FACTORS CONTROLLING THE DIURNAL RHYTHM OF ACTIVITY OF *PERIPLANETA AMERICANA* L.

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(*Received* 7 *October* 1955)

INTRODUCTION

Two main lines of approach are evident in recent work on diurnal rhythms of activity in animals. On the one hand investigators have been determining the form of the activity rhythm of many animals, particularly insects, and the effects of various environmental factors on these rhythms. On the other a small amount of work has been carried out on the internal factors which are involved in the mechanisms of the rhythm. From the results of the former line of investigation it appears that light or darkness is the factor which fixes the rhythm in most cases, while temperature may modify but not impress a rhythm. The most significant feature of these results is that in most animals it has appeared that once an activity rhythm is established by means of alternating light and darkness then that rhythm continues for a period of time under new environmental conditions. This implies the presence of an 'internal clock' which is regulated by the external conditions but can function without regulation for a period varying in different animals from a few days, as in cockroaches, to months or longer, as in some beetles (Park, 1932) and mayflies (Harker, 1953).

A possible 'internal clock' in *Periplaneta americana* L. is described in the following pages, and the way in which it may be regulated by the environment is discussed.

METHODS

The activity of *P. americana* was measured using two different methods. In one an actograph was used, consisting of an oblong box 8 × 4 in. which was balanced on a knife-edge and had a central wire which brushed through a mercury switch so that an electric circuit was closed whenever the actograph tipped. Recordings were made on a smoked drum. The main disadvantage of this method is that only some of the movements of the cockroach are recorded, either movements along the whole length of the box or smaller movements about the central region. Also the cockroach cannot be given much food or water at a time because the balance of the box is upset as it is consumed. Feeding itself is an activity which is not often recorded by an actograph as the animals may only be moving about in a very limited area. The advantage of the method lies in the fact that the movements of the cockroach are perhaps not as likely to be affected by extraneous factors as in other methods.

The second method of recording activity was to attach a fine thread to the pronotum by a tiny drop of wax, the other end of the thread being attached to a light glass capillary tube acting as a lever. The free end of the tube recorded its movements on a smoked drum. This method recorded all the movements of the cockroach, however small, and did not appear to disturb the animal. Food and water could be left in the cage with the animal in large quantities. In this method the disadvantage lay in the difficulty of analysing the smoked drum record. Under normal environmental conditions the period of activity is a definite one and can be seen at a glance, but when the animals are being subjected to experimental conditions some method of assessing the number of movements is essential. The method adopted was to count only vertical marks of 5 mm. and over, and to count any vertical mark as one movement however great its amplitude. Without knowing the direction of a movement the amplitude could not be taken as a measure of the distance moved by the cockroach. In any case no greater accuracy was required than was given by this means. The second method was used for all experiments and a small number of trials for each was run using the actograph as a check.

All experiments except those carried out in natural daylight and darkness were conducted in a light-tight chamber in which the light was provided by a 60 c.p. lamp giving a light of 25 f.c. at the position of the cockroach. The light periods were controlled by a time switch. Temperature was not controlled but was normal room temperature, preliminary experiments having shown that temperature only affected the amount and not the rhythm of activity (cf. Cloudsley-Thompson, 1953). All experiments were repeated at least five times. Only adult males were used for the experiments in case females might be affected by a reproductive cycle.

ENVIRONMENTAL FACTORS AND THE RHYTHM OF ACTIVITY

(*a*) *Alternating light and darkness*

Under conditions of alternating light and darkness *P. americana* exhibits a remarkably constant type of activity rhythm. The few insects which showed any different rhythm were all found to have some major injury or died within a few days.

There is a substantial increase in activity at the beginning of the dark period, and this reaches its peak, on an average, in 2 hr. The peak is only sustained for from 30 min. to 1 hr. and gradually falls off over the following 2 hr. The animals move about very little during the remaining hours of darkness or in the light period, but after about 6 hr. of light activity begins to increase slightly (Fig. 1*a*). The same activity curve is obtained whether the animals are kept in natural daylight and darkness or in artificial light and darkness. The activity of over a hundred cockroaches has at various times been recorded under these conditions.

