RETINOMOTOR AND DIURNAL RHYTHM IN CRUSTACEANS

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(With Plates 6–8 and Five Text-figures)

INTRODUCTION
It is well known that a periodicity in various vital functions is still maintained after the elimination of all periodic environmental factors. Formerly it was believed that these factors were essential to the preservation of such periodicity, and a preponderant role was ascribed to the light perceiving organ. Kiesel (1894), however, was the first to notice a persistent periodicity of luminosity in the compound eye (observable after a short illumination by means of an ophthalmoscope): this occurred even when the animal (Plusia gamma) was kept permanently placed in the dark. The change took place at sunset and was reversed at daybreak. The true significance of this phenomenon was realized only at a later date.

Diurnal rhythms are described not only in relation to vision, but also in relation to other physiological functions, e.g. the periodic alterations in the expansion of dermal chromatophores, as described in various forms of life by different investigators (Gamble & Keeble, 1900, in Hippolyte varians; Young, 1935, in Lampetra planeri; Hogben & Slome, 1931, in Xenopus laevis). However, some of the most interesting phenomena of this kind are the periodic changes in the pigment systems of the compound eye, which continue even when the animal is kept permanently in the dark. (For corresponding phenomena in the vertebrate eye see Welsh & Osborn, 1937; Arey & Mundt, 1941).

Demoll (1911) confirmed the observation of Kiesel. More recently, further investigations have been carried out by Welsh (1930a, 1932, 1936), who observed a diurnal rhythm in the distal pigment cells in Macrobrachium and Anchistiodes (see Text-fig. 3) after elimination of all interfering factors, including light. Bennitt (1932a) noticed a similar rhythm in the proximal pigment cells of Canthus. From these observations it is evident that more than one pigment system is involved in the diurnal rhythm.

The crustacean eye usually contains three pigment systems (see Text-fig. 1), although in some species only two are known. These are:

(a) The distal, so-called 'iris' pigment, which in dark adaptation envelops the crystal cone of each ommatidium.

(b) The proximal, so-called 'retina' pigment, lying in dark adaptation below the membrana fenestra.
Retinomotor and diurnal rhythm in crustaceans

(c) The reflecting pigment or 'tapetum' consisting of an amorphous guanine mass. In dark adaptation this pigment is located above, or distal to, the membrana fenestra. The tapetum is responsible for the appearance of luminosity in the eye when the animal is placed in darkness.

During light-adaptation, the following changes take place in the three pigment systems:

(a) The iris pigment migrates in a proximal direction between the ommatidia.
(b) The retinal pigment migrates distally through the gaps in the membrana fenestra.
(c) The tapetum is retracted through the meshes of the membrana fenestra to give the retinal pigment an opportunity of enveloping the rhabdomes completely.

Fig. 1 illustrates this whole process in a three-dimensional diagram. On the left side of the figure the pigment systems are shown in light-adaptation, whereas on the right side two ommatidia are shown in a state of dark-adaptation. (Compare with Pl. 7, fig. 1, a and b; sections of the eye of L. squilla in light and dark-adaptation respectively.)

When the diurnal rhythm is studied, it must not be forgotten that other important factors besides light play a role in the retinomotor process. Congdon (1907) demonstrated the influence of temperature on the position of the retinal pigment in Palaemonetes and Cambarus; Bennitt (1924) found that in the state of anaesthesia and 'post-mortem' the pigment systems of most Crustaceans assume the position of light-adaptation. Again, Bennitt, co-operating with Dickson Merrick (1932b),

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Text-fig. 1. Three-dimensional diagram of the compound eye in light-adapted state; only two ommatidia in the right half are shown in dark-adaptation. c. cornea; h.c. hypodermal cell; cr.c. crystal cone, distal part of the ommatidia; i.p. iris pigment; r.p. retinal pigment; t. tapetum; o.n. optic nerve fibres; rh. rhabdome, proximal part of the ommatidia.
demonstrated the influence of lack of oxygen upon the position of the retinal pigment in *Cambarus*. The same state of light-adaptation can be demonstrated immediately before death and in overcrowded aquaria.

From the above it is clear that in the study of diurnal rhythm it is absolutely essential to pay attention not only to absolute darkness during observations, but also to constant temperature and sufficient oxygenation of the seawater. [For general aspects of retinomotor phenomena, the reader is referred to the excellent monographs of Exner (1891), of Hess (1912) and to a more recent one by von Studnitz (1940).]

