DIURNAL RHYTHMS OF LOCOMOTOR ACTIVITY IN
THE SCORPION HETEROMETRUS FULVIPES
Studies on the locomotor activity rhythm, one of the indicator processes often chosen for investigating the diurnal rhythms, in different organisms have considerably contributed to the understanding of the problems in this field. The investigations on the activity rhythms of mammals (Szymanski, 1918; Richter, 1922; Browman, 1943; Aschoff, 1951, 1952; Tribukait, 1954; Mayer-Lohmann, 1955) crustaceans (Roberts, 1944; Schallek, 1942; Guyselman, 1957; Bennett, Shriner and Brown, 1957; Naylor, 1958, 1960, 1961, 1963; Bliss and Sprague, 1958; Bliss, 1960) and insects (Gunn, 1940; Mellanby, 1940; Cloudsley-Thompson, 1953, 1960a, 1963b; Harker, 1954, 1955, 1956, 1960a, b; Roberts, 1960, 1962; Nowosielski, 1962; Nowosielski and Patton, 1963; Nowosielski et al., 1964) constituting the large body of literature, particularly add to the understanding of the nature of the rhythms and elucidate some of the mechanisms of the "clocks" regulating the activity cycles.

Many investigations carried out under controlled conditions of light proved the persistence of the rhythms in continuous darkness as well as in continuous illumination, at least for a few cycles. Light is shown to inhibit the activity in rats (Munn, 1950), mantids (Roeder, Tozian and Weiant, 1960) and the cockroach, Blaberus giganteus (Cloudsley-Thompson, 1960a). A loss or disappearance of rhythms in Cambarus (Roberts, 1944) Microtus (Calhoun, 1945) and cockroaches (Gunn, 1940; Cloudsley-Thompson, 1953; Harker, 1956) has been reported. But as against the above observations on the cockroaches, Roberts (1960)
demonstrated the persistence of rhythms both in continuous light and continuous darkness for more than three months and suggested that it may be indefinitely persistent.

The changes in the period of the activity rhythms is another very significant finding in the study of the diurnal activity rhythms and are reported by a number of workers (Johnson, 1939; Stinson, 1960; Aschoff, 1960; DeCoursey, 1960; Hoffmann, 1960; Roberts, 1960; Nowosielski, 1963).

The susceptibility of the rhythms to phase shifts is documented in many publications (see Harker, 1958, 1961; Webb and Brown, 1959) and the reversal of the locomotor activity rhythm under reversed conditions of light and darkness is obtained in cockroaches (Harker, 1956) and crickets (Nowosielski, 1963).

Attempts to force activity rhythms to run in light-dark cycles of less than 24 hours are many. Cloudsley-Thompson (1956) could impose an 18 hour cycle, of 9 hours light and 9 hours darkness, in Anthea venator which does not persist in continuous darkness. Schallek (1942) has shown that Cambarus takes up an 8 hour cycle of 4 hours light : 4 hours darkness. Amongst mammals Johnson (1939) found that the activity of mice in a 16 hour cycle revealed traces of the 24 hour rhythm. Cloudsley-Thompson (1953) reported that an 18 hour rhythm could not be impressed on Periplaneta and Blaps requieni. Such experiments as these are attempted to test the endogenous nature of the rhythm.
A very remarkable feature of activity rhythms that evoked excitement and interest among the research workers in the field of biological "clock" systems is the fact that despite the great dependence of metabolism of the organisms on temperature, the "clock systems" are generally temperature independent. Such temperature independence of the locomotor rhythms has recently been demonstrated in the cricket (Cloudsley-Thompson, 1958), cockroaches (Roberts, 1960) and Carcinus maenas (Naylor, 1963). The functional significance of this temperature independence, within at least a limited ecological range of temperature has been much emphasised.

In contrast to advances made in the study of activity rhythms in crustaceans, insects, mammals and other organisms the available information on arachnids is rather random and cursory. Park (1938) studied the rhythm of activity in an arachnid, the tarantula, Sericopelma rubronilens and demonstrated a 24 hour rhythm of activity in constant conditions. The work on Scorpio, as summarized by Harker (1958) shows the existence of persistent rhythm of activity which could be reversed under the reversed conditions of light and darkness and it returns to the normal phase when returned to continuous light.

The other investigations on the diurnal rhythms of activity of arachnids are those of Cloudsley-Thompson (1957, 1961b, 1962, 1963a). While working with three species of spider, Ciniflo, Cloudsley-Thompson (1957) has reported the daily rhythm
of activity in all the three species. His work on "some aspects of the physiology and behaviour of Diplostomum", a mite, reveals the existence of diurnal rhythms of activity with a peak of activity shortly after 13.00 hours local time (Sudan). In the absence of significant difference in darkness he concludes that the rhythm is endogenous. Similar studies carried out in Galeodes arabs (Cloudsley-Thompson, 1961) shows that the activity, appearing 3 or 4 days after ecdysis, has a normal 24 hour periodicity. By actograph experiments it is shown to be nocturnal in habit.

The only recent work on the diurnal rhythms of motor activity in scorpions is that of Cloudsley-Thompson (1963) who worked on the two buthid scorpions, Leirus quinquestriatus and Entotoxus minax, and a scorpionid, Pandinus exitialis. The rhythm of activity is found to be essentially similar in all the cases with the activity beginning soon after the onset of darkness.

