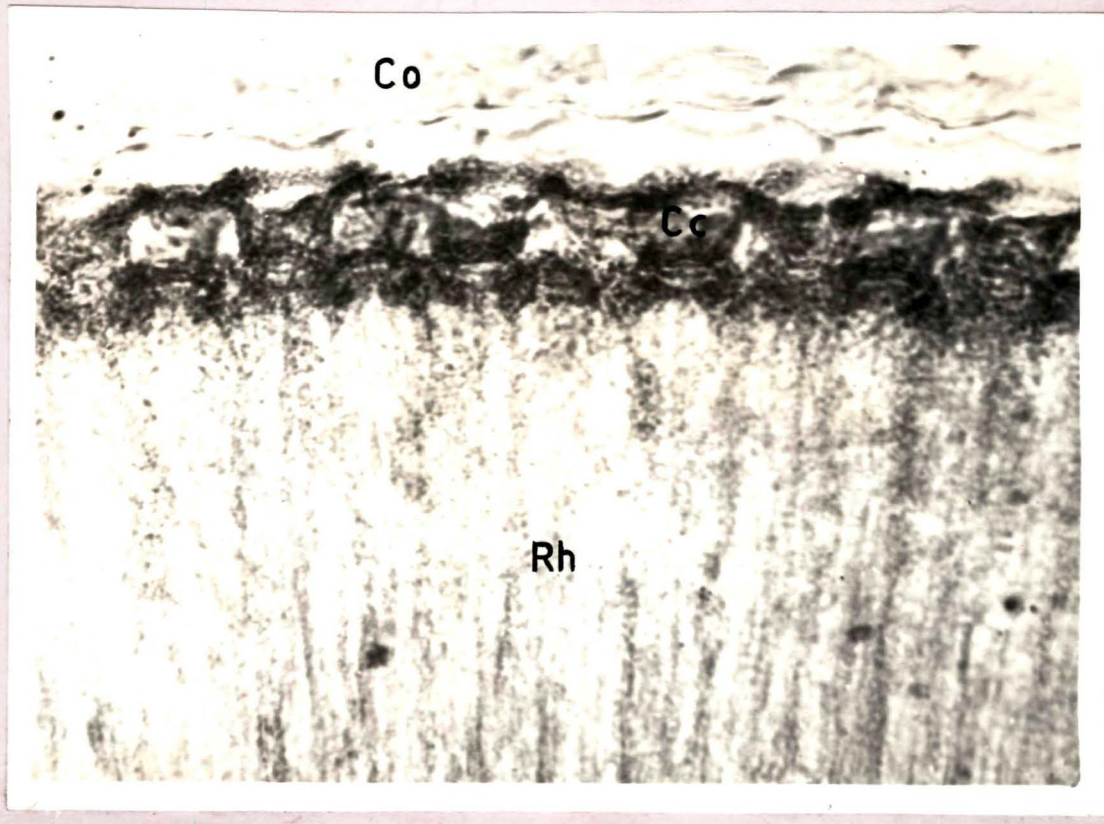

Cc
Rh

EXPLANATION TO FIGURES

Fig. 30. T/S through the eye of dark-adapted house-fly, Musca domestica showing peripheral distribution of the pigment granules.

10 x 40.

Co , Cornea ,
Cc , Crystalline cone
Rh , Rhabdom.

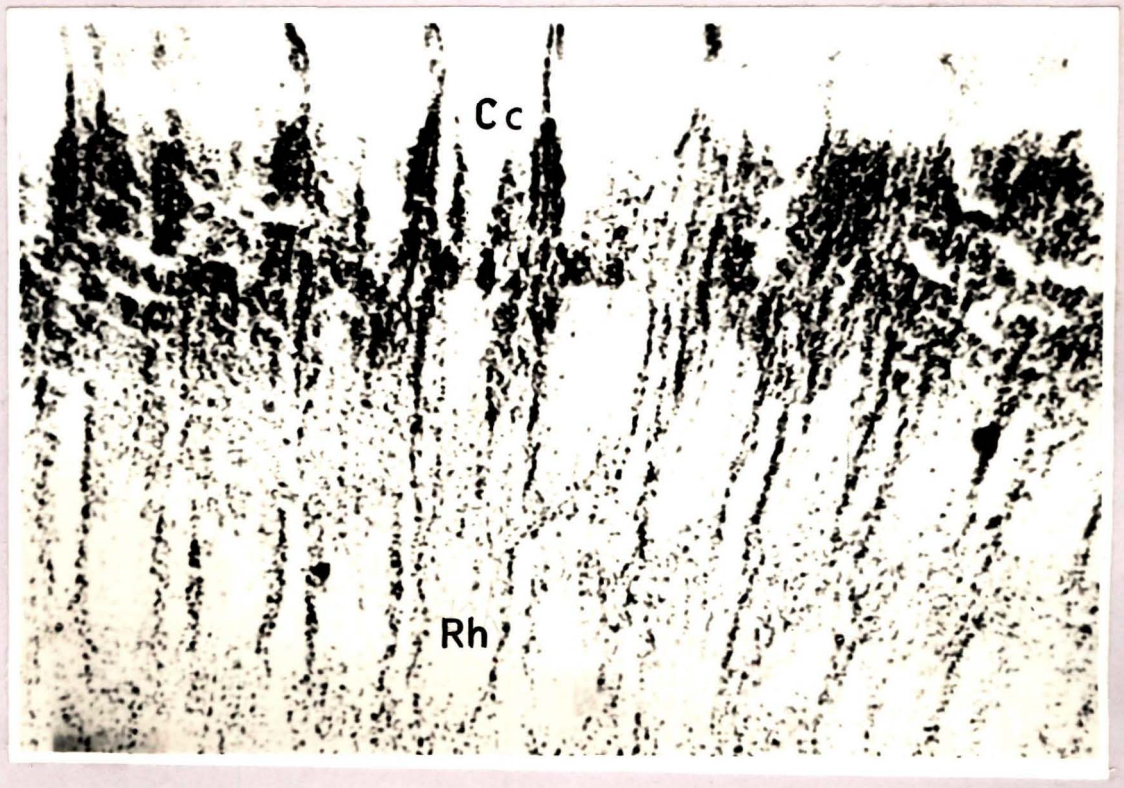

Co
Cc
Rh

EXPLANATION TO FIGURES

Fig. 31. T/S through the eye of light-adapted honey - bee, Apis cerana indica showing the radial movement of pigment granules. 10 x 40.

Cc , Crystalline cone

Rh , Rhabdom.


Cc
Rh

EXPLANATION TO FIGURES

Fig. 32. T/S through the eye of dark-adapted honey bee, Apis Cerana indica showing peripheral distribution of pigment granules.
10 x 40

Dp , Distal pigments.
Rh , Rhabdom.


Dp
Rh

Similarly, it has been found that low -temperature can bring the pigment - granules of a dark -adapted insect towards a position more or less similar to light - adapted forms.

Effect of Light or Darkness on Neurosecretory system

It has been observed that , both in Musca and Apis , when the insects are kept in darkness, there is a large accumulation of neurosecretory materials (NSM) in the form of compact neurosecretory granules,(Figs. 33, and 34) as evidenced from their positive reaction to paraldehyde - fuschsin stain. The granules appear purple in colour.

In contrast to this, when the insects are kept in continuous light, it has been observed that there is a significant reduction in the quantity of the neurosecretory material in the cell-bodies, presumably due to the axonal transport of neuroscretory material. The Cytoplasm now appears lightly stained, and the shape of the cell becomes slightly irregular (Figs. 35 and 36).

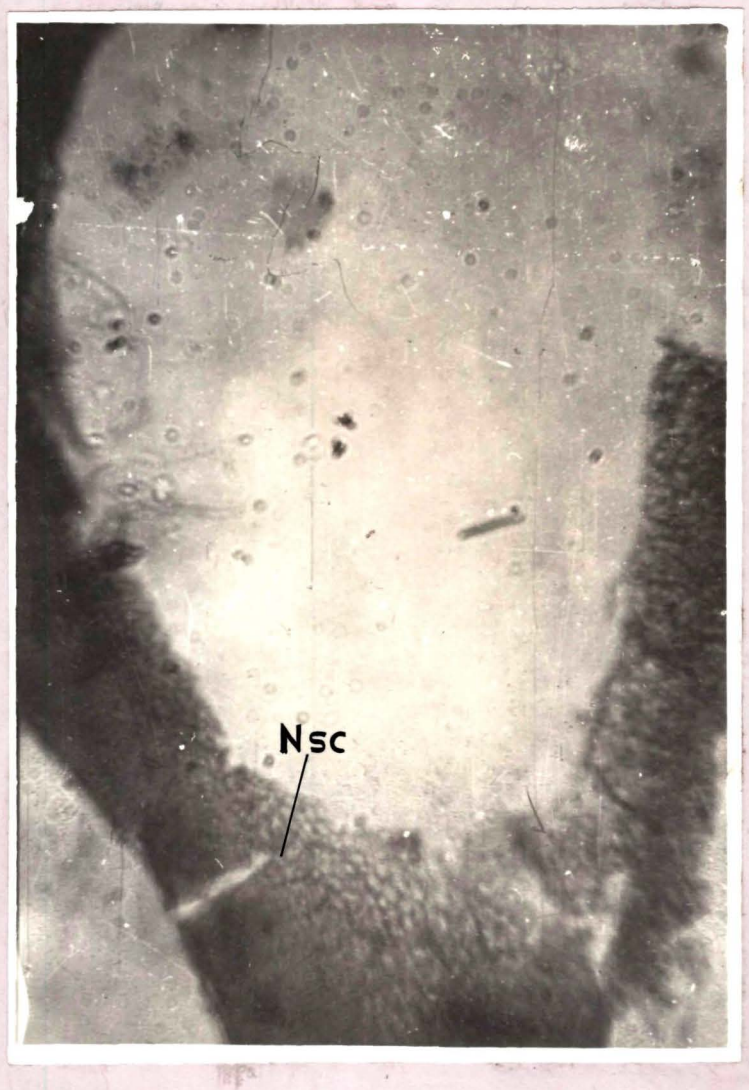
Effect of cyclic AMP . 5 - HT and Colchicine:

Since, our study depends on the examination of the paraffin sections with light - microscope alone, it has not been possible to detect the effect very clearly. However,

EXPLANATION TO FIGURES

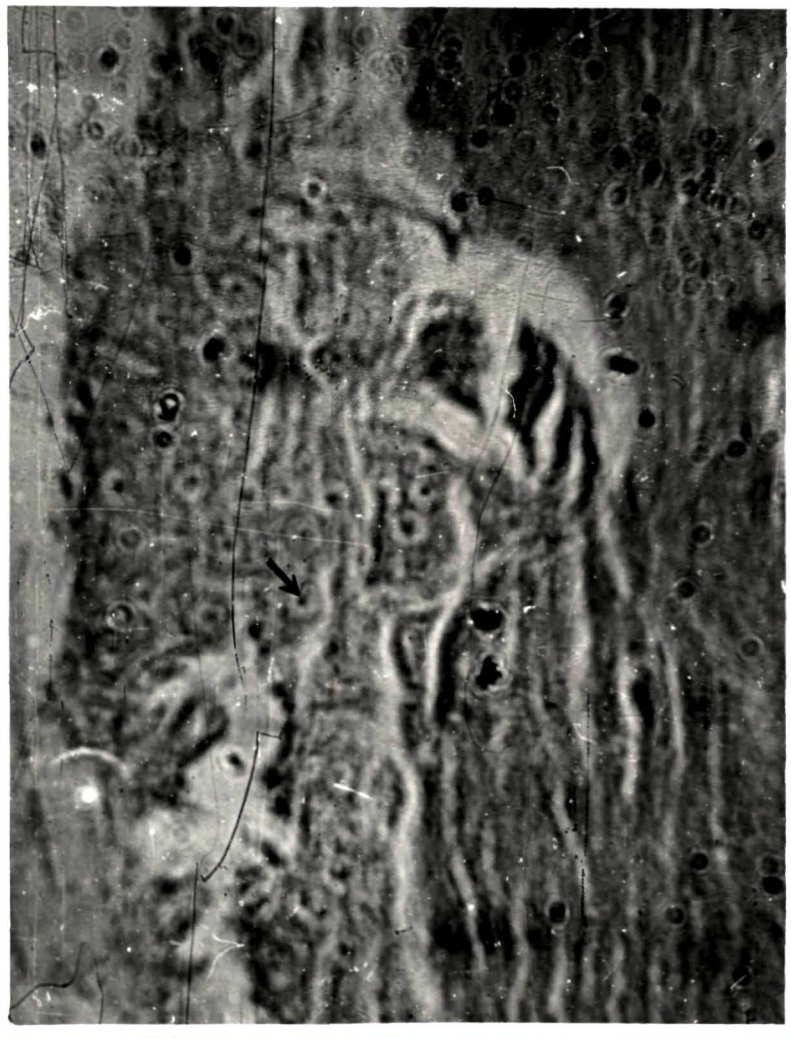
Fig. 33. Longitudinal section passing through the brain of the dark-adapted house fly, *Musca domestica*, showing the large accumulation of neurosecretory materials in the form of compact neurosecretory granules. 10 x 40.

Nsc, Neurosecretory cells.


Nsc

EXPLANATION TO FIGURES.