Retinomotor changes can be studied by histological methods. In addition, a number of related phenomena, which can be studied *in vivo*, also depend on changes in the position of the pigment. These are:

A. *The luminosity of the eye in darkness* on illumination with the ophthalmoscope. Leydig had knowledge of this phenomenon in 1864. It was thought that when the tapetum was not covered by retinal pigment a reflection of the light beam from the ophthalmoscope would occur, whereas this light would otherwise be absorbed. From this it should follow that in cases in which a diurnal rhythm in the retinal pigment or in the tapetum can be demonstrated, a corresponding diurnal rhythm in the luminosity should take place. However, the present investigations have shown that this does not always hold true, and therefore we must assume that this phenomenon is more complicated than is generally believed.

B. *Variations in the diameter of the pseudo-pupil*. The pseudo-pupil—described for the first time by Leydig (1855)—appears as a dark spot around the point at which the visual line meets the surface of the compound eye. When the eye of the observer is moving, the position of the pseudo-pupil changes correspondingly. According to Exner (1891), the appearance of a pseudo-pupil is possible only when the iris pigment is covered by an iris tapetum (see Text-fig. 2). According to the general view, the position of the iris tapetum influences the diameter of the pseudo-pupil. In light-adaptation the pseudo-pupil is small, whereas it is large in the dark-adapted eye. An oval or rectangular form of the pseudo-pupil can be explained either by differences in the position of the iris tapetum and iris pigment around the affected (round or square) ommatidia, or by an astigmatism of the cornea.

C. *Variations in the transillumination picture of the eye* (see Text-fig. 4). This investigation can be carried out only in transparent eyes such as those of *Leander*, which allow the light of a microscope lamp to pass through. The transparent margin around the dark central disk varies in breadth with the position of the iris pigment and the cornea of those ommatidia which lie in the horizontal plane perpendicular to the direction of observation (see Text-figs. 2 and 4). Welsh (1930b) made such measurements in the eyes of *Leander* and of *Anchistioides* in the course of his investigations on the diurnal rhythm (1936).

When studying the problem of the diurnal rhythm, I found it of great importance to compare the results obtained by studying the histological sections with those derived from the experiments *in vivo*, as described below.

The following crustaceans were used in the present investigations. The systematic
diagnoses were made by Dott. Bacci in Naples. The number of bulbi of each species used is given below:

(1) *Leander squilla* 90 bulbi
(2) *Leander elegans* 70 ,,
(3) *Portunus arcuatus* 34 ,, 
(4) *Portunus depurator* 14 ,, 
(5) *Portunus corrugatus* 18 ,, 
(6) *Pachygrapsus marmoratus* 20 ,, 
(7) *Carcinus maenas* 40 ,, 

Text-fig. 2. Showing the influence of the diurnal rhythm on the position of the pigment systems. This influence is most marked during dark-adaptation, see (b). (a) shows the pigment systems in light adaptation during the day and during the night respectively; (b) shows the migration of the pigment in the dark-adapted eye under the influence of the diurnal rhythm in daytime and at night. c. cornea; h.c. hypodermis cell; c.c. crystal cone; i.t. iris tapetum; i.p. iris pigment; r.h. rhabdome; r.p. retinal pigment; m.f. membrana fenestra; t. tapetum; e.s. eye stalk.

The investigations consisted in the examination of the dark-adapted eye:

(a) In vivo. (1) Luminosity of the eye on exposure to a light flash from the ophthalmoscope; (2) measurement of the diameter of the pseudo-pupil by means of an ocular-micrometer; (3) measurement of the dark disk and the breadth of the transparent zone by momentary trans-illumination of the eye of *Leander*. Although it is theoretically possible for a migration of pigment to occur as a result of a short exposure to light, this possibility may be disregarded since the migration process takes place too slowly (Welsh, 1930b). In the *Leander* eye full light-adaptation is not reached in under 50–60 min.

(b) In histological section. We measured the position of the distal 'iris' pigment, the proximal retina pigment and, if present, the reflecting pigment (tapetum) in
relation to the cornea and the membrana fenestra. The histological technique used was a simple one. The animals were killed and simultaneously fixed by immersion in water at 80–90° C. The optic stalks were cut as close to the head as possible.