(*b*) *Reversed light and darkness*

When cockroaches which have previously been in alternating daylight and darkness are transferred to conditions in which the light periods are altered, for example, the times of light and darkness entirely reversed, the old rhythm of activity continues for from 2 to 4 days, but is gradually lost and replaced by a new rhythm with again the peak of activity occurring in the dark period.

A new rhythm can be induced provided that neither the dark nor the light period is of less than 2 hr. duration.

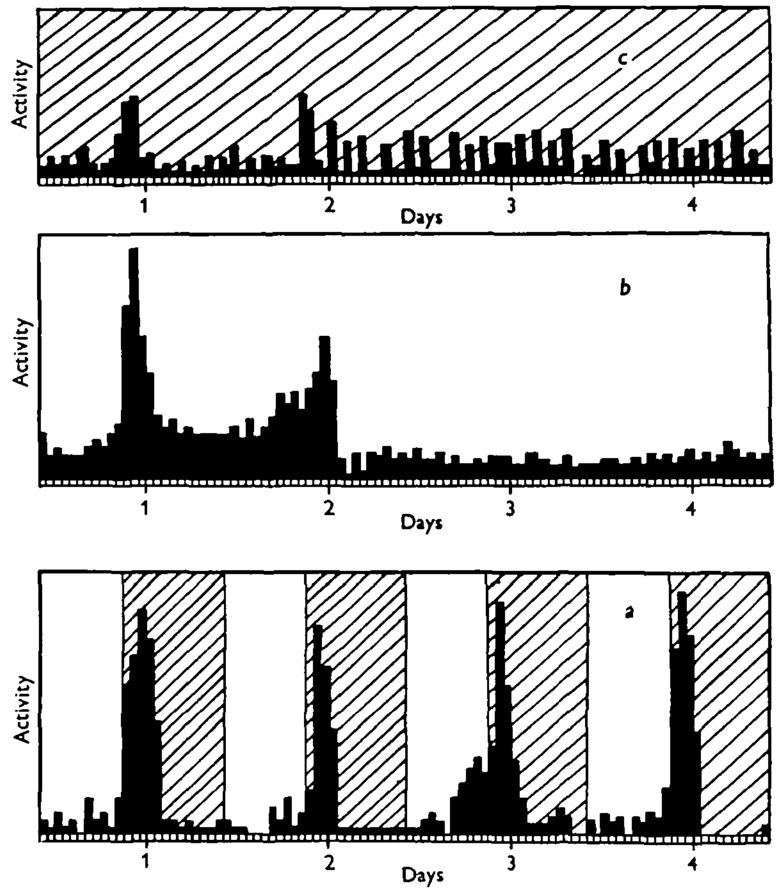

Fig. 1. The activity of *P. americana* at room temperature (*a*) in alternating light and darkness, (*b*) in continuous light, (*c*) in continuous darkness. Hatched areas represent dark periods.

(*c*) *Continuous light or darkness*

When cockroaches are transferred from alternating light and darkness into continuous light or continuous darkness the original rhythm of activity is carried on over a period of 3–5 days, but becomes gradually less marked during that period. After the fifth or sixth day in constant light activity becomes random, but total activity per day remains the same as under the previous conditions. Activity tends to be low but fairly constant (Fig. 1 *b*).

In continuous darkness activity is lower on the first day than it is on the first day in continuous light. For example, in one set of experiments there was an average of 79 movements compared with 186. After the fifth or sixth day in continuous darkness the total activity per day remains the same as under the previous conditions but activity is spasmodic, very high and then very low, and the animals are seldom active in two successive hours (Fig. 1*c*).

When cockroaches are subjected to alternating light and darkness, after having been in continuous light or darkness, they pick up a new rhythm of activity immediately without any initial conditioning period.