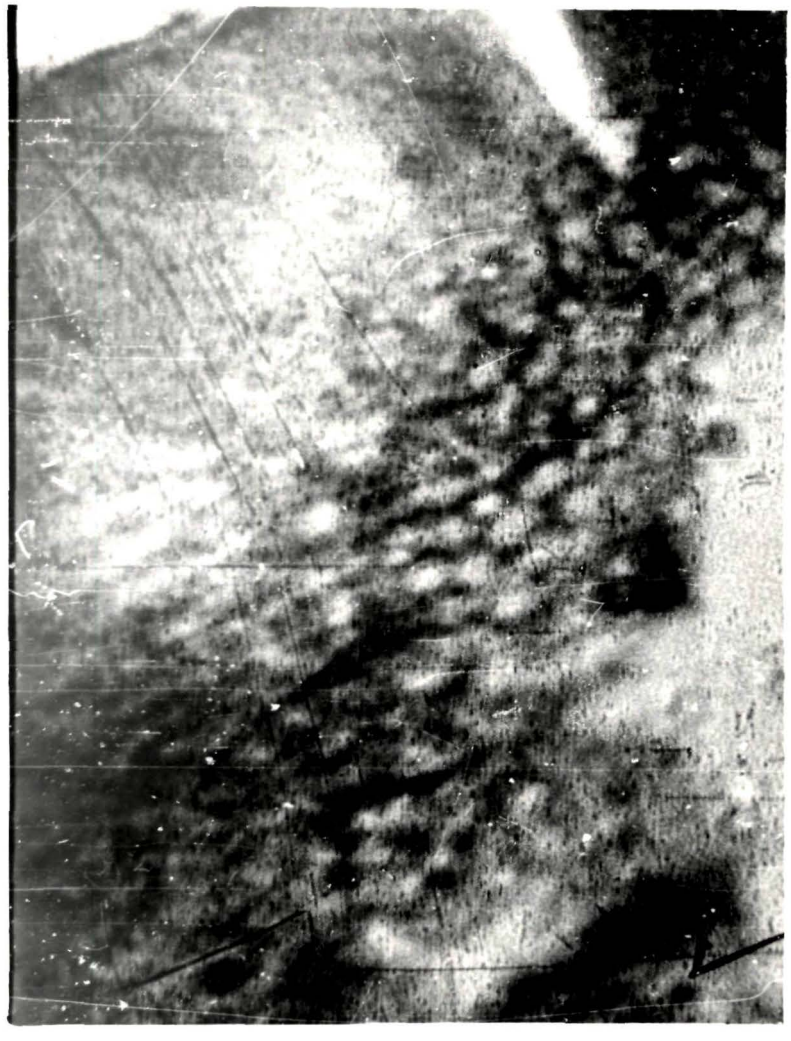
Fig. 34. Longitudinal section passing through the brain of the dark-adapted honey bee, Apis cerana indica, showing the large accumulation of neurosecretory materials in the form of compact neurosecretory granules. 10 x 40.



EXPLANATION TO FIGURES.

Fig. 35. Longitudinal section passing through the brain of light-adapted house fly, Musca domestica, showing the lightly stained cell-cytoplasm, and irregular shape of the neurosecretory cells.

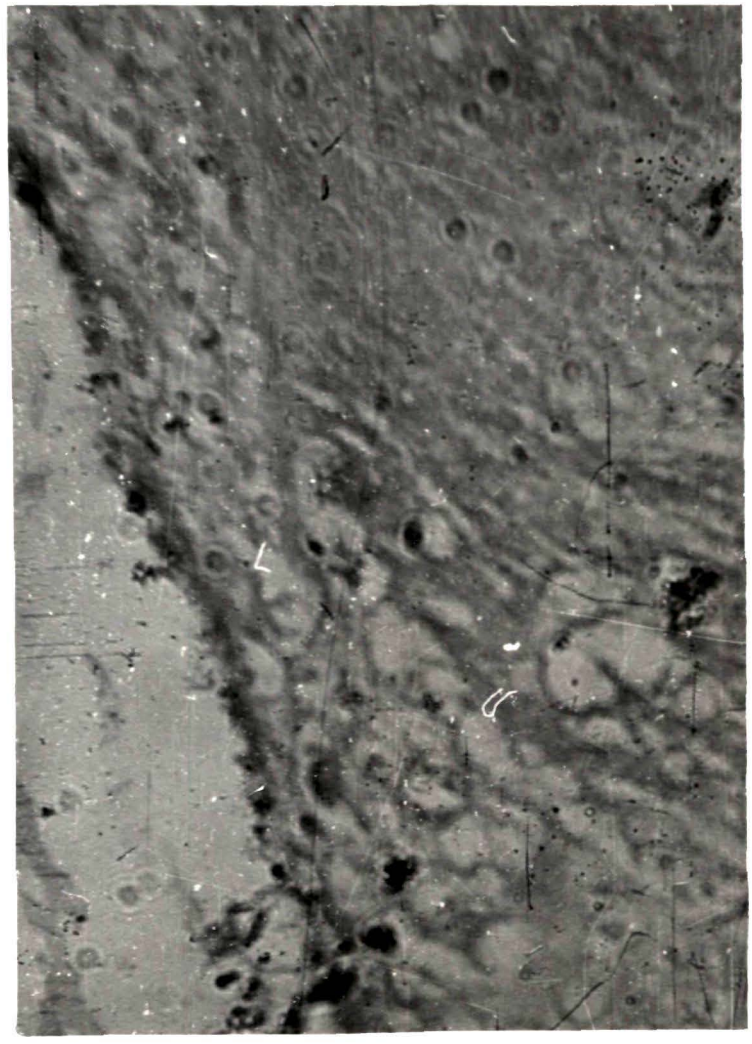
10 x 40.



EXPLANATION TO FIGURES

Fig. 36. Longitudinal section passing through the brain of light-adapted honey bee, *Apis cerana indica* , showing the lightly stained cell-cytoplasm, and irregular shape of neurosecretory cells. 10 x 40.

Nsc, Neurosecretory cells.

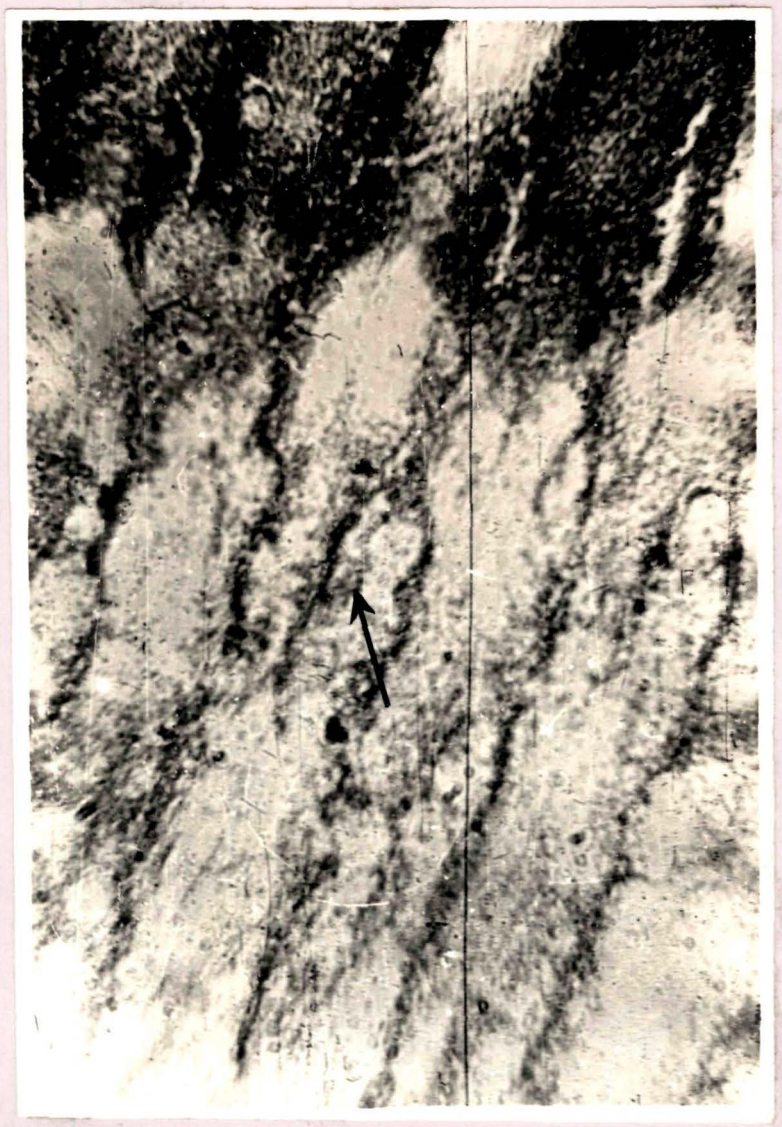


careful observations of a large number of sections and comparison with suitable control reveals that cyclic AMP, 5 - HT and colchicine have some effects on radial pigment migration in the eyes of Musca and Apis. It has been found that these substances can bring the pigment granules from dark -adapted towards a light - adapted condition. It is observed that among the substances used, colchicine has the most pronounced effect on pigment movements. When the insects are dark-adapted and treated with high concentration (10^{-2}M) of colchicine, the dark - adapted condition is not observed in the eyes as compared to the dark control.

In the control insects, it is observed that the pigment granules are present towards the peripheral portion of the retinuala cells whereas in the treated insects the pigment granules are observed towards the rhabdom, a condition more or less similar to the light adapted - forms (Fig. 37).

EXPLANATION TO FIGURES

Fig. 37. T/S through the eye of the dark-adapted and colchicine-treated house-fly, *Musca domestica* 10 x 100., showing the effect of colchicine on bringing the pigment granules from DARK towards LIGHT-adapted condition.



DISCUSSION

Most of the diurnal insects have apposition eyes in which radial movement of pigment granules occurs, whereas pronounced longitudinal pigment migration are characteristic feature of nocturnal or crepuscular species with superposition eyes. The optical function of pigment migration and other photomechanical effects is presumably to control the amount of light in the photoreceptor organelle, i.e. the rhbdomere. The rhabdomere has a higher refractive index than its surrounding medium (Stavenga 1974), and because of this, it acts as optical wave- guide (Snyder 1974). The amount of energy that is propagated in the organelle is a function of the refractive index difference between the organelle and surrounding material and of the organelles physical dimensions and the wavelength of light. As the radiant energy propogates within the organelle, a small fraction is outside the organelle. The pigment granules brought in this manner near the organelle has two effects - it tends to increase the refractive index of the surrounding zone ~~with~~ which "frustrates" total internal reflection and increases the intensity in the surrounding. Secondly, the pigment granules absorve a fraction of the light coming from the organelle.

Additionally, the pigments will absorb light in the retinular cell that propagate outside the rhabdom.

In apposition type of compound eye, on illumination, small pigment granules which are dispersed throughout the visual cells during dark - adaptation move towards the rhabdomere. The granules assembled in the immediate neighbourhood of the rhabdomere constitute a longitudinal pupil.

The mechanism by which the pigment granules alter their position in relation to change in the condition of illumination is not known clearly. In an attempt to study the effect of various factors on migration of the pigment granules, it has been observed in the present investigation that, the pigment migration in the eyes of the house-fly, Musca domestica , and the honey bee, Apis cerena indica is markedly affected by temperature. Similar effect of temperature on pigment migration has been reported in various insects by different authors. O'Farrell (1964) has found that the chromatophores of Diphlebia spp. are sensitive to change in light intensity as well as to temperature.

Day (1941) has found that the distal eye- pigment cells of the moth, Ephestia are substantially sensitive to

change in temperature as well as light intensity. Some similarity may also exist between the diurnal rhythm of sensitivity to temperature change, as in the chromatophores of the dragonfly, Austrolestes annulosus (Veron, 1973) and the diurnal change in the rate of eye-pigment migration observed in other insects (Demoll, 1911; Horstmann, 1934; Jahn and Wulff, 1941).

Srinivasan, Bernard and Stavenga (1977) have found that in butterflies at temperature above the ambient 24 ^{o}C, sensitivity of the pupil is reversibly reduced. Increase in temperature speeds up closing and opening of the pupil. In Nymphalis, a temperature increases of 10^{o}C decreases the half- time of closing by 25% and that of opening by 15%. In general, closing - speed is more temperature - sensitive than opening - speed. In Eurema a temperature increase of 10^{o}C decreases the closing half time by 55%, but there is no measurable change in opening half time. It has been hypothesised in the literature that the light-attenuating granules which mediate the pupillary response are in continuous random motion within the retinular cell, and that this " Brownjan motion" mediates opening of the pupil in the dark. However, the importance of Brownian motion as a mechanism for pupil -

opening is questionable. Stavenga (1977) found that circadian movement of distal pigment, which is observed by examining the psendopupil in crabs, praying mantis, Katydid, Limulus, etc . are also sensitive to temperature. These pigment movements cause the ommaditial aperture to be enlarged at night. The day - adapted state can be established at night by cooling.

The physiological control of eye- pigment migration in insects has long been a subject of debate. Many early workers believed that eye- pigment migration is under direct nervous control (Demoll, 1911). Some authors (Friza 1928; Uchida, 1934) have suggested that control is more likely to be hormonal. Collins (1935) has concluded that the moth, Capocypsa, has independent effectors, whilst Day (1941), after finding that cutting the Optic tract in the eyes of Ephestia prevents repeatable migration, has supported the concept that the control is by nerves. However, by showing that normal, reversible pigment migration can take place in isolated eyes, Tuurala (1954) has proved that at least in some moths, pigment cells are either independent effectors or are under some local controls. Veron (1974) has suggested that in the dragonflies, Austrolestes annulosus (Selys) (Lestidae) and Ichnura heterosticta (Burm)

(Coenargrionidae). eye pigment cells act as independent effectors during proximal migration but the control of distal pigment migration appears to be complex. He has observed that the distal pigment migration in Austrolestes and Ichnura is significantly reduced in the eyes and in head chromatophores of decapitated insects. Very little migration occurs in isolated eyes and none has been observed in isolated pieces of eyes. He has also observed that distal pigment migration occurs at similar rates in both distal eye-pigment cells and epidermal chromatophores.