Text-fig. 3. (a) Records of the movements of the distal pigment cells of four animals kept in constant illumination (open circles), except for last 2 days, and of four animals kept in constant darkness (solid circles). Experiment started 13 June and ended 30 June. Noon and midnight are indicated by the letters N and M. In the sketch of an ommatidium A is the extreme peripheral or night position of the distal pigment cells, B the inner or day position, in constant darkness. The points as plotted are averages of the measurements of the distance from the cornea to the outer boundary of pigment. Each unit, 10 μ. Time of sunset during course of the experiment about 7.35 p.m. (b) The measurements made on the eyes of animals kept in constant darkness and shown plotted over a period of 15 days are here shown in one 24 hr. period. Such a plot makes it possible to construct a curve representing the daily movements of the distal pigment cells. One of the co-ordinates shows the hours of the day beginning at noon, the other the distance of the distal pigment cells from the outer boundary of the cornea, each unit being equal to 10 μ. (From Welsh, 1936.)

The eyes were placed successively in alcohol 96 %, alcohol 70 %, diaphanol (to soften the hard chitinous covering of the eyeball), alcohol 70 %, dioxan, dioxan-paraffin and paraffin.
The investigations were carried out on the unstained preparations. Two sections of each eye, including the optic nerve, were made. The two ommatidia most centrally situated were used for measurement. The presence of a tapetum was determined by means of dark-ground illumination. The iris and retinal pigment do not reflect, whereas the tapetum is visible as a brilliant white structure (see Pl. 6, fig. 1 b.)

The investigation of the diurnal rhythm was carried out under conditions of constant temperature (with a maximal variation between day and night of 0-3°), constant darkness and in well-ventilated aquaria. Specimens were taken before and after sunset.

At the same time investigations into the retinomotor changes in general were carried out under conditions of (a) sunlight; (b) diffuse daylight; (c) post-mortem (after natural death); (d) darkness during the hours of daylight ('day-darkness'); (e) darkness during the hours of night ('night-darkness'). As far as possible, the eye was examined with the ophthalmoscope; the pseudo-pupil was measured and investigated by trans-illumination before death and the histological examination was performed thereafter as soon as possible.

There was no significant difference in the position of the pigment of animals killed (a) after 1 hr. exposure to sunlight, (b) after some hours in diffuse daylight, and (c) after natural death.

We are thus able to confirm Bennitt's (1932 b) findings that, irrespective of whether the animal has died naturally in sunlight or in darkness, the final position of the pigment is as found in the light-adapted state. The same holds true in all the Crustaceans investigated; and not only for the iris pigment, but also for the retinal pigment and the tapetum, if this is present. The influence of oxygen-lack to these findings will be dealt with later.

Text-fig. 2 a and b summarize in a diagram the migration of the three pigment systems in light- and dark-adaptation. The influence of the diurnal rhythm on the position of the pigment in the light- and dark-adapted eye is now discussed.
In adaptation to *normal illumination during the day* we find—as illustrated in Text-fig. 2a left half—the iris pigment (i.p.) and the tapetum (t.) situated in the extreme proximal position, whilst the retinal pigment (r.p.) is in the extreme distal position. The iris tapetum being in actual contact with the iris pigment, closely follows the movement of the latter in spite of an anatomical connexion between iris tapetum and tapetum (see Pl. 7). While the crystal cones of the ommatidia are deprived of iris pigment, the rhabdomes, on the other hand, are almost completely enveloped by a layer of retinal pigment.

During *illumination at night* (‘light at night’) (Text-fig. 2a, right half) the iris pigment lies more distally, indicating an initial change towards the dark-adapted position (Text-fig. 2b), although the positions of the retinal pigment and tapetum are unchanged. The effect of light changes upon the positions of retinal pigment and tapetum is much greater than the effect of the diurnal rhythm during constant illumination. The iris pigment, on the other hand, is influenced more by the diurnal rhythm.