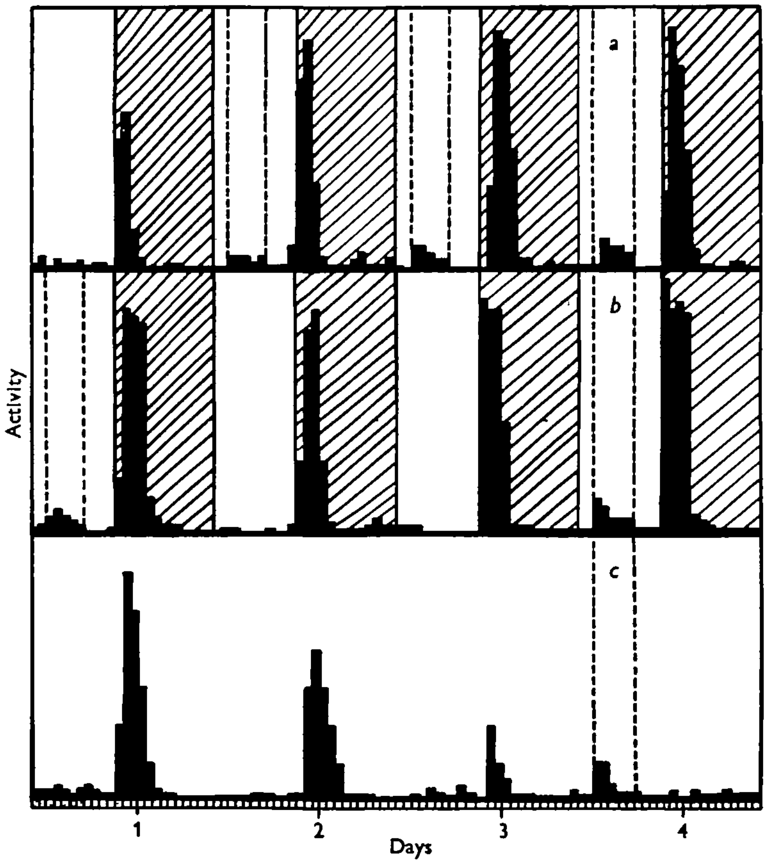

Fig. 2. (*a*) The activity of *P. americana* in alternating light and darkness when food is obtainable only in the light period. The cockroaches had been starved for 4 days previous to the experiment. (*b*) The activity after 3 weeks of above conditions when food was not given at the usual time. (*c*) Activity after food had been introduced at usual time for 5 days, and then animals transferred to continuous light and starved. Period when food present shown between dotted lines. Dark period hatched.

(*d*) *Feeding in relation to activity*

During the active period while the animal is in darkness most of the feeding takes place. The relationship between the hunger cycle and the rhythm of activity was investigated as follows. Cockroaches were starved for 4 days and then food and water were placed in the cage from 12 noon to 5 p.m. when it was removed. This was continued for a period of 3 weeks, the animals meanwhile being in alternating light and darkness, the dark period of which lasted from 8 p.m. to 5 a.m. The animals showed a normal activity rhythm throughout with the peak of activity occurring during the dark period. They showed a slight increase in activity when the food was in the cage, but when the food was not introduced at the usual time there was no increase in activity. Therefore even if it appeared that a rhythm was formed it was not a true one which could be carried over under new environmental conditions, as was shown when the cockroaches were moved to constant light conditions (Fig. 2).

It follows from these experiments that the activity rhythm is not likely to be the expression—at least, not the direct expression—of a hunger cycle.

INTERNAL FACTORS AND THE RHYTHM OF ACTIVITY

(a) *Parabiotic experiments*

Cockroaches were joined together in parabiosis following the method used by Bodenstein (1953), the notum being the point of contact. The legs were removed from the uppermost cockroach to prevent it clinging to the side of its cage or its food.