On the basis of the results of these experiments, Veron (1974) has suggested that distal migration of the pigment granules in the compound eyes are probably under the neurosecretion.

The existence of a relationship between the elaboration of chromatotropins and neurosecretion is rendered probable by the results of Dupont- Raabe(1956, 1957) establishing the presence of neurosecretory cells in the tritocerebrum of phasmidae, a region in which the nerve- centres have been shown by pharmacodynamic test to be particularly rich in chromatotropins.

Quite a number of other regions in the central nervous system of insects as well as the corpora cardiaca

contain chromatotropins. B. Scharrer (1959) noted that there are indications of the elaboration of some of these substances by neurosecretory cells.

Gabe (1966) has suggested that, since all segments of the central nervous system contain neurosecretory cells, it possible that the chromatotropins of the corpora cardiaca have the same charecteristics as those of the cerebral ganglion (Dupont - Raabe,1952); Removal of the corpora cardiaca does not effect colour-adaptation in phasmidae (Dupont- Raabe 1951) and section of the nervi corporis cardiaci lead to the disappearence of all chromatotropic activity from extracts of corpora cardiaca (Cameron, 1953).

In the present study regarding the effect of light or darkness on the state of activity of neurosecretory cells, it is found that in the dark-treated insects there is a large accumulation of neurosecretory material, whereas in the light - adapted ones there is reduction in the quantity of the neurosecretory material in the cell- bodies of median neurosecretory cells. Presumably during light - adaptation axonal transport of neurosecretory material takes places. It may be suggested that in the dark-treated insects the rate of discharge of neurosecretory material

is slower, whereas in light-treated ones the rate of production of neurosecretory material is slower than the rate of discharge.

In general, there are two basic types of neurchormones present in the neurosecretory system of insects. One type is represented by compounds of relatively low molecular weight. These are catecholamines (dopamine, adrenaline, non-adrenaline) or biogenic amines like 5- hydroxytryptamine (Gersch 1970). The other type is charecterized by compounds of relatively large molecular weight such as peptides or proteins.

It is well established that catcholamines and the biogenic amines like 5- hydroxytryptamine are responsible for physiological phenomena which take place rapidly and their liberation is so to speak instantaneous . In this context, it is of interest of mention that pigment migration in the compound eye of insects is also a very rapid process. Franceschini and Kischfeld (1969) have observed that pigment granule movements in Musca on light adaptation are very rapid and occur even in a few seconds. The palisade in Locusta requires 15 minutes of dark -adaptation to develop fully (Walcott, 1974). In cockroach Periplanata americana, where both palisade and pigment

granule changes have been observed, anatomical light-adaptation requires 10 minutes of exposure to light (Butler ,1971). Thus in any attempt to study the effect of neuroscretion in pigment migration of the compound eyes of insects the involvement of catecholamines or biogenic amines, like 5 - hydroxytryptamine seems to be important.

With this view, the effect of 5-hydroxytryptamine on the migration of pigment granules on the compound eye of Musca and Apis has been tested. It has been found that 5- hydroxytryptamine can bring the pigment granules from a dark-adapted position towards a light-adapted condition. In this context, it is worth-mentioning that 5-hydroxy-tryptamine has been reported to have melanin-aggregating action (Scheline 1963; Scott 1965).

In addition to this, the result obtained in the present investigation regarding the effect of colchicine on pigment migration is of significance. It has been observed that when high concentration of colchicine is applied to the dark-adapted insect, histological preparations of the eyes appear like light-adapted ones in relation to the position of the pigment granules. Similar effect of colchicine has been reported on pigment granule

movements in Limulus photosceptor by Miller and Cawthan (1974). They have observed that high concentration of colchicine (10^{-2}M) causes the pigment granules in the retinular cells to move to the light - position. In addition to this, they have reported the presence of radially oriented microtubles in the retinular cells. The microtubules have been easily detected in the retinular cells of colchicine treated materials. Becuase colchicine specifically binds the tubuline protein of the microtubule's subunit(Shelansk and Taylor 1968), they concluded that the radially oriented microtubules of the retinular cell mediate the light and dark - induced pigment migration in Limulus.

In case of cell-movements in compound eye of arthropods also, there are sub-cellular structures which could affect some of the movements. In Lethocerus, for example, microtubules occur in great numbers in all the cells which move (Walcott 1971 a). In the cone cells and primary pigment cells, the microtubules are highly oriented in the directions of movement. Similar orientations are seen in the cone cells of a megalopteran (Walcot and Harridge, 1971).

GENERAL DISCUSSION

GENERAL DISCUSSIONS

The compound eyes of arthropods are of considerable interest in elucidating the visual system of animals.Compound eyes are image-forming eyes which are particularly efficient in detecting movement in their total visual fields. In addition, many of the arthropods exhibit orientation relative to the direction of vibration of polarized light, which suggests the existence of a polarized light analyser with the eye. . If we are to understand how the compound eye functions in response to a light stimulus it becomes important to study its structure and chemistry.

In the present investigation, some attempts have been made to study certain very important aspects of arthropod vision and some interesting observations have been made.

While studying the histochemistry and bio-chemistry of the lens-cuticle, a protein-carbohydrate complex,"Acid mucopolysaccharde" has been detected in the compound eyes of the house fly, Musca domestica, the honey bee, Apis Carana indica, the fresh-water prawn, Macrobrachium birmanicum and the marine prawn, Metapinneus sp. Mucopolysaccharides have been detected in certain specialised arthropod cuticles and different roles for the substances have been suggested in different types of cuticles. Meenakshi and Scheer (1959) and Sundara Rajulu(1969) have suggested that acid mucopolysaccharides of the cuticle of Hemigrapsus nudus

and Cingalobolus bugnioni respectively may have important roles in clacification of the cuticle. Krishnan(1965) has suggested that acid mucopolysaccharides may be associated with S-S bonding of the cuticle in the scorpion, Palamneus swammerdami. The presence of acid mucopolysaccharides in the sub-cuticle of insects such as Philosamia cynthia (Taylor and Richards,1963), has been assumed to indicate the region where the chitin-protein complexes are formed. Due to the high water-binding capacity of the acid mucopolysaccharides, their occurrence in intersegmental cuticle of the queen of the termite, Odototermes obesus has been suggested to impart to the extreme flexibility of the cuticle(Sannasi,1969). Now lens-cuticle is a modified cuticle and hence it is reasonable to presume that the mucopolysaccharides present in the lens may play some important roles in the visual phenomenon of the arthropod. Since in the present investigation, studies have been performed in various aspects of arthropod vision, it has not been possible to study the mucopolysaccharides alone of the compound eyes in detail. Thus it would be of great significance, if one can study the mucopolysaccharides of the compound eye in detail and to find out whether the function is mechanical or optical.

Another interesting observation which we have been made is the detection of ascorbic acid in the compound eye. As already mentioned in the relevent section, ascorbic acid may play some important roles in energy generation of the

Photoreceptor. In addition to this, an important point, which can be mentioned here is that ascorbic acid may have some role in synthesis mucopolysaccharides which are specifically present in the len cuticle. Evidence for and against ascorbate stimulation of sulfated glycosaminoglycans are found in the literature. Human skin fibroblasts in tissue culture produce more sulfated polysaccharides in the presence of ascorbate(Dische,1947). Kafoed and Robertson (1966) on the other hand, suggest that ascorbate is not required for chondroitin sulfate synthesis in guinea pig tracheal cartilage. Lavietes(1971) has shown that the presence of ascorbate in the culture medium does affect the distribution of the chondroitin sulfate synthesized.

With these reports in view, it can be suggested that, it will be of significance of study whether there is any such relationship between mucopolysaccharides and ascorbic acid in the compound eye of arthropods.

Fluorescence in the compound eyes of arthropods is another aspect where some interesting observations have been made. In this context, detection of resilin in the lens-cuticle of the house-fly, Musca domestica , the honey bee, Apis cerana indica, the fresh-water prawn, Macrobrachium birmanicum and the marine prawn, Metapinneus sp. is of significance. In addition to this the qualitative studies of pteridines in the compound eyes would certainly serve as spring board for future study.

Another interesting observation made on the compound eye of house-fly, honey-bee and the fresh-water prawn is the study of the enzyme adenosine triphatase(ATPase). The mechanism of enhancement of metabolism which supplies the necessary energy for the mechanism of stimulus processing and conversion into nervous excitation by the sensory cells appears to involve cation exchange process(Bonting,1969). The role of (Na^+- K^+)- stimulated ATPase of cell-membrane in active cation transport has been well-established (Skou,1965). Therefore it is possible that this enzyme is involved in the visual process, in which cation transport plays a role.

In addition to all these an important observation made on the visual system of arthropods is the effect of certain factors on the light-stimulated pigment migration in the compound eye . It has been observed that temperature has a significant effect on the migration of screening pigments. Colchicin, 5-hydroxy tryptamine and cycle AMP, applied topically on the head and eye, have marked affect on migration of the screening pigments in the compound eye. In addition to this it has been observed that neuosceretion may play some very important roles in pigment-migration. But in spite of all these, it is not possible to tell exactly, which factor is responsible for pigment migration.

Considering all these, it can be concluded that much research still remains before one can completely understand

how compound eyes are structured for performing such a complicated physiological phenomenon of vision.

SUMMARY

SUMMARY

In the present investigation, certain physiological, biochemical and histochemical studies have been performed on the compound eyes of the house-fly, Musca domestica, the honey bee, Apis cerana indica, the fresh-water prawn, Macrobrachium birmanicum and the marine prawn Metapinneus spp. which can be summarised as follows :-

1. In the lens-cuticle of the above mentioned arthropods, a protein-carbohydrate complex, acid mucopolysaccharides have been detected by histochemical tests. An attempt has also been made to extract the mucopolysaccharides from the compound eye of these arthropods. After extration, the sugar components of the crude mucopolysaccharide extract have been analysed by means of paper chromatography following acid hydrolysis of the mucopolysaccharide extract. With a view to know the nature of the mucopolysaccharide, it has been subjected to paper electrophoresis in various buffer systems and the Rf value has been compared with some standard mucopolysaccharides such as chondroitin 4-sulfate, chondroitin-6 sulfate. keratan sulfate and heparan sulfate. It has been found that the mucopolysaccharide extracted from the compound eye of all these arthropods behaves like sulfated mucopolysaccharides,- more particularly like chondroitin-4-sulfate, in the electrophoretic field. The significance of occurrence of this substance in relation to arthropod vision

is discussed.

2. In an attempt to study the significance of reducing substances in generation of energy in the photoreceptor, the compound eyes of Musca, Apis, Macrobrachium and Metapinneus have been subjected to the study for detection of ascorbic acid. The histological preparations of the compound eye of these arthropods have been subjected to histochemical tests for detection of ascorbic acid. In addition to this ascorbic acid turn over in the compound eye of each of these arthropods has been estimated colorimetrically. It is known that ascorbic acid in the biological system occurs not only in free form(AA)., but also it occurs in the bound form, or ascorbigen (ASG). It is also known that ascorbic acid is acted upon continuously by a number of oxidizing enzymes. Further, chinoy(1967,1969) has reported that in living system endogenous as well as exogenously added ascorbic acid forms a complex with macro-molecules like proteins and nucleic acids. Isherwood and Mapson(1962) have suggested that in a tissue, the actual concentration of ascorbic acid represents the excess, formed in synthesis over that used in the compound eyes has been studied by simultaneous determination of (i) free form of ascorbic acid, (ii) bound form of ascorbic acid or ascorbigen(ASG), (iii) enzymic utilization of ascorbic acid(AAU) and(iv) complexing of ascorbic acid with macromolecules.

3. It has been known that the compound eyes of arthropods show blue or blue-gree fluorescence when irradiated with U- V light in a darkened room. In the present study, attempts have been made to detect some fluorescent substances from the compound eye and to find out the significance of their occurrence. In this context, the detection of resilin in the lens-cuticle is of significance. Resilin has been detected by staining the histological preparation with light Green-Toluidine blue combination, by analysing the amino-acids and detecting the two fluorescent amino-acids, di-tri- tyrosine by paper chromatography and by studying the swelling properties of the lens-cuticle.

In addition to this, pteridines has been studied in the compound eyes by extracting them and then by alalysing with paper chromatography and examining the chromatograms with U-V light.

4. While studying the enzymology of the compound eye, the knowledge of which is very scanty in the existing literature, we made an attempt to study the adenosine tri-phosphatases system (ATPase system). Both the $(Na^{+}- K^{+})$ - activated and Mg^{2+} - activated ATPase have been studied in the compound eyes of house-fly, Musca domestica, honey-bee, Apis cerana indica and the fresh-water prawn, Macrobrachium birmanicum. The ATPase system of the eye-homogemate of Macrobrachium birmanicum has been subjected

to a detail study. The effect of substituting Mg^{2+} by other di-valent cations especially calcium has been investigated. The effect of increasing concentration of Na^{+} and K^{+} on the ATPase activity has also been done. In addition to this, relation between the enzyme and the co-substrates and the effect of bicarbonate on the ATPase activity has been carried out.

5. Last but not the least important observation, which has been made in the present investigation is the studies on the factors affecting the migration of screening pigments in the compound eye. An attempt has been made to study the effect of temperature on pigment migration in compound eyes. It has been observed that temperature has marked effects on migration of screening pigments. When light-adapted insects are exposed to high temperature, it is found that the histological preparations of the eyes appear like dark-adapted ones, in relation to the position of the migratory pigment granules. Similarly, it has been found that low temperature brings the pigment-granules of a dark-adapted insect towards a position more or less similar to light adapted forms.