When the animal is adapted to *dark during the night* (‘night-darkness’ position), the changes in position of the three pigment systems become evident. The iris pigment and tapetum migrate to the distal part, whilst the retinal pigment moves proximally (see Text-fig. 2b, right half). As the rhabdomes are no longer covered by the retinal pigment, and as the latter is replaced completely by the light-reflecting tapetum, we observe a red glow of the pseudo-pupil. If the animal is kept in the dark during the hours of daytime (pigment in ‘day-darkness’ position; Text-fig. 2b, left half) these changes are not so marked; under the influence of the diurnal rhythm the iris pigment and tapetum move less distally and the retinal pigment moves less proximally. The glow of the pseudo-pupil now disappears because the reflecting tapetum is covered once again by the retinal pigment, and its glow will reappear only after sunset, when the influence of the diurnal rhythm is eliminated and final dark-adaptation has been established once again.

In some of the Crustaceans examined, it was obvious that the pigment changes, as described in Text-fig. 2, were incomplete. In *Leander*, for example, the tapetum is situated to a large extent above the membrana fenestra, even in extreme light-adaptation, i.e. in sunlight, whilst in *Leander* in dark-adaptation the retinal pigment is not found wholly under the membrana fenestra. Nevertheless, in general the changes of the pigment follow the scheme as outlined in Text-fig. 2a and b.

**EXPERIMENTS**

We tried to answer the following questions:

(A) Does a diurnal rhythm occur in the compound eyes of all species of Crustaceans?

(B) Are the same elements concerned in this periodicity of pigment migration in different species of Crustaceans when all light has been excluded, and does such a periodicity correspond with sunset and sunrise?

(C) Does a correlation exist between the position of the migrating pigment, the
Retinomotor and diurnal rhythm in crustaceans

Size of the pseudo-pupil and the glow of the eye? If so, can the existence of a diurnal rhythm be deduced by measuring the variations in size of the pseudo-pupil?

(D) What is the anatomical and physiological basis of the process of the diurnal rhythm?

Questions A and B

Although I was able to demonstrate a periodicity in each of the species of Crustaceans investigated, the changes may not in all cases take place in the same pigment system (as found in the investigations of Welsh, 1935, and Bennitt, 1932a).

Under optimal conditions and during a period of 14 days we found:

(1) In Leander squilla

(a) No marked difference between the position of the iris pigment in 'night-darkness' and 'day-darkness' as determined by means of trans-illumination in vivo and measured in the histological preparations. In various sections of eyes fixed under 'day-darkness' the iris pigment had shifted over a distance of about 10 μ, compared with the eyes of animals killed under 'night-darkness'. (Compare with this: Text-fig. 3, which shows the diurnal rhythm of the iris pigment in Anchistiotides (Welsh, 1936), where migration took place over a distance of about 90 μ.)

(b) We could not discover a periodicity of any importance in the migration of retinal pigment in histological section, whereas

(c) A marked periodicity was demonstrated in the position of the tapetum; when fixed in the position of 'day-darkness' it was found that the distal border had moved about 15 μ proximally, measured from the membrana fenestra; simultaneously a diffuse distribution of the tapetum under the membrana fenestra was seen. (Compare Pl. 7b with c; sections of the eye of Leander squilla in 'night-darkness' and 'day-darkness' respectively.)

(2) In Leander elegans

The changes in pigment positions are in principle the same as in L. squilla. The investigation was carried out over a period of 9 days. A difference of 10 μ was measured between the distal border of the tapetum in 'night-darkness' and 'day-darkness' positions (see Pl. 8).

(3) In Portunus arcatus

(a) I could not observe a movement in the iris pigment of an amount sufficient to establish with certainty the occurrence of a diurnal rhythm.

(b) In some preparations a detectable movement in the retinal pigment through the membrana fenestra in the distal direction could be demonstrated in 'day-darkness', whilst in other preparations under exactly the same conditions a significant migration of about 45 μ took place.

(c) An absence of the tapetum has been noted.
4) In *Portunus depurator*

(a) A marked diurnal rhythm occurs in the position of the iris pigment; the pigment migrates from the 'night-darkness' to the 'day-darkness' position over a distance of 50 μ, measured from the cornea.

(b) The retinal pigment shows also a significant diurnal rhythm. When the 'day-darkness' position is reached we found a migration through the membrane likewise over a distance of 50 μ.

(c) A tapetum could not be demonstrated.

5) In *Portunus corrugatus* we found

(a) A marked diurnal rhythm in the iris pigment (as in *P. depurator*) but only an average of 15 μ, whereas

(b) In *P. arcuatus* and *P. depurator* the diurnal rhythm resulted in a migration of the retinal pigment between 'night darkness' and 'day-darkness' positions over a distance of about 80 μ.