Most of these studies are only preliminary and more emphasis is laid on establishing the ecological significance of activity rhythms.

The work reported in this chapter, on the diurnal rhythms of locomotor activity in the Scorpion, Heterometrus fulvipes, essentially consists of a considerably detailed study of the nature and the pattern of activity rhythms (a) in normal day-night conditions, (b) under conditions of continuous darkness and (c) continuous illumination, (d) under controlled conditions
of light and darkness of 12 : 12 hours and (e) on exposure to light dark cycles of less than 24 hours. In addition studies on the reversal of activity rhythm and the temperature characteristics of the same are included.

METHODS

The type of apparatus used for recording the locomotor activity under the various experimental conditions and the principle involved are similar to those used by Naylor (1958). Using similar apparatus the locomotor activity of the animals is recorded automatically on a smoked paper on which the activity can be registered continuously at a stretch for two full days at one speed and about ten days at another speed.

Transparent plastic boxes of 9 x 6 x 4 cms in dimensions, are used as animal chambers. The boxes are suspended about their median transverse axis in such a way that as the animal moves or walks along the length of the chamber, the chamber tilts to one side and an attached lever makes nearly a vertical line or a mark on the smoked paper.

The speed at which the paper moves is constant and known and therefore the hourly activity is ascertained. The number of flicks in a given hour is considered proportional to the locomotor activity of the animal. This set up enables the recording of activity in four animals at a time.

The activity records thus obtained are preserved safely by coating the belt of smoked paper with varnish diluted with
turpentine in 1:2 proportion. The records are then analysed and the activity of the animals during each hour is determined by counting the number of vertical marks recorded. The hourly activity is plotted in block histograms for a thorough study of the patterns of activity. In all cases the animals were not fed during the course of recording the activity but only when at least a day's break in recording is given.

The conditions required for different experiments are provided as follows:

**Locomotor activity in normal day and night conditions**

The recording equipment is set up in a room which is spacious and well ventilated. One of the sides of the room is completely fitted with transparent glass shutters and ventilators that are constantly kept open. Such a room as this is exposed to all the cyclical variations in the physical factors such as light, temperature and humidity by its being in almost direct communication with the atmosphere outside. The activity of the scorpions whose size and sex are noted already is recorded as previously described under these natural (normal) day and night conditions. The daily variations in temperature and humidity are recorded using thermo-hygrometer for correlation purposes.

**Locomotor activity in continuous darkness and continuous illumination with other variables following the natural periodicity**

The activity is recorded in the same room which communicates
with outside as above and constant conditions are provided with respect to light and darkness alone, whereas the other variables in nature are not controlled. The scorpions are placed in opaque animal chambers for recording the activity in continuous darkness. Two 60 watt bulbs are used for providing the continuous illumination. The animals are exposed to the continuous light by placing them in transparent animal chambers.

**Controlled conditions of light and darkness of 12:12 hours**

These experiments were conducted to study whether the pattern of rhythm exhibits any differences when compared to that under natural day-night conditions. During day time (6.00 A.M. to 6.00 P.M.) the natural day light is substituted by the artificial light from the two 60 watt electric bulbs and during nights (6.00 P.M. to 6.00 A.M.) the darkness is provided by switching the lights off. Thus the 24-hour cycle of light and darkness, approximately corresponding to the natural day with the light period coinciding with day and dark period with the night, is provided.

**Locomotor activity in continuous darkness and continuous illumination at constant temperature**

These recordings are done in a dark room, that is thermally insulated using thermocole where the light conditions can be regulated as desired. Another advantage of using this room for these experiments is that fairly constant conditions of temperature (± 1°C) and humidity are maintained inside. For recording
the activity in continuous darkness this dark room is best made use of and for continuous illumination two 60 watt electric bulbs at approximately 2 meters distance were utilized as the source of light.

Reversal of rhythm

Light dark cycles of 24 hours duration (L:D, 12:12) are just reversed by making the day time dark and illuminating the night time using the above source of light. In other words a light regime of 12 hours, from 6.00 P.M. to 6.00 A.M., alternated with a dark period of 12 hours duration from 6.00 A.M. to 6.00 P.M.

Light dark cycles of less than 24-hours

In these studies attempts were made to force the locomotor rhythms to run in 12 hour and 18 hour cycles of light and darkness. While trying the 12 hour cycles of 6 hours of light and 6 hours of darkness care was taken to see that the dark period would not reinforce the normal rhythm by its falling during the normal active period of the scorpions (Harker, 1958). Therefore the 12 hour cycles would consist of 6 hours of light from 6.00 A.M. to 12 noon followed by a dark period of 6 hours from 12 noon to 6.00 P.M. The second 12 hour cycle would start with a 6 hour light period from 6.00 P.M. to 12 mid-night followed by a dark period of 6 hours upto 6.00 A.M.

Similarly the activity of scorpions, subjected to 18 hour cycles of 9 hours of light alternating with 9 hours of darkness
was also recorded. Thus for every three natural cycles the animals are subjected to four artificial cycles.

Effects of temperature on the locomotor activity rhythm

The temperature characteristics of the locomotor rhythms are studied by recording the activity at two temperatures namely $20^\circ C \pm 1^\circ C$ and $30^\circ C \pm 1^\circ C$ in continuous darkness. The period of the rhythm at these two temperatures are compared for evaluating the effects of temperature on the locomotor activity rhythm.