In addition to this, a preliminary study on the effect of light and darkness on neurosecretion of these insects have been performed in view of the suggestions by Veron(1973) regarding the influence of neurosecretion in pigment migration.

It has been observed that in insects kept in darkness, there is a large accumulation of neurosecretory materials (NSM) in the form of compact neurosecretory granules. When the insects are kept in continuous light, it has been observed that there is a significant reduction in the quantity of neurosecretory materials in the cell-bodies, presumably due to the axonal transport of neurosecretory materials.

Finally, we have also studied the effect of cyclic-AMP, 5- hydroxytryptamine and colchicine on the movement of screening pigments. It has been observed that among these substances, colchicine has some effect on pigment mogements. When the insects are dark-adapted and treated with 10^{-2}M of colchicine, the dark-adapted condition is not observed in the eyes as compared to the dark control; rather it mimics the light-adapted condition.

REFERENCES

AARONSON, S. and RODRIGUEZ, E. (1958): Relationship between purines and folic acid-vitamins in the micrococcus, Gaffkya homari J.Bact., 75,660-665.

ADAMS, R.G., and HAGGINS, W.A.(1960): The ionic composition of squid photoreceptors. Biol. Bull., Woods Hole. 119, 300 - 301.

ALBERT, A.(1954): The pterdines. In L. Zechmeister, Fortschritte der chimie organischer Naturstoffe" Springer- Verlag, Berlin, 351-403.

_____(1955) : Pteridine studies: Part VII. The degradation of 4, -6- and 7- hydroxy pteridine by acid and alkali. J. chem.soc., 1955, 2690-2699.

_____(1957) : Transformation of purines into pteridines. Biochem J., 65, 124- 127.

ANDERSEN, S.O.(1964): The cross-links in resilin identified as di-tyrosine and tri-tyrosine. Biochim. biophys. Acta. 93, 213-215.

_____(1966) : co-valent cross-links in a structural protein, resilin. Acta physiol. Scand. 66 , Suppl. 263.

_____, (1971a): Resilin: In " Comprehensive biochemistry (ed. by Florkin, M and Stotz, E.H. 26 c, 633- 657. Amsterdam: Elsevier.

_____,(1971b) : phenolic compounds isolated from insect hard cuticle and their relationship to the sclerotization process. Insect. Biochem. 1, 157- 170.

_____, (1976): Cuticular enzymes and sclerotization in insects. In " The insect integument"(Edit. by H.R.HEPBURN) Elovier Scientific Publishing co., Amsterdam.

_____, and BARRET, F.M.(1971) : The isolation of Ketocatechols from insect cuticle and their possible role in sclerotization. J. Insect physiol 17, 69-83.

_____, and WEIS-FOGH, T. (1964): Resilin: A rubber-like protein in Arthropod cuticle.

ANSETH , A. (1961): Studies on corneal polysaccharides. III. Topographic and comperative biochemistry. Exp. Eye Res. 1, 106 - 115.

_____, FRANSSON, L.-A.(1970) : The importance of the polysaccharides for the normal function of the corneal stroma. Acta ophthal. 48, 455 - 464.

ASSHURST, D. and COSTIN, N. (1971a): Insect mucosubstances- I. The mucosubstances of developing connective tissue in the locust, Locusta migratoria. Histochem J. 3, 279 - 295.

_____, and COSTIN, N.(1971 b): Insect mucosubstances.-III. The mucosubstances of the central nervous system. Histochem. J. 3, 297 - 310.

_____, and RICHARDS, A. (1964) : The histochemistry of the connective tissue associated with the central nervous system. of the pupa of a moth, Galleria mellonella L. J. Morph. 114 , 237-245.

BACCHUS, H. (1950) : Cytochemical study of the adrenal cortex of the rat under salt-stresses. Am. J.physiol. 163, 326 - 331.

BAILY, K. and WEIS-FOGH,T. (1961): Amino acid composition of a new rubber-like protein, resilin. Biochem. biophys. Acta , 48 , 452 - 459.

BAKER , J.R. (1946) : The histochemical recognition of lipine. Quart. J. microse.Sci. 87,441 -470.

BALAZS, E.A. (1961) : In " The structure of the eye(G.K. smelser, ed). pp. 293-310. Academic Press. New York and London.

_____,(1965): Amino sugar containing macro-molecules in the tissues of the eye and ear. In: The Amino Sugars(eds. E.A.BALAZS, R.W. JEANLOZ), Vol.2A,pp 401 - 460. New York. Academic Press.

BALDWIN, W. and SALTHOUSE,T.(1959): Dermal glands and mucin in the moulting cycle of Rhodnius prolixus stal. J. Insect.Physiol.3 , 345 - 348.

BARNES, M.J. and PARTRIDGE, S.M.(1968): Isolation and characterization of a glycoprotein from human thoracic aorta. Biochem. J. 109, 883 - 896.

BARNETT, S.A. and BOURNE, G. (1941): The distribution of ascorbic acid(Vitamin C) in the early stages of the developing chick embryo. J. Anat. 75 , 251 - 264.

_____, and FISHER, R.B. (1943) : Experiments on the silver nitrate method for the histological demonstration of ascorbic acid(Vitamin C). Brit. J. Exp. Biol. 20 , 14-15.

BARANY , E.H. and LANGHAM , M.E.(1955): Acta. Physiol.Scand. 34, 99 - 115. cited in "Biochemistry of the eye" edit by C.N.GRAYMORE. Academic press. London(1970).

BAUGH , C.M. and SHAW, E.(1963): The role of pyrimidines in the biogenesis of pteridines. Biochem,biophys. Res. commun., 10 , 28 - 33.

BECKER, E. and SCHOPFF, C. (1936): Uber neue pterine. Justus Liebigs Annln.chem., 524, 49 -123.

BELLOWS, J.G. and ROSNER, L. (1936) : Arch.Opth. 16,1001. cited in "Biochemical studies on the vertebrate lens. 1. Quantitative study of L.-ascorbic acid and Glutathione by RAWAL et al. (1977): VIDYA B- Science XX(2) 172-178.

BENNET- CLARK, H.C. (1963): Negative pressures produced in the pharyngeal pump of the blood-sucking bug, Rhodnius prolixus. J. exp. Biol. 40, 223 - 229.

_____, and LUCEY, E.C.A.(1967): The Jump of the flea: a study of the energetics and a model of the mechanism. J. Exp. Biol. 47, 59-76.

BENNIT, R. (1932): Physiological interrelationship in the eyes of decapod crustacea. physiol. Zool. 5, 49 - 69.

BERA, B.C., FOSTER, A.B., and STACEY, M.(1955) J. chem.Soc. 1955, 3788. cited in "Occurrence of Acid mucopolysaccharides in the cuticle of scorpion palamneus swammerdami". Curr.Sci, 34, 4, 120-121.

BERENSON, G.A., RADHAKRISHNAMURTHY, B., FISKIN, H.F. DESSAUER, H., ARQUEMBOURG, P (1966): Individuality of glycoproteins in human aorta. J. Atheroclerosis. Res. 6 , 214 - 223.

BERMAN, E.R.(1969) : Mucopolysaccharides(glycosamine glycans) of the retina: identification, distribution and possible biological roles. Bibl. Ophthalmol No.79 , 5 - 31.

_____, and BACH, G. (1968): The acid mucopolysaccharides of cattle retina. Biochem J. 108, 75-88.

_____, and VOADEN, M.(1970): The vitreous body. In : Biochemistry of the eye(ed. C.N.GRAYMORE),pp. 373 - 471. New York, Academic press.

BERNARD, C.G., MILLER, W.H., and Moller, A.R.(1965): The insect corneal nipple array. A biological, broad-band impedance transformer that acts as an anti-reflection coating. Acta physiol. Scand. 63, (Suppl. 243.).

BERNARD, G.D. (1971): Evidence for visual function of corneal interference filters. J. Insect Physiol. 17, 2287 - 2300.

BONNER, W.D.(1957) : Soluble oxidases and their functions. Ann. Rev. plant Physiol. 8, 427 - 452.

BONTING, S.L. (1970): Sodium Potassium activated adenosine triphosphatase and cation transport. In: Membranes and ion transport, Vol. I.(E.E.Bittae,ed.),pp. 257 - 363. London : Wiley- Interscience 1970.

_____, and BENGHAM, A.D.(1967): On the biochemical mechanism of the visual process. Exptl. Eye Res. 6 , 400 - 413.

_____, CARAVAGIO, L.L., NAOMI M. HAWKINS (1963): Studies on Sodium potassium activated adenosine triphosphatase, VI. Its role in cation transport in the lens of cat, calf and rabbit. Arch.Biochem Biophys. 101, 47 - 55.

_____, CARAVAGIO, LL. and CANDY, M.R.(1964). Sodium-potassium activated adenosine triphosphatase. X. occurrence in retinal rods and relation to rhodopsin.. Exp. Eye. Res. 3 , 47.

BOREL, J.P. , DESANTI, M., CLOGNE, M., BOCHET, C.(1970): Constitution de 6 glycoproteins associ'ees an collagene dans le derme de lapin. C.R.Acad. Sci.(Paris) 270, 2714 - 2716.

BOURNE, G. (1944): Intracellular localization of Vitamin C. Nature 153 , 254 - 255

BOWES, J.H., ELLIOT, R.G., MOSS, J.A. (1958): The extraction of soluble protein from skin by alkaline solutions. In: Recent advances in Gelatin and Glue Research (ed. J.H. Bowes), pp. 71-75. New York: Pergamon Press.

BOWNDS, D., DAWES, J., MILLER, J. and STAHLMAN, M.(1972): Phosphorylation of frog photoreceptor membrane induced by light. Nature, New Biol. 237 , 125 - 127.

BRENNER- HOLZACH, O. and LEUTHARDT, F.(1959): Untersuchungen Zue Biosynthese der pterine bei Drosophila melanogaster. Helv. chim. Acta, 42, 2254 - 2257.

_____, and LEUTHARDT, F; (1961): Untersuchung Uber die Biosynthese der pterine bei Drosophila melanogaster. Helv.chim.Acta, 44 1480 - 1495.

BROWN, F.A., HINES, L.N., and FINGERMAN, M.(1952): Hormonal regulation of the distal retinal pigment of Palacemonetes, Biol. Bull. 102: 212 - 225.

BROWN, H.M., MEECH, R.W., KOIKE, H., HAGIWARA, S(1969): Current-voltage relations during illumination: photoreceptor membrane of a barnacle. Science 166, 240.

BUCHANAN, J.M., and HARTMAN, S.C. (1959): Enzymic reaction in the synthesis of purines. Adv. Enzymol., 21 , 199 - 261.

_____, and SONNE, J.C., and DELLUVA, A.M.(1948): Biological precursors of uric acid.II. The role of lactate, glycine and carbondioxide as precursors of the carbon chain and nitrogen atom 7 of uric acid. J. biol. chem.c173 , 81-98.

_____, LARRABEE, A.R., ROSENTHAL,S. and CANHOU, R.E. (1964): The role of 5 - methyl tetrahydro folate in the enzymatic synthesis of methionine-methyle. In " Pteridine chemistry", (W. pfleiderer and E.C. TAYLOR, eds). Pergamon Press, Oxford,343-373.

BUCK, M.G., and JADUNAISKY, J.A.(1975): Stimulation of ion-transport by ascorbic acid through inhibition of 3: 5' - cyclic AMP Phosphodiesterase in the corneal epithelium and other tissues. Biochemica et Biophysica Acta , 389, 251 - 260.

BUTENANDT, A. and REMBOLD, H. (1958): Über den Weiselzellengutsaft der Honigbiene.II. Isolierung von 2 - Amino - 4 hydroxy - 6 -(1,2,- dihydroxypropylpteridin). Hoppe - Seyler's Z. physiol. chem., 311, 179 - 183.

_____, SCHIEDT, U.,BIEKERT,E., and KORNMAN,P.(1954): Über Ommochrome, I.Mitteilung: Isolierung von xanthommatin, Rhodommatin and Ommatin C aus den schulpfsekreten von Vanessa urticae. Justus Liebigs chem. 586 , 217 - 228.

BUTLER,R.(1971): The identification and mapping of spectral cell types in the retina of periplanata americana. Z. Vergl. physiol.72.67.

CAMERON, M.L. (1953): Secretion of an orthodiphenol in the corpus cardiacum of the insect. Nature, 172, 349-350.

CARLSON, S.D. and PHILIPSON, B. (1972): Micro spectro photometry of the dioptric apparatus and compound rhabdom of the moth (Manduca sexta) eye. J. Insect physiol. 18, 1721 - 1731

CASSIER, (1965): Le compartement phototropique du ciquet migrateur (Locusta migratoria migratorioides R and F): bases sensorielles et endocrines. Annls. Sci nat. (Zool), (12)7,

CHINOY, J.J (1967): Studies on cellular and molecular aspects of floral induction - a review. Indian J. pl. physiol. 11, 202 - 220

_____, (1969): A new concept of flowering on the basis of molecular and submolecular events occuring in shoot apex and the leaf of wheat. Indian J. pl. physiol. 12 , 67-80

_____, GURUMURTI, K., and SINGH, Y.D. (1973): Inductive effect of low does of gamma radiation on the biosynthesis of ascorbic acid and sulfhydril compounds . Indian J. plant physiol. 16 , 42-49

_____, SINGH, Y.D. and GURUMURTI, K. (1971): Effect of water stress on paramagnetic behaviour, ascorbic acid turnover and enzymic activity in Cicer arietinum J. Indian Bot. Soc., 50: 462 - 76

_____, SINGH, Y.D. and GURUMURTI, K (1974): Biosynthesis of ascorbic acid and mobilization pattern of macromolecules during water stress in germinating cicer seedlings. Biologia plant 16 , 301 - 307

_____, SINGH, Y.D. and GURUMURTI, K. (1976): Colourimetric determination of ascorbic acid turnover in plants Indian. J. plant. physiol. XIX, 2. 122 - 130.