(c) Contrary to the finding of Exner (1891), I was able to demonstrate the presence of a tapetum; there was no significant alteration between 'night-darkness' and 'day-darkness' positions.

6) In *Pachygrapsus marmoratus*

(a, b) No clear diurnal rhythm in the position of the iris-pigment nor in that of the retinal pigment was demonstrable, whereas

(c) In the tapetum, by contrast, a definite diurnal rhythm could be shown, with a migration of the pigment of about 60 μ in a proximal direction, when the state of 'day-darkness' is reached.

7) In *Carinus maenas*

We could not detect:

(a) A diurnal rhythm in the iris pigment, although

(b) In the retinal pigment a definite diurnal rhythm could be shown. This pigment, which in the 'night-darkness' state is totally under (proximal to) the membrana fenestra, moves distally after sunrise (in the state of 'day-darkness') passing between the ommatidia and joins the iris pigment. A precise measurement is not possible, but the migration is roughly 130 μ (see Pl. 6, fig. 2a, b).

*Question C*

According to Welsh (1936) the transparent zone, demonstrable by trans-illumination of the transparent eye, narrows and broadens in accordance with the movement of the iris pigment, and its breadth depends on the distance between the distal border of the iris pigment and the cornea (see Fig. 4).

Besides this correlation between the breadth of the transparent zone and the position of the iris pigment which I was able to show, I also found a correlation
between the position of the iris pigment measured in histological section and the
diameter of the pseudo-pupil measured \textit{in vivo}. 

In the third place I demonstrated the relationship \textit{in vivo} between the size of the
pseudo-pupil and the breadth of the transparent border zone of the eye of \textit{Leander};
measurements were made during a momentary trans-illumination and are summarized
in Table 1. The data of both series of observations (\textit{a}) and (\textit{b}) show, according to
Fischer (1946), a correlation in the form of a linear regression.

\begin{table}[h]
\centering
\begin{tabular}{cccccc}
\hline
(a) & 1130 & 1575 & 1600 & 1100 & 1405 \\
(b) & 160 & 370 & 310 & 140 & 250 \\
& 880 & 1150 & 1450 & 205 & 130 \\
\hline
\end{tabular}
\caption{Diameter (in \(\mu\)) of the dark centre of the compound eye during trans-illumination. (b) Diameter in \(\mu\) of the pseudo-pupil. Both measured in nine animals.}
\end{table}

(On account of the shape of the ommatidia of \textit{Leander} (four-sided pyramids, with
their bases against the cornea) the pseudo-pupil presents a square aspect. When
measurements are made in an astigmatic part of the cornea, the pseudo-pupil
becomes rectangular. The average length of the sides of the rectangle is taken to
represent the diameter of the pseudo-pupil.)

Our investigation made it clear that this relationship between the size of the
pseudo-pupil and the position of the iris pigment holds not only for the translucent
eye, but also for the non-translucent eye of some of the other species of Crustaceans
(\textit{Portunus, Pachygrapsus} and \textit{Carcinus}). In the latter the failure of the trans-illumin-
ation is due to the pigment in the hypodermis (Text-fig. 2). This pigment has
absolutely no connexion with the pigment systems concerned with the retinomotor
function.

Although for several weeks we measured the pseudo-pupil in \textit{C. maenas} \textit{in vivo}
under diurnal rhythm conditions, no significant differences were noticed between
the night and day recordings. This is in complete agreement with our findings on
the histological preparations of the control animals, where there was no significant
migration of the iris pigment.

Where the size of the pseudo-pupil and the breadth of the transparent zone of the
eye both depend on the position of the iris pigment—as we have shown before—trans-
illumination and measurement of the pseudo-pupil are valuable means of indicating
\textit{in vivo} the presence or absence of a diurnal rhythm in the iris pigment system.

We mentioned on p. 180, that periodic changes in the positions of the retinal
pigment or of the tapetum can produce a diurnal rhythm in the luminosity of the
eye. This latter change can be observed in the dark during the day and at night by
means of an ophthalmoscope. We could demonstrate such a rhythm in the lumin-
osity of the eye only in \textit{Portunus arcuratus, P. depurator} and \textit{Pachygrapsus marmoratus}.