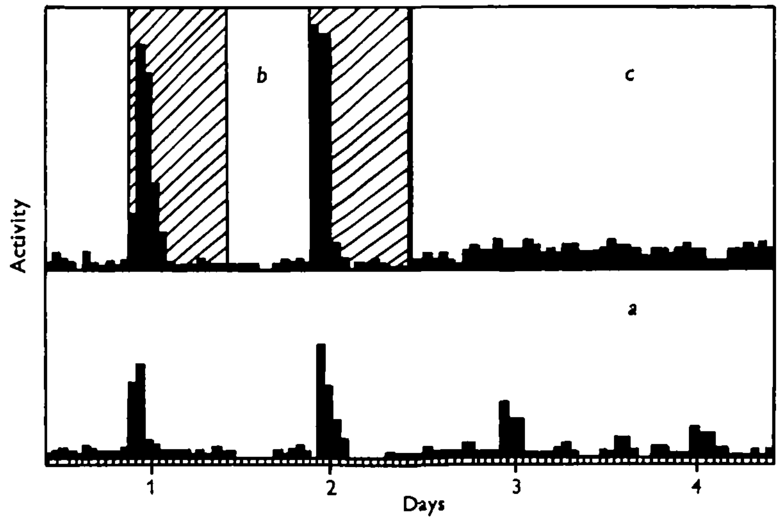

Fig. 3. (*a*) The activity of a parabiotic pair of cockroaches in continuous light. The lower, mobile cockroach had previously been in continuous light and had shown the arrhythmic activity recorded in (*c*), the upper cockroach had previously been in alternating light and darkness and had shown the rhythm of activity recorded in (*b*). Dark periods hatched.

A cockroach which had previously been in alternating light and darkness, and had a normal rhythm of activity, was joined in parabiosis with another which had been in constant light for the whole of its life, and showed no rhythm of activity. The arrhythmic cockroach was the lower and mobile one of the pair. The pair were left in constant light and their activity measured. They were found to show a rhythm similar to that previously shown by the uppermost, immobilized cockroach (Fig. 3).

If left in constant light this rhythm was gradually lost, as is that of any cockroach in constant light.

If two cockroaches were joined together in the reverse position, the mobile one being that from conditions of alternating light and darkness, then the pair still showed a normal rhythm of activity.

Painting over the eyes and ocelli has been found to cause a loss of rhythm (Cloudsley-Thompson, 1953). Two cockroaches which had previously been kept in constant light and were arrhythmic were joined together in parabiosis, the eyes and ocelli of the mobile one were painted over and the pair placed in alternating light and darkness. A normal rhythm of activity developed (Fig. 4). Controls were tested in which both cockroaches of the pair had had the eyes and ocelli blackened, in this case no rhythm was developed.

It would appear from this set of experiments that a secretion, carried in either the blood or tissues, is involved in the production of the diurnal rhythm.

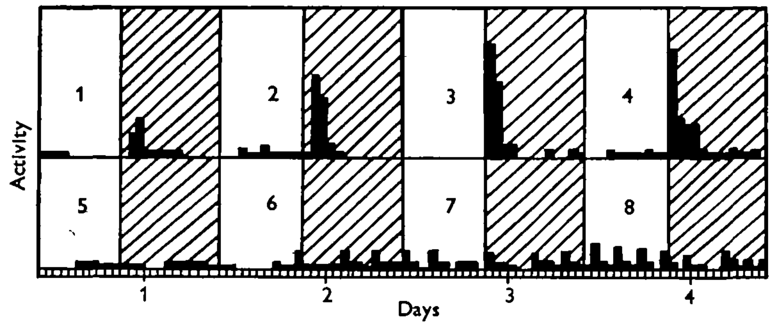

Fig. 4. The activity of a parabiotic pair of cockroaches in alternating light and darkness. Both had previously been in continuous light and the eyes and ocelli of the lower, mobile one, were blackened.

(*b*) *Neurosecretory organs in relation to the activity rhythm*

P. americana will go on moving about quite actively for up to 10 days after decapitation. The activity of headless cockroaches has been measured and found to show no rhythm (Fig. 5*a*).

Pairs of sub-oesophageal ganglia taken from cockroaches with normal activity rhythms were implanted, one each, into headless cockroaches. The implant was made in the abdomen just lateral to the dorsal heart. The implanted cockroaches were placed in continuous light. Their subsequent activity is shown in Fig. 5*b*. On the first day after the operation the headless implanted cockroaches showed a normal rhythm of activity. This disappeared during the second and third day, although in four experiments it continued for 4 days. The appearance of this normal rhythm after implantation was not altered by the light conditions; cockroaches were tested in continuous light and darkness, and in reversed light and darkness, and still showed the rhythm of activity previously shown by the donor of the sub-oesophageal ganglia.