CLEMENTS, A.N., and POTTER, S.A. (1967): The fine structure of the spermathicae and their ducts in the mosquito, Aeder aegypti. J. Insect physiol. 13, 1825 - 1836

COLES,G.C.(1966): Studies on resilin biosynthesis. J.Insect physiol. 12, 679 - 691

COHEN, L.H., and NOWELL,W.K.(1960): Glucose catabolism of the rabbit retina before and after development of visual function. J. Newrochem. 5: 253

CRAMMER,J.L.(1948): Paper chromatography of flavin nuclestides. Nature, Lond, 161, 349-350

CRESWELL,R.M.,MAURER,H.K., STRAUSS,T. and BROWN,G.B.(1965): Purine N - oxides.XIV. A total synthesis of a pyrimidine N - oxide, a pteridine 1 - N - oxide, and Xanthine 3 - N - Oxide J.Org.chem.,30, 408 - 410.

CURRAN,R.C.(1964): The histochemistry of mucopolysaccharides Int.rev. cytol. 17.

DAY,M.F.(1941): Pigment migration in the eyes of the moth Ephestia Kuhniella zeller. Biol.Bull,Woods Hole 80, 275 - 291.

_____, (1949): The distribution of ascorbic acid in the tissues of insects. Aust. J. Sci.Res. B 2, 19 - 30

DEMOLL,R.(1911): Uber die wanderung des Irispigments in facellerauge. Zool. Jb.(Physiol). 30 , 169 - 180.

DENNELL,R. and MALEK,S.R.A.(1956): The cuticle of cockroach, periplanata americana. The chemical resistance of impregnating material and the self-tanning of its protein component. Proc. R.Soc. Ser. B 144 , 545 - 556.

DE PONT.J.J. H.H.M. and BONTING,S.L.(1971): Biochemical aspects of the visual process._____ XII. Sodium — potassium —— activated adenosine triphosphatase in the retina of the cuttle fish,(Sepia officinalis).

DESCIMON,H.(1965): Identification de la sepiapterine dans les ailes des pieridae (Lepidoptera rhopalocera) Bull. Soc., chim . biol., 47, 1095.

DETHIER,V.G.(1963): The physiology of insect senses, Methuen, London. and New York.

DEY, SUDIP (1976): Acid mucopolysaccharides in the lens-cuticle of insects. M.Sc. dessertation, North-Eastern Hill University.

_____, RAGHUVARMAN,A., and MICHAEL ,R.G.(1978): Acid mucopolysaccharides in the lens- cuticle of the fresh water prawn, Macrobrachium birmanicum (crustacea) and the marine horse-shoe cral, Limulus polyphemus (Xiphosaura) and their physiological significance.IV All India Symp. on Comp. Animal physiol. and Endocrinol.

DI BENEDETTA,C., BRUNNGRABER,E.G., WHITNEY,G.,BROWN,B.D., ARO,V.(1969): Compositional patterns of sialofuco hexosaminoglycans derived from rat brain glycoproteins. Arch.Biochem. Biophys. 131 , 404 - 413.

DISCHE, Z. (1947): J. biol chem 167, 189. cited in LAVIETES,B.B. (1971): Kinetics of matrix synthesis in cartilage cell cultures. Exp. cell. Res. 68 , 43 - 48.

_____, (1970) : Biochemistry of connective tissue of vertebrate eye. Int. Rev. Conn. Tissue Res. 5 , 209 - 279.

DRUJAN, B.D., and ALI, A.M. (1972) ATP metabolism in light and dark adapted retinae. In VIth Int. congr. Photobiol ., Bochum 1972, Book of Abstracts (Edited by Schenk G.O.)., P. 208.

DUNCAN,G., DE PONT, J.J.H.H.M., BONTING,S.L.(1971): Biochemical aspects of the visual process. X. Distribution and movements of ions in the dark-adapted retina in the cultle fish(Sepia officinalis). Pflugers Arch. 322 , 264.

DUPONT- RAABE, M.(1951): Etude morphologique et cytologique due cerveau de quelques phasmides. Bull.Soc. Zobl. France, 76 , 386 - 397.

_____, (1952): Contribution a l'etude du role encocrine due cerveau et notamment de la pars intercerbralis chez les phasmides. Arch. Zool.exp. gen. 89 , N.R. 128 - 138.

_____, (1956a): Mise en évidence , chez les phasmides, d'une troiseme paire de nervi Corporis cardiaci, Voie possible due cheminement de la substance chromactive tritocerebrate vers les corpora Cardiaca. C.R. Acad. Sci. Paris., 243 , 1240 - 1242.

_____ , (1956b): Quelques donnees relatives aux phenomenes de neurosecretion chez les phasmides. Ann. Sci. Nat. Zool. (11), 18 , 293 - 303.

_____, (1957) : Les mechanismes de l'adaptation chromatoque chez les Insects. Arch. Zool. exp. gen. 94 , 61 - 294.

EGUCHI, E. (1965) : Rhabdom structure and receptor Potentials in single crayfish retinular cells. J.cell. Comp. physiol. 66 , 411.

ENOMOTO, N. , NAKAGAWA, H., MATSUDA, K., and TOMIYASU, Y. (1966): Sialic acids in the external mucous substance and the cartilage of fishes. Bull. Japan Soc. Sci. Fisheries 32, 442 -445.

EPHRUSSI, B., and HEROLD, J.L. (1944): Eye pigments of Drosophila.I. Methods of extraction and quantitative estimation of the pigment components. Genetics, 29 , 148 - 175.

EXNER, S. (1891) : Die physiologic der facettierten Augen von Krebsen und Insekton. Liepzig.

FARBER, S., DIAMOND, L.K., MERCER, R.D., SYLVESTER, R.F.Jr. and WOLFE, J.A. (1948): Temporary remissions in acute leukemia in children produced by Folic acid antagonist , 4 - aminopteroyl glutamic acid (aminopterin). New Engl. J. Med., 238 , 1787-1789.

FARBER, S.J. and SCHUBERT, M.(1957): The binding of cations by chondroitin sulfate. J. Clin. Invest. 36 , 1715 - 1722.

FEARON, W.R. (1946): An introduction to biochemistry. Williams Heinman, London, 1946.

FIDDICK, R. and HEATH, H. (1966): Expl. Eye Res. 5 , 829. cited in " Biochemistry of the eye edit by C.N.GRAYMORE. Academic Press. London.(1970).

FINGERMAN, M.(1957): Regulation of the distal retinal pigments of the dwarf Cray fish Cambarellus shufeldti J.Cell. comp. physiol. 30 ,357-370.

_____,(1959) : Dark-adapting and light-adapting hormones controlling the distal retinal pigment of the prawn, Palaemonetes Vulgaris. Biol.Bull., 116 , 30-36.

_____, NAGABHUSANAM, R., and PHILLPOTT, L.(1962): Photomechanical responses of the proximal pigment in palaemonetes and Orconectes. Ibid. 123, 121-138.

FISKE, C.H., and SUBBAROW, Y.(1925): The colourimetric determination of phosphorus. J. Biol.chem. 66 , 375 - 400.

FORREST, H.S. (1969) : The effect of isoxanthopterin on nucleic acid synthesis in developing eggs of milkweed bug, Oncopeltus fasciatus(Dallas).IVth Int.Congr. Pteridines., Toba, Japan.Abst.P.64.

_____, and Mitchell, H.K. (1954a):Pteridines from Drosophila.II.structure of the yellow pigment.J.Am. chem. Soc. , 76 , 5636 -5658.

_____, and MITCHELL, H.K. (1954b); pteridines from Drosophila.II. Structure of the yellow pigment. J. Am. chem. Soc., 76, 5658 - 5662.

_____, and McNUTT, W.S.(1958) : Isolation of a new pteridine from Eremothecium ashbyu and some observations on its structure. J. Am. chem.Soc., 80 , 739 - 748.

_____, HATFIELD, D. and VAN BAALEN, C. (1959): Charecterization of second yellow compound from Drosophila melanogaster. Nature, Lord., 183 , 1269 - 1270.

_____, HANLY, E.W., and LAGOWSKI, J.M. (1961): Biochemical differences between the mutants rosy-2 and maroon-like of Drosophila melanogaster. Genetics, 46 , 1455 - 1463.

FRANK, R.N. and GOLDSMITH, T.H.(1965): Adenosine triphosphatase activity in the rod outer segments of the pigs retina. Archs Biochem. Biophys. 110 , 517 - 525.

FRIZA, F. (1928): Zur frage der Farbung und Zeichnung des facettierten Insektenauges. Z. vergl.physiol. 8, 289 - 336.

GABE, M. (1966): Neurosecretion-- Translated by R.Crawford. Pergamon Press, Oxford, London. Edinburgh, New York. Toronto. Paris. Brawschweig.

GEMNE, G. (1966): Ultrastructural ontogenesis of Cornea and Corneal nipples in the compound eye of insects. Acta physiol. Scand. 66 , 511 - 512.

_____,(1971): Ontogenesis of Corneal surface ultrastructure in nocturnal Lepidoptera. Phil. Trans. R.Soc. (B) 262 , 343 -363.

GERSH, M., and STURZEBECHER, J.(1970): Experimentelle stimulierung der Zellularen Aktivitat der prothorakaldrusen von periplanata americana durch den Aktivationsfaktor. J.Insect. Physiol. 16, 1813 - 1826.

GIRI, K.V., and NIGAM, V.N.(1954): Circular paper chromatography of Carbohydrates. J.Indian.Inst. Sci. 36 , 49 - 63.

GIROUD, A., and RAKTO-RATSIMAMANGA, A.(1936): Distribution of vitamin C in invertebrates. Bull Soc.Chem. Biol. 18 , 375 - 383.

GLICK, D. and BISKIND , G.R.(1936): Arch.Opth. 16 , 990. Cited in " Biochemical studies on the vertebrate lens 1. Quantitative study of L-ascorbic acid and Glutathione by RAWAL et al. (1977): VIDYA B-Science XX(2) 172 -178.

GOLDSMIDT, M. (1924): Arch. Opth., 113 , 160. Cited in "Biochemical studies on the vertebrate lens.1. Quantitative study of L-ascorbic acid and Glutathione by RAWAL et al. (1977): VIDYA B-Science XX(2), 172 - 178.

GOLDSMITH, T.H. (1958a): The visual system of the honey bee. Proc. nat. Acad. Sci. 44 , 123 - 126.

_____, (1958b): On the visual system of the bee (Apis melligera). Ann. N.Y. Acad. Sci. 74 , 223 - 229.

_____, (1961): Light and Life (Ed.by McELROY, W.D. and GLASS, B) Johns Hopkins Press, Baltimore.

_____, and FARNANDEZ, H.R. (1968): The sensitivity of housefly photoreceptors in the mid ultra violet and the limits of the visible spectrum. J.exp. Biol. 49 , 669 - 677.

_____, and WARNER, L.T. (1964): Vitamin A in the vision of insects. J.Gen. Physiol. 47 , 433-441.

GREGG, T.G. and SMUCKER, L.A.(1965). pteridines and gene homologies in the eye colour mutants of Drosophila hydei and Drosophila melanogaster. Genetics , 52 , 1023 - 1034.

GUPTA, B.L.and SMITH, D.S.(1969): Fine structural organization of the spermatheca in the Cockroach, Periplanata americana. Tissue and Cell 1 , 295 - 324.

GUREVICH, A.A.(1963): Catalytic action of peroxidase in the induced reaction of O-dinitrobenzene by ascorbic acid. Dokl. Biol.Sci.,145 , 674-677

HACKMAN, R.H., and GOLDBERG, M. (1971): Studies on the hardening and darkening of insect cuticle. J.Insect. Physiol. 17 , 335 - 347.

HADORN, E. (1962): Fractionating the fruit fly. Scient. An. 206 , 100 -110.

_____, KUHIN, A. (1953): Chromatographic und fluorometrische underouchungen Zur biochemischen polyphanic Von Augen farb -Genen bei Ephestia Kuhniella. Z. Naturf., 8b , 582 -598.

_____, MITCHELL, H.K.(1951): properties of mutants of Drosophila melanogaster and changes during development as revealed by paper chromatography.

HAGERMAN, D.D.(1964): pteridine cofactors in enzymatic hydrooxylation of steroids. Fed.Proc.Fed.Amm. Soc. 23, 480.

HAGGINS, W.A. (1965): Electrical signs of information flow in photoreceptors. Cold Spring Harbour Symp. on Quantitative Biology, 30 , 404 - 418

_____, and JENNINGS, W.H. (1960): Energy transfer with special reference to biological systems. Disc. Faraday Soc. 27, 180 - 190

_____, ZONANA, H.V. and ADAMS, R.G. (1962): ocal membrane current in the outer segments of squid photoreceptors Nature 194 , 844 - 847

HAMA, T. and OBIKA, M. (1960): Pteridine synthesis in the amphibian neural crest cell. Nature, Lond., 187 , 326 - 327.

_____, MATSUMOTO, J. and OBIKA, M. (1960): Localization of pterins in the goldfish - skin melanophores and erythrophores. Proc. Japan Acad., 36, 346-351

HANDSCHUMACHER, R.E. and WELCH, A.D. (1960): Agents which influence nucleic acid metabolism. In: "The Nucleic Acid" (E.Chargaff and J.N.Davidson, eds). Academic Press, New York and London, 3, 453 - 526.

HASTINGS, A.B. and PANESTIL, D.D. (1963): Effect of CO_2 concentration on Glucose ^{14}C metabolism by rabbit Kidney cortex and medulla in vitro. Biochem . Z, 338, 276 - 282.