Exner's (1891) explanation of the luminosity of the compound eye depends upon
the light-reflecting tapetum being covered by the retinal pigment. Accepting this
hypothesis, the diurnal rhythm in the luminosity of the eye can be explained by
assuming such a rhythm in either or both tapetum and retinal pigment. However,
neither *Portunus arcuatus* nor *P. depurator* possess a tapetum, though both display a diurnal rhythm in their retinal pigment. On the other hand, *P. corrugatus* possesses a tapetum, and exhibits a rhythm in the migration of the retinal pigment over a distance of 80 μ, thus providing ideal circumstances for the appearance of a diurnal rhythm in luminosity; but we never observed in this species any trace of luminosity of the pseudo-pupil. On the other hand, in *Leander* we observed an intense and invariable red glow of the pseudo-pupil, both in 'night-darkness' and 'day-darkness'. *Leander* shows the same ocular structure and the same diurnal rhythm in the tapetum as *Pachygrapsus marmoratus*, but *Pachygrapsus* shows a marked diurnal rhythm in luminosity which *Leander* does not.

From these facts, it will be clear that the explanation of this phenomenon is more complicated than was formerly believed. One can but state that the periodic changes in the luminosity of the pseudo-pupil in vivo are not conclusive evidence for the existence of a diurnal rhythm in tapetum or retinal pigment.

**Question D**

The cause of the diurnal rhythm has been the subject of speculation by many writers. For example, Hogben & Slome (1931) have ascribed it to hormonal influences, whilst Welsh (1936) has stressed the 'eyestalk hormone' of Kleinholz (1934) as an important aetiological factor in the rhythm of the distal pigment system. Bennitt (1932) ascribed the periodic changes to nervous factors, acting through the influence of periodic alterations of oxygen tension upon the cells of the different pigment systems concerned. Periodic changes in the metabolism have been suggested by Menke (1911). According to Bennitt (1932 a-c) the aetiological factors could exert their actions via nervous or vascular pathways. From the diversity of opinions expressed by various experimental workers, the reader will appreciate that up to the present no definite conclusion has been reached as to the aetiology of this interesting problem. However, we must concede that changes in the metabolism of the pigment systems concerned may be a factor of importance.

Not much work has been done on the metabolism of Crustaceans and only a few facts are available (Henze, 1910; Cohnheim, 1911-12).

In *Carcinus* Cohnheim (1911-12) found, inter alia, an oxygen consumption of 7.4-10.1 mg./100 g./hr., and a carbon dioxide production of 15-27 mg./100 g./hr., with a respiratory quotient of 1.34-1.18.

Continuous estimations of metabolic rate in Crustaceans, a necessary requirement for our purpose, have not yet been made.

Since *C. maenas* shows a marked diurnal rhythm in the retinal pigment system (p. 186), and one specimen is large enough to allow a reliable determination of the metabolic rate, I chose this animal for the experiment.

The continuous metabolism estimations were performed by means of the diaferometer of Noyons (1937). Two series of experiments were done under different conditions:

1. The animal in diffuse daylight (during the hours of daytime) and in darkness (during the hours of night).
Retinomotor and diurnal rhythm in crustaceans

(2) The animal permanently in the dark under conditions suitable for the study of the diurnal rhythm.

We thought it of interest to see if it was possible to demonstrate the presence of a diurnal rhythm in the production of carbon dioxide, when such measurements were made over a sufficient period of time. The results are shown in Text-fig. 5. From our data it is evident that during the 24 hr. period, even in constant darkness, the production of carbon dioxide is greater during the night than during the hours of daylight, thus indicating a diurnal rhythm in metabolism.

Text-fig. 5. Curve showing the continuous determination of the metabolism in *Carcinus maenas*. Measurements are shown in one 24 hr. period. From the data obtained a curve can be constructed representing the daily variations in the production of carbon dioxide. On the abscissa time is plotted in hours from 0 to 24. The relative carbon dioxide production is plotted on the ordinate. First series (open circles): the animal is kept in dimmed daylight during the day and in darkness during the night; second series (filled circles): animal in continuous darkness. Note that there is no marked difference between these two series.

The sea-water could not be changed in the course of the experiment; consequently the animals showed signs of intoxication, probably due to the presence of their own excreta. For this reason it was impossible to continue with the investigations beyond a period of 3 days.