Sub-oesophageal ganglia were implanted into entire cockroaches, one being placed in each. These cockroaches under conditions of alternating light and darkness continued to show a normal activity rhythm, but the level of the activity during the dark period was considerably lower than before the operation (Fig. 6*a*). There were present in the animals two sub-oesophageal ganglia after the operation,

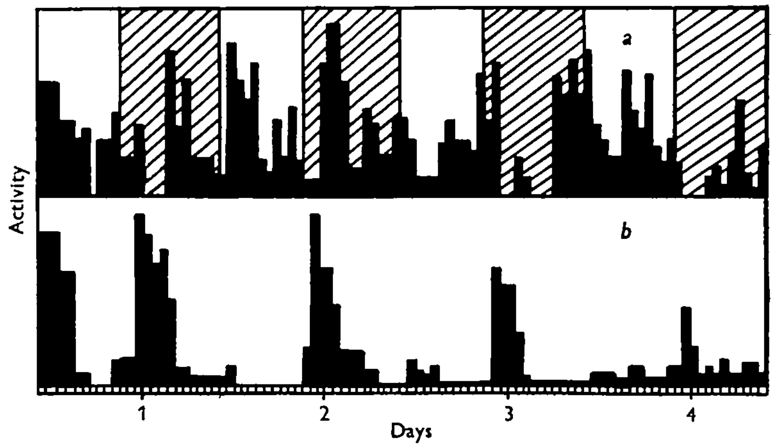

Fig. 5. (*a*) The activity of a headless *P. americana* in alternating light and darkness. (*b*) The activity in continuous light of a headless cockroach into which had been implanted the sub-oesophageal ganglia from a normally rhythmic cockroach.

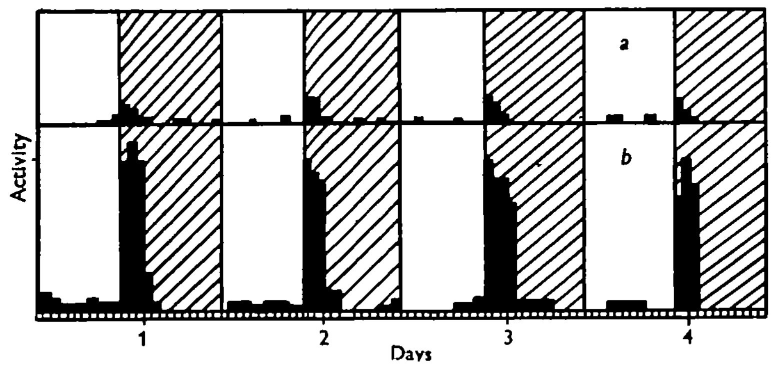

Fig. 6. (*a*) The activity of an entire *P. americana* into which had been implanted two sub-oesophageal ganglia from normally rhythmic cockroaches. (*b*) The activity of a headless cockroach into which had been implanted two sub-oesophageal ganglia.

therefore as a control experiment sub-oesophageal ganglia from normal animals were implanted two at a time into headless cockroaches. No depression of activity was noted during the active period in these animals (Fig. 6*b*).

Sub-oesophageal ganglia from animals with a normal rhythm were implanted, one per animal, into entire cockroaches which had been in constant light; that is, into cockroaches with the sub-oesophageal ganglia present, but which were not showing a rhythm of activity. These animals took up a rhythm of activity with a normal level of activity being reached in the active period.

The corpora allata were removed from animals with normal activity rhythms. This did not appear to affect the rhythm. When the corpora cardiaca were removed from normal cockroaches there was found to be no change in the rhythm while the cockroaches were in alternating light and darkness, but when placed in constant light there was no carry-over of the rhythm (Fig. 7). The total activity in the absence of the corpora cardiaca is considerably higher, however, than in a normal cockroach, and it is possible that this is masking any diurnal rhythm.