HEATH, H. (1962): Expl. Eye Res. 1 , 362 cited in " Biochemistry of The Eye. (edit. C.N.GRAYMORE) Academic Press, London, New York. (1970).

_____, and FIDDICK, R. (1964): Nature. Lond. 202 , 1128 cited in " Biochemistry Of The Eye "(edit. C.N. GRAYMORE) Academic Press. London, New Yourk (1970)

_____, and FIDDICK, R. (1965 a): Biochem J. 94, 114 Cited in " Biochemistry Of The Eye "(edit. C.N. GRAYMORE) Academic Press. London, New York (1970).

_____, and FIDDICK,R.(1965 b): In "Biochemistry of the retina " (C.N.GRAYMORE,ed). P.14 Academic Press. London and New York.

_____, RUTTER, A.C., and BECK,T.C.(1962): Vision Res. 2, 431. cited in " Biochemistry of The Eye (edit. C.N.GRAYMORE) Academi Press, London, New York (1970).

HENDLER,E.D.,TORETTI,J., EPSTEIN,F.H.(1971): The distribution of Sodium —— potassium activated adenosine triphosphatase in the medula and cortex of the kindney. J.clin. Invest. 50,1329

HENKES,H.E.(1946): Opthalmologica, 112(3), 113.Cited in " Biochemical studies on the vertebrate lens. 1. Quantitative study of L - ascorbic acid and Glutathione by RAWAL et al., VIDYAB-Science XX(2) 172 - 178

HERRING,G.M.(1972): The organic matrix of bone. In: The Biochemistry and physiology of bone (ed.E.H. BOURNE) 2nd Ed., Vol. 1, PP.127 - 189.New York: Academic Press.

HESS,C.(1920): Neues Zur Frage nach cinem Farbensinne bei Bienen. Naturwissenschaften 8, 927 - 929

HEYMANN,H., CHAN,F.L., and CLANCY,C.W.(1950): Partition chromatography of red eye pigment of Drosophila melanogaster J. Am. Chem Soc., 72, 1112 - 1117

HAFFMAN,C and LANGER,H.(1961): Dic Spektrale Augenmpfindlichkeit der Mutante " Chalky " Von calliphora erythrocephala. Naturwissenschaften 48,605

HOME,E.M.(1973): Comparative ultrastructure of certain insect eyes. D. Phil. Thesis, University of Oxford.

HOPKINS,F.G.(1889): Note on a yellow pigment in butterflies. Nature, Lond., 40 , 335

_____, (1891):Pigment in yellow butterflies. Nature, Lond., 45 , 197

_____, (1895): The pigments of the pieridae; a contribution to the study of excretory substances which function in ornament. Phil. Trans. R.Soc., 186 B, 661 - 682

HORRIDGE,G.A.(1969): The eye of the firefly, Photuris. Proc. R.Soc. (B) 171 , 445 - 463

HORSTMANN,E. (1935): Die tagesperiodeschen pigmentwanderungen in Facettenauges von Nachschmetterlingen. Biol.Zbl. 55, 93 - 97.

HUBBY,J.L. and THROCKMORTON,H.(1960): Evolution and pterine metabolism in the genus Drosophila Proc. natn. Acad. Sci., U.S.A., 46 , 65 - 78.

HUGHES,R.E., HURLEY,R.J., and JONES,P.R.(1971): Retention of ascorbic acid by the Guinea pig eye lens. Exp. Eye Res. 12 , 39 - 43

HUNT,S., GRANT,M.E., LIEBOVICH,S.J.(1970): Polymeric collagen isolated from squid (Loligo peallii) connective tissue. Experientia, 26, 1204 - 1205

HUXLEY,A.F.(1968): A theoretical treatment of reflection of light by multilayer structures.J.Exp. Biol. 48,227 - 245

IMMS,A.D.(1957): Text book of Entomology,Methuen,London.

ISHERWOOD,F.A. and MAPSON,L.W.(1962): Ascorbic acid metabolism in plants. Part II. Biosynthesis Ann. Rev. plant physiol., 13, 329 - 350.

JAENICKE,L. (1961): Die Folsaure in stoffwechsel der Einkohlenstoff - Einheiten. Angew.Chem., 73, 449 - 460

JAHN,T.J. and WULFF,V.J.(1941): Retinal pigment distribution in relation to a diurnal rhythn in the compound eyes of Dytiscus. Proc. Soc. exp. Biol. Med. 48, 656 - 660

JANADO,M. and DUNSTONE,J.(1972): A glycoprotein from bovine nasal cartilage. J. Biochem.(Tokyo) 71 , 635 - 643

JEANLOZ,R.W (1970): Mucopolysaccharides of higher animals In : The Carbohydrates (eds.W. PIGMAN,D.HORTON, A. HERP), Vol 2B, PP. 589 - 625. New York: Academic Press.

JENSEN, M. and WEIS-FOGH , T. (1962): Biology and Physics of locust flight. V. Strength and elasticity of locust cuticle . Phil, Trans. B 245 , 137 - 169

JOHNSON,S.W. (1936): Biochem. J. 30, 1430 - 1437. Cited in " Biochemistry of the eye (edit C.N. GRAYMORE. Academic Press. London. New York(1970).

JOLY,P. (1940): Recherche, Par voice chimique, des principales vitamines et hormones Cetoniques dans le sang de reine de Bellicositermes n natalensis (Hav) CR.Soc. Biol., 134,408-410.

KARRER,P. (1950): " Organic Chemistry ". Elsevier Publ.Co., New York.

KASBEKAR,D.K. and DURBIN,R.P.(1965): An adenosine triphosphatase from frog gastric mucosa. Biochem. biophys. Acta 105, 472 - 482

KATCHALSKY,A.(1964): Polyelectrolytes and their biological interactions. Biophys. J. 4 , 9 - 41.

KATZ,A.I. and EPSTEIN,F.H.(1967): The role of Sodium Potassium activated adenosine triphosphatase in the reabsorption of Sodium by the Kidney .J. clin. Invest. 46 , 1999

KAUFMANN,S. (1957): The enzymatic conversion of phenylalanine to tyrosine. J. biol. chem. 226, 511 - 524

_____, (1958): Participation of tetrahydrofolic acid in the enzymic conversion of Phenylalanine to tyrosine. Biochim. biophys. Acta, 27, 428 -429

_____,(1959): Studies on the mechanism of enzymatic conversion of phenylalanine to tyrosine .J.biol. chem. 234, 2677 - 2682

_____, (1961): The nature of the primary Oxidation product formed from tetrahydropteridines during phenylalanine hydroxylation. J. biol chem., 236, 804 - 810

_____, (1962): On the structure of phenylalanine h hydroxylation cofactor. J.bil.chem., 239 PC 2712 - 2713.

_____, (1963): The structure of the Phenyl alanine hydroxylating cofactor. Proc. natn. Acad. Sci., U.S.A., 50 , 1085 - 1093

_____, (1964): Studies on the structure of the primary oxidation product formed from tetrahydro pteridines during phenylalanine hydroxylation J.biol. chem., 239

_____, (1967): pteridine cofactors. In " Annual Review of Biochemistry" (P.D. Boyer,A. Meister, R.L. Sinsheimer and E.E.Snell eds.) Annual Review , Inc., Palo Alto, California, 36, 171 - 184

KAY, R.E. (1969): Fluorescent materials in insect eyes, and their possible relationship to ultraviolet sensitivity. J. Insect Physiol. 15, 2021-2038.

KEELEY, F.W., LA BELLA, F., QUEEN, G. (1969): Dityrosine in a non- hydroxyproline, alkali - soluble protein isolated from chick aorta and bovine ligament. Biochem. Res. commun. 34 , 156-161

KENDREW, J.C. (1954): Structure proteins. I. In "The proteins, chemistry, Biological activity, and Methods" (H. Neurath and K Bailey, eds.) 2, B, PP. 845 - 950. Academic Press. New York.

KIDDER, G., and DEWEY, V.C. (1968): A new pteridine from Tetrahymena. J.biol. chem. 243 , 826 -833

KINNE-SAFFRAN, E. and KINNE, R.(1974): Presence of bicarbonate stimulated ATPase in the brush border microvillus membranes of the proximal tubule. Proc. Soc. exp. Biol.(N.Y.). 146, 751 - 753.

KINOSHITA,J.H.(1964): Arch.Opth. 72, 554. Cited in "Biochemical studies on the vertebrate lens. 1. Quantitative study of L-ascorbic acid and Glutathione by U.M.BAWAL and G.N.RAO,(1977); VIDYA B SCIENCE XX(2);172 - 178.

KINSEY, V.E.(1947): Am. J. Opthal. 30 , 1262-1266. Cited in "Biochemistry of the eye" (edit. C.N.GRAYMORE) Academic press. London. New York.(1970).

_____, and JACKSON,B.(1949): Am. J. Opthal. 32 , 370 - 378. Cited in " Biochemistry of the eye" (edit. C.N.GRAYMORE) Academic Press. London. New York (1970).

KIRSCHFELD, K and FRANCESCHINI, N.(1969): Kybernetic, 6, 13 - 22. Cited in "Visual adaptation in butterflies". by D.G.STAVENGA, Natare , 254 435 - 437.

KLEINHOL Z, L.H.(1936): Crustacean eye stalk hormones and retinal pigment migration. Biol. Bull. 70 , 159 - 184.

KOBAYASHI, T.K. and PEDRINI, V. (1973) : proteoglycans-collagen interactions in human costal cartilage. Biochem . Biophys. Acta 303 , 148 - 160.

KOFOED, J.A. and ROBERTSON, W.B.(1966): Biochem biophys. acta 124 , 86. Cited in LAVIETES, B.B. : Kinetics of Matrix synthesis in cartilage cell cultures. Exp. Cell. Res. 68, 43 - 48.

KRISHNAN, G.(1970): chemical nature of the cuticle and its mode of hardening in Roperipatus weldoni Acta histochem. 37 , 1 -17.

KRISHNAN, N.(1965) : Occurrence of acid mucopolysaccharides in the cuticle of scorpion Palamneus swammerdami. " Curr. Sci." 34 , 4, 120-121.

KUCK, J.F.R., Jr. (1961): Archs opthal., N.Y. 65, 840. cited in " Biochemistry of the eye" (edit. C.N.GRAYMORE). Academic Press. London, 1970.

KUHN, A. and EGELHAAF, A. (1959): Der rote Augenfarbstoff von Ephestia Kuhniella und ptychopoda ein Pterinpigment. Z.Naturf., 14b, 654 - 659.

KUHN, H, and DREYER, W.J.(1972): Light dependent Phosphorylation of rhodopsin by ATP. FEBS Lelt, 20 , 1-6.

LANGER, H. (1960 a) : Uber den chemischen Aufban des facet-tenauges von Calliphora erythrocephala Meig. und scine veranderungen mit dem Imaginalatter. Z. vergl. physiol. 42, 595-626

_____, (1960b): Die wirkung von Licht auf den chemischen Grundaufbau des Auges von Calliphora erythrocephala. Meig., J.Insect physiol., 4 , 283 -303.

_____, (1962) : Untersuchungen uber die Grobe des stoffwechsels isolierter Augen von Calliphora erythrocephala Meig. Biol. Zbl. 81, 691-720.

_____, (1963) : The phosphate metabolism of the faceted in light and darkness. Proc. XVIth Internat. congr. Zool. 2 , 37.

_____, (1964) : Der phosphatstoffwechsel des facet tenauges im Dunkeln und im Licht. Helgolander Wiss. Meeresunters. 9, 251 - 2.

_____, (1974) : Aktivitaten von ATPasen im Fliegen-Auge. In 25 coll. Ges. Biol.chem. Mosbach 1974 (Edited by Jaenicke L). pp. 29-32. Springer, Berlin.

_____, and THORELL, B (1966): The functional organization of the compound eye. (Ed. by BERNHARD, C.G.) Pergamon Press, Oxford.

LANGHAM, M.E. (1950) : J. Physiol., Lond. 111 , 388-393. Cited in "Biochemistry of the eye (edit. by C.N.GRAYMORE) Academic Press. London. New York (1970).

LAVIETES, B.B. (1971): Kinetics of matrix synthesis in Cartilage cell-cultures. Exp. Cell. Res. 68 , 43 - 48

LEWIN, S. (1973): The ascorbate system and the formation of adenosine 3' , 5' - cyclic mono phosphate. Biochem. Soc. Trans., 2 , 400 - 403.

LILLIE, R.D. (1954): Histopathologic technique and Practical histochemistry. Mc Graw Hill Book company, New York- Toronto- Sydney - London.

LLOYD, D.J. and GARROD, M (1946): The rubberlike condition of the fibres of animal skin. Fibrous Proteins. In " The Society of Dyers and colourists Symposium". P. 24 - 29.

LOCKE, M. (1969): The localization of a peroxidase associated with hard cuticle formation in an insect, Calpodes ethlius stoll. (Lepidoptera: Hesperidae). Tissue and cell, 1 555 - 574.

LOWRY. O.H., BESSEY, O.A. and CRAWFORD, E.J. (1949): Photolytic and enzymatic transformations of pteroul-glutamic acid. J. biol. chem., 180 , 389 -398.

_____, ROSEBROUGH, N.J., FARRA, L. and RANDAL, T.J. (1951): Protein measurement with the Folin-phenol reagent. J. biol. chem. 198, 265 - 275-

MALLORY, F.B. (1938) Pathological techniques, Saunders, Philadelphia.

MARGOLIS, R.K., and MARGOLIS, R.U.(1970): Sulfated glycoproteins from rat brain glycoproteins. Biochem. 9 , 4389 - 4396.