The series of experiments were made: First series (open circles): the animal was kept in diffuse daylight during the day; in darkness at night. Second series (solid circles): the animal was kept in complete darkness.

Before we started with the second series, the animal was adapted to constant darkness for a period of several days. In both series a constant temperature and adequate oxygenation of the sea-water were maintained.

We have demonstrated—at least in *Carcinus*—a diurnal metabolic rhythm in terms of a diurnal rhythm in the production of carbon dioxide, which is about
30% smaller by day than by night. Does this help to explain the diurnal retinomotor rhythm? The work of Bennitt (1924, 1932b) bears upon this problem. Bennitt demonstrated—and the present experiments have confirmed—that under conditions of anaesthesia or of oxygen deficiency there is a migration of pigment towards the light-adapted state, even if the animal is kept in the dark. Anaesthesia and oxygen deficiency are likely to be associated with reduced metabolic rate. Thus it appears that if the metabolic rate is lowered, either in the course of the natural diurnal rhythm or by experimental interference, then there is a tendency for the pigment to migrate towards the light-adapted state, even in the dark. This evidence suggests that the diurnal retinomotor rhythm may be explained as an inevitable consequence of the diurnal metabolic rhythm.

SUMMARY

The demonstration of the existence of a diurnal rhythm in the migration of the pigment in Leander, Porttmus, Pachygrapsus and Carcinus justifies the supposition that in many species of Crustaceans such a rhythm exists in the retinomotor changes of one of the pigment systems.

These periodic changes in the position of the pigment, despite the exclusion of all light, correspond with sunrise and sunset. Even if the animal is kept in complete darkness during the hours of daytime, the pigment systems concerned exhibit a partial migration towards the position of light-adaptation; when the night returns the pigment system resumes its maximum state of dark-adaptation and is found in the same position as in the previous night.

The measurement of the size of the pseudo-pupil is of value in proving, in vivo, the existence of a diurnal rhythm in the iris pigment. The existence of a periodic change in the luminosity of the living eye when examined with the ophthalmoscope is of no value in ascertaining the occurrence of a diurnal rhythm in the retinal pigment or in the tapetum.

In C. maenas it was possible to demonstrate a periodic variation in metabolism, in terms of a diurnal rhythm in the production of carbon dioxide. This production was about 30% smaller during the day than during the night.

The diurnal retinomotor rhythm may depend on a periodic hypoxaemia of the cell systems concerned.

I wish to express my thanks to Prof. J. Jongbloed and Dr A. Punt of the Physiological Laboratory at the University of Utrecht for their kind help and advice in the use of the diaferometer.

REFERENCES


Fig. 1

Fig. 2

HENKES—RETINOMOTOR AND DIURNAL RHYTHM IN CRUSTACEANS
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HENKES—RETINOMOTOR AND DIURNAL RHYTHM IN CRUSTACEANS
Retinomotor and diurnal rhythm in crustaceans


EXPLANATION OF PLATES

PLATE 6

Fig. 1. Microphotograph of a section through the compound eye of Leander elegans in the light-adapted state: (a) with normal illumination; (b) with dark-ground illumination. Compare (a) with Text-fig. 2, left half. In dark-ground illumination only the tapetum and iris tapeture are demonstrable. For abbreviations see Text-figs 1 and 2.

Fig. 2. Diurnal rhythm in the eye of Carcinus maenas. (a) shows the dark adapted eye during the night; (b) shows the dark adapted eye in day-time. Note the marked migration of the retinal pigment in darkness; in day-time it moves between the ommatidia in a distal direction and comes in apposition with the iris pigment (‘day-dark position’). No tapetum can be demonstrated.

PLATE 7

Micro-photographs of the compound eye of Leander squilla in normal illumination and under dark-ground illumination: (a) in light-adaptation; (b) in dark-adaptation during the night (‘night-darkness’); (c) in dark-adaptation during the day (‘day-darkness’). Note that in (c) the tapetum is shifted proximally in the direction of light-adaptation (a).

PLATE 8

Photographs showing the diurnal rhythm in the compound eye of Leander elegans in normal and dark-ground illumination. Note the marked difference in the position of the tapetum between (a) ‘night-darkness’ and (b) ‘day-darkness’.