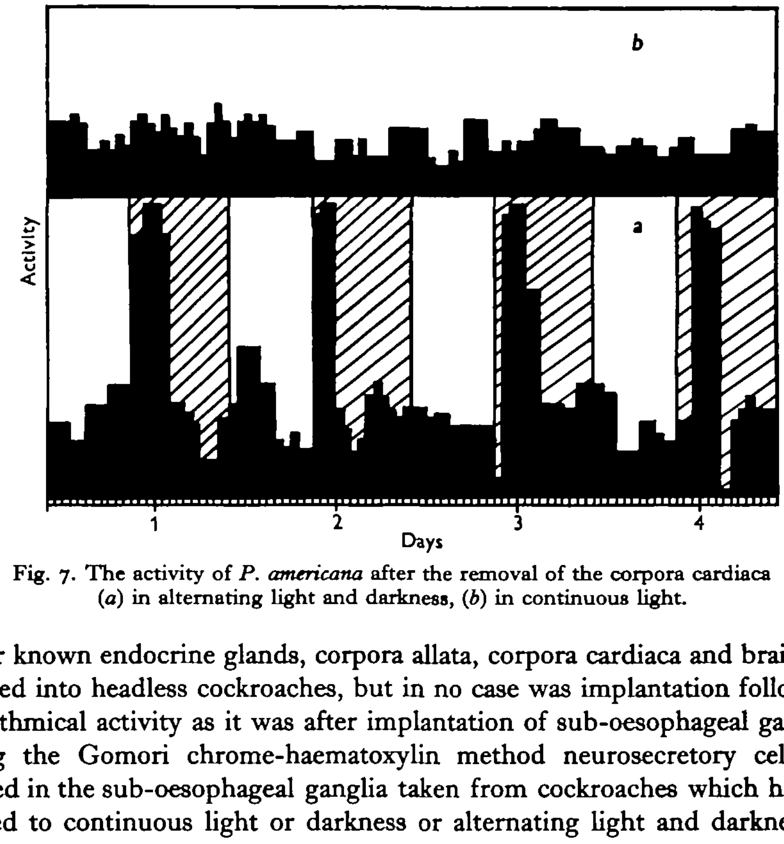

Fig. 7. The activity of *P. americana* after the removal of the corpora cardiaca (*a*) in alternating light and darkness, (*b*) in continuous light.

Other known endocrine glands, corpora allata, corpora cardiaca and brains were implanted into headless cockroaches, but in no case was implantation followed by any rhythmical activity as it was after implantation of sub-oesophageal ganglia.

Using the Gomori chrome-haematoxylin method neurosecretory cells were examined in the sub-oesophageal ganglia taken from cockroaches which had been subjected to continuous light or darkness or alternating light and darkness. No differences in their content could be detected.

(*c*) *The ocelli*

It would appear possible that the sub-oesophageal secretion might be governed by some sensory organ which is sensitive to light or darkness.

Cloudsley-Thompson has shown that blacking out the eyes and ocelli will cause a loss of rhythm. The compound eyes alone were covered in a group of ten animals which had previously been in constant light and were arrhythmic. They were then placed in alternating light and darkness and their activity measured. They all showed a normal rhythm after 2 days, and there was a carry-over of the rhythm when they were transferred back to continuous light. The optic nerves were cut in another series of animals from continuous light, and six out of ten animals showed a rhythm under alternating light and darkness, and this continued when the animals were removed to continuous light.

The ocelli alone were painted over in another series of animals which had been in alternating light and darkness. The cockroaches then showed a gradual loss of the original activity rhythm and an active phase appeared in the light period. The new rhythm was not one which could be carried over under new conditions. The peak of activity during the light phase was eliminated when the compound eyes were also blackened (Fig. 8).

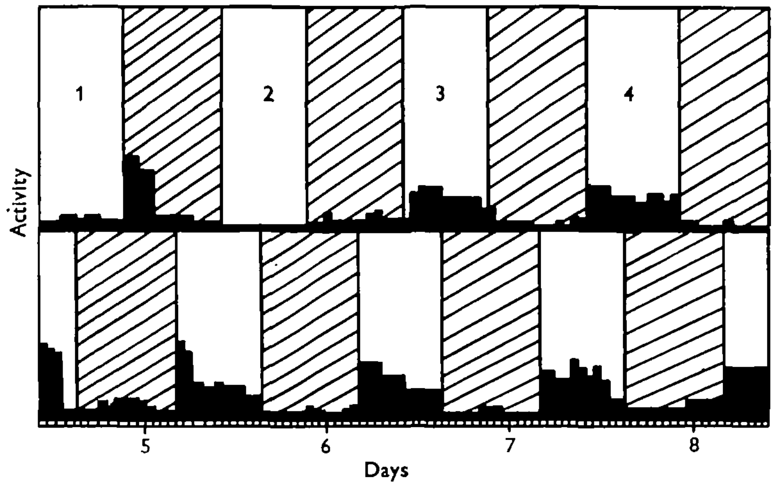

Fig. 8. The activity of *P. americana* in alternating light and darkness after the ocelli had been blackened.