MARSHALL, A. (1966a): Histochemical studies on a mucocomplex in the malpighian tubules cercopoid larvae. J.Insect physiol. 12, 925 - 932.

MASUDA, T. (1955) : The formation of flavine adenine denucleotide in the culture of Eremothecium ashbyii . Pharm.Bull., Tokyo , 3, 434.

_____(1956a): chem. pharm. Bull., Tokyo, 3 , 434. Cited in " Isolation, and Separation of pteridines"- MATSUMOTO et al , Exp. In Physiol. and Biochem., 4 , 289 - 363(1971).

_____, (1956b) : Isolation of some new substances produced by Eremothecium ashbyii. Pharm. Bull., Tokyo, 4 , 72.

_____, KISHI, T. and ASAI, M (1957): Application the chromatography. XXXIII V- compound isolated from the mycelium of Eremothecium ashbyii. Pharm. Bull., Tokyo, 5, 598

_____, KISHI, T., ASAI, M., and KUWADA, S.(1959): Application of chromatography. XXXV. Biochemical significance of 6-methyl-7-hydroxyribolumazine in the culture of Eremothecium ashbyii. chem. Pharm. Bull. Tokyo., 7 , 361

MATHEWS, M.B. (1959): Macromolecular properties of isomeric chondroitin sulfates. Biochim. biophys. Acta, 35 , 9-17.

_____, (1975): Connective Tissue-Macromolecular structure and Evolution. Springer- Verlag Berlin. Heidelberg. New York.

MATSUBARA, M., KATOH, S., AKINO, M and KAUFMAN, S, (1966): Sepiapterin reductase. Biochim. biophys. Acta., 122, 202 - 212.

MATSUMOTO, J. (1965a): Role of pteridines in the pigmentation of chromatophores in cyprinid fish. Jap. J. Zool., 14, 45-91.

_____, BAGNARA J.T., and TAYLOR, J.D.(1971): Isolation and Separation of pteridines from Animals. Exp. in physiol. and Biochem. 4 , 289- 363.

MAURICE, D.M.(1969) : The cornea and sclera. In: The Eye (ed. H.Davson), 2nd Ed., Vol. 1., pp. 489-600. New York: Academic Press.

Mc CULLAGH, K.G., DEROUETTE, S., ROBERT, L.(1973): Elephant aortic tissue.II. Amino acid analysis, structural glycoproteins, and anti genicity. Exp. Mol.Pathol.18, 202- 213.

Mc NUTT, W.S.(1964): The enzymatic conversion of pteridines into xanthine-8-carboxylic acid. In: " Pteridine chemistry"(W.pfleiderer and E.C.Taylor,eds). Pergamon Press, Oxford, 427-441.

MEENAKSHI, V.R., and SCHEER, B.T.(1959):Acid mucopolysaccharides of the crustacean cuticle. Science, 130 , 1189-1190.

MERKER, E.(1929): Die Fluoreszenz in Insekten auge, die floreszenz des chitins der insekten und seine DurchlassigKeit fur ultraviolettes Licht. Zool. Jb. (Physiol) 46,483 - 574.

METCALF, R.L.(1943): The storage and interaction of water soluble vitamins in the malpighian system of pteriplanata americana (L). Arch Biochem. 2, 55 - 62.

MILLER, W.H. and CAWTHON, D.F.(1974): Pigment granule movement in Limulus photoreceptors. Invest. opthalmol. 13 , 5, 401 - 405.

MOAT, A.G. and FRIEDMAN, H(1960): The biosunthesis and interconversion of purines and their derivatives. Bact. Rev., 24 , 309 - 339.

MULLER, JOHANNES, (1829): Cited in WIGLESWORTH, V.B.: The principles of Insect Physiology. English Language book Society and Methuen & Co. Ltd. pp. 188-(1965).

MULLER, H.K. and BUSCHKE, W.(1934): Arch. Augenheilk. 108 , 368 - 390. Cited in "Biochemistry of the eye"(edit. C.N.GRAYMORE) Academic Press. London. New York.(1970).

MUSTAFA, M. and KAMAT,D(1970): Mucopolysaccharide histochemistry of Musca domestica -1. A report on the occurrence of a new type of KOH-labile alcianophila. Histochemie 21, 54 - 63.

NAGABHUSANAM,R.and SAROJINI,R.(1970): Physiology of retinal pigments in the compound eye of Diogenes bicristimanus, Broteria- Ciencias Naturals. XXXIX(LXVI) Numero 1-2; 87 -108.

NAWA, S.(1960): The structure of the yellow pigment from Drosophila. Bull.chem. Soc. Japan. 33, 1555 - 1560.

NEVILLE, A.C.(1963 b): Daily growth layers in locust rubber-like cuticle, influenced by an external rhythm. J. Insect. physiol. 9 , 177 -186.

_____, (1970): Cuticle ultra-structure in relation to the whole insect. Symp. R. ent. Soc. Lond. 5, 17 - 39.

_____, (1975): Biology of Arthropod cuticle. Springer-verlag. Berlin - Heidelberg-New York.

_____, and LUKE, B.M. (1971): Form optical activity in crustacean cuticle. J.Insect. Physiol, 17 , 519-526.

_____, and ROTHSCHILD, M.(1967): Fleas-insects which fly with their legs. Proc. R.ent. Soc. Lond.(c) 32, 9-10.

NOLTE, D.J. (1952): The eye pigmentary system of Drospphila. III. The action of eye color genes. J.Genet .., 51 , 142 - 186.

OBIKA, M.(1963): Association of pteridines with amphibian larval pigmentation and their bio-synthesis in developing chromatophores. Devl. Biol. , 6 , 99 - 112.

O' FARRELL, A.F.(1964): On physiological colour change in some Australian odonata. J.ent.Soc. Aust. 1, 5-12.

OGSTON, A.G.(1966 a) : On water binding. Federation Proc. 25 , 986 - 989.

_____, (1970): The biological function of the glycosaminoglycans, In: Chemistry and Molecular Biology of the intercellular matrix. (ed. E.A. BALAZS), Vol.3., pp- 1231-1240. New York. Academic Press.

_____, and WELLS, J.D.(1972): The osmotic properties of sulfoethyl-sephadex. A model for cartilage. Biochem. J. 128 , 685-690.

OOSAWA, F.(1971): Polyelectrolytes. New York. Dekker.

PANTIN, G.F.A.(1948): Microscopical techniques for Soologists. Cambridge University Press, London.

PARKER, G.H.(1932): The movements of retinal pigment. Ergebn. Biol. 9 , 239 -291.

PEARSE, A.G.E.(1968): Histochemistry: Theoretical and applied. Vol. 1, J & churchill Ltd., London.

PFLEIDERER, W.(1963): Pterorhodin, ein neus naturliches schmetterlingspigment. Z. Naturf., 18 b , 420.

_____, and RUKWIED, M. (1961): Pteridine, XIII. Zur struktur des Leukopterins. Chem. Ber., 94 , 118 - 124.

_____, LOHRMANN, R., REISSER, F. and SOLL, D.(1964): Synthese und struktur von : pteridine-GlyKosiden und ihre mogliche biologische Bedeutung. In "pteridine chemistry" (W.Pfleiderer and E.C. TAYLOR, eds). Pergamon Press. Oxford., P. 87 - 108.

PIRIE, A.(1946): Biochem. J. 40 , 96. Cited in "Biochemistry of the Eye"(C.N.GRAYMORE, eds). Academic Press. London.New York(1970).

PODESTA, H.H. and BAUCKE, J.(1938): Albercht V. Graefes Arch. Ophthal. 139 , 720 -731. Cited in "Biochemistry of the Eye(edit. by C.N.GRAYMORE) Academic Press. London, New York. (1970).

PURECELL, E.F., LERNER, L.H., and KINSEY, V.E.(1964): Archs. Ophthal., N.Y. 51 , 1-6. Cited in " Biochemistry of the eye"(edit. by C.N. GRAYMORE) Academic Press. London. New York.(1970).

PURRMAN, R. (1940a): Uber die Flugelpigmente der Schmetterlinge. VII. Synthese des Leukopterins und natur des Guanopterins. Justus Liebigs Annln. chem., 182 - 190.

_____, (1940 b): Uber die Flugelpigmente der Schmetterlinge. X. Die Synthese des Xanthopterins. Justus Liebigs Annln.chem., 546, 98 -102.

_____ , (1941) : Konstitution und Synthese des sogenannten Anhydroleukopterins. Justus Liebigs Annln. chem. , 548 , 284-292.

_____, (1943) : Die Farbstoffe der Schmetterlinge. Angew chem., 56 , 258 - 258.

_____ , and EULITZ, F. (1948): Uber die Flugelpigmente der Schmetterlinge. XVI. Zur Kenntins des Erythropterins. Justus Liebigs Annln.chem., 559 , 186 -199.

RACKER, E. (1962) : Adenosine triphosphatase and Oxidative phosphorylation. Fed. Proc. 21 , 54.

RADHAKRISHNAKURTHY, B. and BERENSON, G.S.(1973): Structures of glycopeptides from a glycoprotein from bovine aorta. J. biol. chem. 248, 2000 - 2006.

RAUEN, H.M. and STAMM, C.V.(1952): Beitrage Zur Analyse der pterine. Hoppe-Seyler's Z. physiol. chem., 289 , 201 - 210.

RAVEN, D.J., EARLAND, C. and LITTLE, M.(1971): Occurrence of dityrosine in Tussah silk fibroin and Keratin. Biochem biophys. Acta, 251 , 96 -99.

RAWAL, U.M. and RAO, G.N. (1977): Biochemical studies on the vertebrate lens. 1. Quantitative study of L-ascorbic acid and Glutathione. Vidya B -Sciences XX(2), 172 - 178.

RASMUSSEN, I.E. (1954): Differenze sistematiche e biochemiche nel genere Drosophila. Boll. Zool 21, 339 - 343.

REMBOLD, H.(1961): Zum pteridin-stoffwechsel der Taufliege, Drosophila Melanogaster. Angew chem. 73 , 543 - 544.

RICE, M.J. (1970): Function of resilin in Tse tse fly feeding mechanism. Nature, Lond. 228 , 1337 - 1338.

RICHARDS, A.G.(1951): " The integument of arthropods" University of Minnesota Press. Minneapolis.

RICHARDS, C.M. and BAGNARA, J.T. (1967) Expression of specific pteridines in neural crest transplants between pleurodeles and Axolotl. Dev. Biol. , 15, 334 - 347.

RILLEY, M.V. (1964): The effects Sodium ions on glucose metabolism of ciliary body and retina In: Graymore, C.N., editor, Biochemistry of the retina, New York, Academic Press., Inc. pp 149 - 154.

RIVERA, M.E. (1974): The ATPase system in the compound eye of the blowfly, Calliphora erythrocephala (Meig). comp. Biochem. physiol., 52 B. 227 - 234.

ROBERT, A.M. ROBERT,B., and ROBERT,L(1970): Chemical and physical properties of structural glycoproteins. In: chemistry and Molecular Biology of intercellular matrix (ed. E.A. BALAZS),pp. 237 -24. New York. Academic Press.

ROSNER,L., FARNER, C.J. and BELLOWS, J.G.(1938): Arch. Opth. 20 , 417. Cited in "Biochemical studies on the vertebrate lens.1. Quantitative studies of L-ascorbic acid and Glutathion: RAWAL et al.(1977): VIDYA B- Science XX(2), 172-178.

RUBIN, P.S. and HOWARD, J.R.(1950): Conf. on Metabolic interattions, Trans, 2nd conf. col.2., p. 155.

SANNASI, A.(1969): Ph.D. Thesis, Madras University, Madras.

_____ (1970) Resilin in the lens_cuticle of the firefly, Photinus pyralis Linnaeus. Experientia , 26 , 154.

SCARPELLI, D.G. and GRAID, E.L.(1963) : The fine localization of nucleoside triphosphatase. J. Cell Biol. 17 , 279 -288.

SCHARRER, B. (1959) : The role of neurosecretion in neuroendocrine integration. Comparative Endocrinology(A.GORBMAN Ed.) PP.134-148. Wiley, New York.

SCHMIDI, G.H. (1969): Photo active substances in different predisposed eggs and young larvae of Formica-Polycena Foerster. IVth Int. Congr. Pteridines., Toba, Japan., Abstr.P.76.

_____, and VISCONTINI, M.(1962): Fluoreszierende stoffe aus roten Waldameisen der Gattung Formica(Ins.,Hym). Helv. chim.Acta., 45, 1571 - 1575.

SCHMIDI, U: and DUBACH, U.C.(1971): Na-K-stimulated adenosine triphosphatase: Intracellular localization within proximal tubule of the rat nephron. pfluegers. Arch. Eur. J. physiol. 330, 265.

SCHOPF, C., and BECKER, E. (1933) : Uber das vorkkommen der pterine in wespen und schmetterlinger und uber eigige neue Beobachtungen am Leukopterin und xanthopterin. Justus Liebigs Annln chem., 507 ,266 - 296.

_____, and BECKER,E. (1936): Uber neue pterine. Justus Liebigs Adnln.chem., 524, 49-123.

_____, and REICHERI, R. (1941(: Zur Kenntnis des Leukopterins. Justus Liebigs Annln chem., 74 , 153.

SCOTT, J.A.(1970): Resilin in the sound organs of pyralidae and cicadidae(Lepidoptera: Homoptera) Pan-Pacif. Ent. 46 , 225 -231.

SEKOGUTI, Y.(1960): On the ATPase activities in the retina and rod outer segments. J.Cell. comp. physiol. 56 , 129 - 136.

SHELANSKI, M.L. and TAYLOR, E.W.(1968): Properties of the protein subunit of central pair and outer doublet microtubules of sea urchin flagella. J.Cell Biol., 38A, 304.

SKOU, J.C. (1965): Enzymatic basis for active transport of of Na^+ and K^+ across cell membrane. Physiol. Rev. 45 , 596 - 617

_____, (1972): The relationship of the ($Na^+ + K^+$) activated enzyme system to transport of Sodium and potassium across the cell membrane. Bioenergetics, 4 , 203.

SMYTH, J.D., BINGLEY, W.J., and HILL, G.R. (1944): The distribution of vitamin C in Myetotherus cordiformis Ehrenberg, Opisthroglyphe ranae J. Exp. Biol. 21, 13 - 16

SRINIVASAN, M.V., BERNARD, G.D. and STAVENGA, D.G. (1977): Effects of temperature on the pupillary respons e of the butterfly eye. Biol. Bull. 153, 2, 445-446

STACKHOUSE, H.L. (1966): Some aspects of pteridine biosynthesis in amphibians. Comp. Biochem. Physiol., 17, 219 - 235.

STAVENGA, D.G. (1974): J. Comp. physiol. 91, 417 - 426. Cited in " Visual adaptation in butterflies " by D.G. STAVENGA: Nature , 254 , 5499, 435 - 437

_____, (1977): Optic of compound eyes and circadian pigment movements studied by pseudopupil observations in vivo. BIOL. BULL. 153, 2, 446

STIEVE, H. (1965): Interpretation of the generator potential in terms of ionic processes. Cold. Spr. Harb. Symp. Quant. Biol. 30 , 451

STUART, A. and WOOD, H.C.S. (1962): The biosynthesis of xanthopterin. Proc. chem. Soc., 1962 , 151 - 152

SUGIURA, K., and GOTO, M (1968): Biosynthesis of pteridines in the skin of the tadpole, Rana catesbeiana. J. Biochem., Tokyo, 64 , 657 - 666

SUNDARA RAJULU, G. (1969): Presence of acid mucopolysaccharide in the cuticle of a milliped Cingalobolus bugnioni. Curr. Sci. 38, 345 - 346

_____. (1971): Presence of resilin in the cuticle of the centiped Scolopendra morsitans L. Indian J. Exp. Biol. 9 , 122.

SUZUKU, K. (1962): polyphenolic derivatives in rhabdomeres of the compound Eye. Nature 191, 994

SUZUKU, M. (1974): Structural proteins in the egg envelopes of dragonflies, Sympetrum infuscatum and S. frequens. Insect. Biochem. 4, 99 - 111

SWAMY, B.G.L.and SRINIVASAYA, M. (1942): Studies in insect nutrition. Symptomatology of avitaminosis in Corcyra Caephalonica Staint. A histoligical study. Curr. Sci. 11, 147 - 148

SZENT GYORGYI, A. (1960): Introduction to a submslecular Biology ". Academic Press. New York.

TAIRA, T. (1961): Enzymatic reduction of the yellow pigment of Drosophila. Nature, Lond., 189, 231 - 232.

TARTTAR, A. (1940): Harnsaure und Hypoxanthinals pigmentbestandteile der Flugel von ptieriden. Hoppe - Scyler's Z. physiol. chem., 266, 130

THURM, U. (1963): Die Begeihungen Zwischen mechanischen Reizgrossen und stationaren Erregungszustanden bei Borstenfeld - Sensillen Von Bienen. Z. Verql. Physiol. 46, 351 - 382

_____(1964): Mechanoreceptors in the cuticle of the honey bee: fine structure and stimulus mechanism. Science, Wash. 145, 1063 - 1065

TIETZ, A.M., LINDBERG, M. and KENNEDY, E.P. (1963): A new enzyme system requiring a pteridine cofactor. Fedn Proc. Fedn Am. Socs exp. Biol., 22, 296

TIMPL, R., WOLFF, I., WEISER, M. (1969): Acidic structural proteins of connective tissue. I. Solubilization and preliminary chemical charecterization. Biochim. Biophys. Acta , 194, 112 - 120.

TRAVIS, D.F. (1963): Structural features of mineralization from tissues to macromolecular levels of organization in the decapod crustacea Ann. N.Y. Acad. Sci. 109, 117 - 245.

TREVELYAN, W.E., PROCTER, D.P., and HARRISON, J.S. (1950): Detection of sugars on paper chromatograms Nature, Lond. 166 , 444 - 445.

TRUJILLOCENOZ, O. and BERNARD, G.D. (1972): Some aspects of the retinal organization of Sympycnus lineatus Loew (Diptera, Dolichopodidae). J. ultrastruct Res. 38 , 149 - 160

TUURALA, O. (1934): Histologische und physioligische untersuchungen uber die Photomechanischen Erscheimungen in den Augen der Lepidopteran. Suomal Tiedeakat. Toim. (A) 24, 1 - 69

UCHIDA, H. (1934): Colour changes in the eye of a long - horned grasshopper, Homorocoryphus linessaus in relation to light. J. Fac. Sci. Tokyo Univ. 3 , 517 - 525.

UMEBACHI, Y. (1962): Yellow pigments in the wings of the papilionid butterflies. VII. consideration of the nature and distribution of the wing pigment of the papilionid butterflies.

URIST, M.R., SPEER, D.P., IBSEN, K.J., STRATES, B.S. (1968): Calcium binding by chondroitin sulfate. Calcif. Tissue Res. 2 , 253 - 261.

UYEDA, K. and RABINOWITZ, J.C. (1963): Fluorescence properties of tetrahydrofolate and related compounds. Analyt. Biochem., 6, 100 - 108.

VADGAMA, M. and KAMAT, D. (1971): The occurrence of hyaluronic acid and / or hyaluronidase labile substances in the salivary gland cells of insects with diverse feeding habits. Acta Histochem. 40 108 - 115.

VERON, J.E.N. (1973): The physiological control of the chromatophores of Austrolestes annulosus (Odonata). J. Insect. Physiol., 19, 1689 - 1703.

_____, (1974): physiological colour changes in odonata eyes. A comparison between eye and epidermal chromatophore pigment migrations. J. Insect. physiol., 20, 1491 - 1505

VIEIRA, E., and SHAW, E. (1961): The utilization of purines in the biosynthesis of folic acid. J. biol. chem. 236 , 2507 - 2510.

VISCONTINI, M., SCHOLLER, M., LOESER, E., KARRER, P. and HADORN, E (1955b) Isolierung fluoreszierender stoffe ans Drosophila melanogaster. Helv. chim. Acta., 38, 1222 - 1224.

_____, HADORN, E., and KARRER, P. (1957): Fluoreszierende Stoffe aus Drosophila melanogaster: die roten Augenfarbstoffe. Helv. chim. Acta., 40, 579-585

_____, and MOHLMAN, E. (1959a): Fluoreszierende stoffe aus Drosophila melanogaster. 12. Mitt. Die gelb fluoreszierenden pterine: Sepiapterin and isosepiapterin. Helv. chim. Acta, 42, 836 - 841.

_____, and STIERLIN, H. (1961): Isolierung Struktur und synthese von pterinen aus Ephestia Kuhniella ZELLER. Helv. chim. Acta 44, 1783-1785

_____, and STIERLIN, H, (1962): Fluoreszierende stoffe aus Ephestia Kuhniella ZELLER. 3. Mitt. Isolierung und strukturen von Erythropterin, Ekapterin und Lepidopterin. Helv. chim. Acta 45, 2479 - 2487

_____ , and STIERLIN, H., (1963): Fluoreszierende stoffe aus Ephestia Kuhniella ZELLER. Synthese von Erythropterin, Ekapterin und Lepidopterin. Helv. chim. Acta., 46, 51 - 56

VITTUR, F., PUGLIARELLO, M.C., DE BERNARD, B. (1972a): Some properties of proteoglycans of pre-ossesus cartilage. Biochim. Biophys. Acta. 257 389 - 397.

_____, PUGLIARELLO, M.C., DE BERNAD, B. (1972b): The calcium binding properties of a glycoprotein isolated from pre-ossesus cartilage. Biochem. Biophys. Res. Commun. 48, 143 - 152.

VLADESCO, R. and STEFANESCO, H. (1939): C.r Seame. Soc. Biol. 132, 169 - 171. Cited in "Biochemistry of the eye (edit. C.N. GRAYMORE) Academic Press. London (1970).

WAGH, P.V. and ROBERTS, B.I. (1972): Purification and charecterization of a glycoprotein from the intimal region of procine aorta. Biochemistry 11, 4222 - 4227.

WALCOT, B. (1969): Movement of retinula cells in insect eyes on light adaptation. Nature, Lond. 223, 971 - 972.

_____, (1971a): Cell movement on light adaptation in the retina of Lethocerus. (Belostomatidae Hemiptera). Z. vergl. physiol. 74, 1

_____, (1974): Anatomical changes during light-adaptation in insect compound eyes, in: The compound eye and vision in insects, Horidge, G.A. editor. New York., Oxford University Press.

WALTHER, J.B. and DODT, E. (1959): Die Spektralsensitivitat von insektenkomplexaugen in ultraviolet bis 290 mμ. Z. Naturf. 14b, 273 - 278.

WATERMAN, T.H. (1966): Systems analysis and the visual orientation of animals. Am. Scient. 54 15 - 45.

WEBER, K.M. and SCHORATH, G. (1971): Histochemische untersuchungen am Auge der SchmeiBfleige Calliphora erythrocephala. Meig.1. Mitteilung: ATPase, saure phosphatase, alkalische phosphatase und Glucose -6- phosphatase. Hischemie, 28, 137-144

WEIS-FOGH, T. (1960): Resilin: A rubber - like protein in Arthropod cuticle. Adv. Insect. Physiol. 2, 1 - 65

_____, (1961a): Thermodynamic properties of resilin, a rubber-like protein. J. Molecul. Biol. 3, 520 - 531.

_____, (1961b): Molecular interpretation of the elasticity of resilin, a rubber-like protein. J. Molecul. Biol. 3, 648 - 667

WELLS, J.D. (1973b): Salt activity and osmotic pressure in connective tissue .I. A study of solutions of dextran sulfate as a model system. Proc. Roy. Soc., 183 SeriesB, 399 - 419.

WELSH, J.H. (1939): The action of eye-stalk extracts on retinal pigment migration in the crayfish Cambarus bartoni. Biol. Bull. 77, 119-125.

WEYGAND, F and WALDSCHMIDT, M. (1955): Uber die biosynthese des Leucopterins, untersucht mit ^{14}C - markierten Verbindungen am KohlweiBling. Angew. chem., 67, 328.

_____, SCHLIEP, H.J., SIMON, H., and DAHMS, G. (1959): Uber die Biogenese des Leukopterins. Angew chem. 71, 522

_____, SIMON, H., DAHMS, G., WALDSCHMIDI, SCHLIEP, H.J. and WACKER, H. (1961): Uber die Biogense des Leucopterins. Angew .chem. 73 ,402-407

_____, SIMON,H., KEIL, K.D., and MILLAUR,H.(1964): pterine aus 2,4,5 - Trianino - 6 - hydroxypyrimidin und Zucken.chem.Ber., 97, 1002 - 1023.

WIELAND,H., and PURRMAN,R.(1940): Uber die Flugelpigmente der Schmetterlinge. VI. Uber Leukopterin and Xanthopterin. Justus Liebigs Annln chem., 544, 163 - 182.

_____, and SCHOPF,C. (1925): Uber den gelben Flugelfarbstoff des Zitronenfalters. (Gonepteryx rhamni). Ber. dt. chem.Ges., 58, 2178 - 2183.

WIGLESWORTH,V.B.(1965): The principles of insect physiology. English Language book Society and Methuen & Co. Ltd. PP. 25 - 47.

WINKLER,B.S., and RILEY,M.V.(1977): Na^+- K^+ and HCO_3- ATPase activity in retina: dependence on Calcium and Sodium . Invest. Ophthalmol. visual Science. 16,12, 1151- 1154

_____, SIMSON,V., and BENNER,J.(1977): Importance of bicarbonate in retinal function. Invest. Ophthalmol. visual Sci. 16: 766- 768

WOLFF,I., FUCHSWANS,W., WEISER,M.,FURTHMAYER,H.,and TIMPL, R. (1971): Acidic structural proteins of ee connective tissue. characterization of their heterogenous nature. Eur.J. Biochem. 20, 426 - 431.

WOLF,G., VARANDANI,P.T.(1960): Studies on the function of vitamin A in the muco polysaccharide biosynthesis. J.biol.chem.225, 995- 1008.

WOLKEN,J.J.(1968): The photoreceptors of arthropod eyes. Symp.Zool. Soc. Lond. No.23, 113 - 133.

_____, BOWNESS, J.M., and SCHEER, I.J. (1960): The visual complex of the insect: retinene in the house fly, Biochem. biophys. Acta, 43, 531 - 537.

WOLLMAN, E., GIROUD, A., and RATSIMAMANGA, R. (1937): Synthese de la vitamin C chez un insecte orthoptere (Blattella germanica) was elevage ascptique. C.R. Soc. Biol. 124, 434 - 435.

ZIEGLER, I. (1961c): Zur genphysiologischem Analyse der pterine in Insecktenauge(Drosophila melanogaster und Calliphora erythrocephala) Z. Vereblchre, 92, 239 - 245.

ZIEGLER- GUNDER, I. (1956a): Untersuchungen Uber die photolabilen pterine in der Haut der Amphibien und in den Augen von Drosophila melanogaster. Z. Naturf., 11b, 493 - 550.

_____, (1956b): Untersuchungen uber die purin - und pterinpigmente in der Haut und in den Augen der WeiBfische. Z. Veral. physiol., 39, 163 - 189.

_____, (1956c): pterine: pigmente und Wirkstoffe in Tierreich. Biol. Rev. Camb. phil. Soc., 31, 313 - 348.

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