SUBSIDIARY RHYTHMS

In the course of these experiments there has been observed another rhythm of activity subsidiary to the main diurnal rhythm. All the activity records of intact animals, and those of some of the operated or beheaded animals, show peaks of activity at regular intervals varying between 30 and 60 min. These are particularly obvious in the less active periods, but are not so evident when the animals are at the peak of their activity. This rhythm closely approximates to a similar periodicity in the oxygen consumption of resting cockroaches (Roeder, 1953).

DISCUSSION

The type of rhythm which has been studied in this work is one which can persist for a time without any reinforcement by the extrinsic conditions originally necessary for its establishment. That is, there appear to be concerned in the rhythm not only outside governing factors, but also an 'internal clock' with an ability to keep its own time.

It would seem fairly clear from the experiments that at least part of the mechanism of the internal clock is an endocrine secretion released by the sub-oesophageal ganglia, and that this is governed by the extrinsic factors of light and darkness through the medium of the ocelli. The sub-oesophageal ganglia themselves appear to be the 'retainers' of the rhythm in that they can be completely isolated (implantation experiments) and yet continue to secrete at definite times. This may indicate a rhythm of exhaustion of secretion, but so far experiments have revealed no differences in extracts taken from the sub-oesophageal ganglia at various times.

The mechanism through which the secretion acts is not known, nor is it by any means sure that activity occurs at the time of secretion. Should the secretion be inhibitory then the animal may be active only in its absence, and there is some evidence for it being inhibitory, at least in high concentration, under certain conditions. This is evident when the sub-oesophageal ganglia from normally active cockroaches are implanted into similarly active intact animals, the level of activity being considerably lowered in the subsequent active phases. On the other hand, there is no evidence of inhibition when two sub-oesophageal ganglia are implanted into a headless animal. Perhaps this indicates that other factors are involved in the intact animal in addition to a direct secretion from the sub-oesophageal ganglia, a view also supported by the results of the experiments in which the corpora cardiaca were removed.

If the secretion promotes the active phase, then, since activity continues for only a limited time in the dark period, either the secretion ceases to be effective or is exhausted, or a fatigue product is produced as the result of activity. This last possibility would also account for the slight increase in activity after about 6 hr. in light when the fatigue product would be decreasing.

Light acting through the compound eyes is probably stimulatory to a certain degree as was shown in experiments in which the ocelli alone were darkened. Cloudsley-Thompson (1953, p. 707) has shown that after a period of continuous darkness light causes a great increase in activity.

The reception by the ocelli of the extrinsic factor concerned in the rhythm may be taken as added evidence for the secretion taking place during the dark period (implying also that it causes activity). For Hoyle (1955) has shown that there is a continuous discharge of nerve impulses in the circum-oesophageal commissures when the ocelli are in darkness, and it is suggested that it is in this way that the sub-oesophageal ganglia receives its external control.

SUMMARY

1. By using a direct method of recording activity the diurnal rhythm of activity of *Periplaneta americana* L. has been investigated in detail under various conditions of light and darkness.

2. It has been found that there is no direct connexion between a hunger cycle and the activity rhythm.

3. By means of experiments in which cockroaches were joined together in parabiosis it has been shown that a rhythm can be relayed from one insect to another, and is postulated that a secretion carried in either the blood or tissues is responsible for the transmission.

4. Implantation of sub-oesophageal ganglia from cockroaches with a known rhythm into headless cockroaches has been found to result in the appearance of the known rhythm of activity in the latter.

5. Effects of other endocrine organs on the activity rhythm has been investigated.

6. The ocelli have been found to be directly connected with the establishment of the rhythm by the external factors of light and darkness.

7. A rhythm of activity subsidiary to the main diurnal rhythm is described.

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