STUDIES ON THE PHYSIOLOGICAL ADJUSTMENT OF THE HEARTBEAT FREQUENCY *IN VITRO* AND *IN VIVO* AND ACETYLCHOLINE LEVELS IN ACCLIMATIZED *PERIPLANETA AMERICANA* (L.)

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ABSTRACT

The phenomenon of acclimatization was studied at the upper and lower threshold levels of temperatures, *i.e.* 33°C and 15°C, in cockroaches. Dramatic physiological changes occurred in the first twenty four hours at both the acclimatization temperatures. *In vitro* studies on the heartbeat frequency revealed a variation during the course of acclimatization at both the temperatures. However, when the frequencies stabilized, a unique feature was a decreased heartbeat frequency of insects acclimatized to 33°C was close to that of insects reared at ambient temperature (control experiments). The acetylcholine levels varied during the course of acclimatization stabilizing to levels very close to normal at both the temperatures. The effect of the neurotransmitter and its activation at different temperatures is also discussed. The period of acclimatization was found to be 25 days at 33°C and 35 days at 15°C for both the sexes.

INTRODUCTION

INSECTS are known to be able to acclimatize to different temperatures as a result of which the position of their chill-coma points and thermal death points may be altered[1–4]. For each insect species there is a threshold temperature below which acclimatization does not occur, and this threshold temperature may be far above the chill-coma temperature or below the thermal death point. Two types of acclimatization, development and physiological, have been demonstrated[5], however, insects reared at a particular temperature throughout their life histories are not true acclimatized insects[6]. Temperature acclimatization would therefore be different from the temperature at which the insects are reared[7,8].

Metabolic changes, tissue composition and enzyme system have been studied in insects to probe into the mechanism of acclimatization[9–12], but the data are not systematic. Also the period required for acclimatization has only been partly investigated by different workers[13–18], but the parameters reported are different for each author. The neuro-transmittor acetylcholine is known to be present in high concentrations in insects when compared with vertebrates and other invertebrates. But very little is known about the titre of this important factor in relation to temperature acclimatization. Since acetylcholine regulates receptor mediator changes in the transmembrane potential of nerve and muscle cells, it warrants further research. Hence this investigation was undertaken to study the phenomena of acclimatization with respect to different parameters and the period required for physiological stabilization at different temperatures.

MATERIALS AND METHODS

The cockroach *Periplaneta americana* L. reared at 28°C under standard laboratory conditions was used in all the experiments. Groups of 25 each were picked up from stock colony and were kept at 33°C and 15°C in temperature controlled cabins for acclimatization. The threshold levels of temperatures at the upper and lower range were carefully examined before selecting these temperatures for acclimatization.

In vitro techniques for the study of heartbeat frequency (Hbf)

The heart preparations of normal and acclimatized cockroaches were studied employing the semi-isolated heart-technique[19,20] at their acclimatization and normal temperatures. Five to ten different heart preparations were used for each test and the average values were tabulated and mapped.

In vivo experiments on Hbf.

Cockroaches were fixed in depressions carved in a wax plate, with its dorsal side up, the wings spread and pinned so that they did not cover the abdomen. After 15–20 min when the insect had settled, the Hbf was recorded with the help of a binocular microscope and spotlight.

Chemical determination of acetylcholine (Ach)

Acetylcholine chloride was estimated colorimetrically[21,22]. The haemolymph of the insects was collected by the centrifugation method[23]. For each determination ten males and ten females were used and samples were prepared in triplicates. The average of 3–5 replicates of each assay were recorded at different acclimatization temperatures and at control temperature of 28°C.

RESULTS

Hbf in control insects in vivo and in vitro

The average heartbeat was 155/min in males and 134/min in females both *in vivo* and *in vitro* studies; hence further work was restricted to *in vitro* technique.

In vitro studies on Hbf of cockroaches acclimatized to 33°C

The Hbf of the male cockroaches dropped to 125/min in 24 hours of acclimatization at 33°C, followed by irregularity which lasted for 25 days after which the Hbf stabilized, figure 1.

Unlike in males the Hbf of the females increased to 160/min after the first 24 hr at 33°C. The Hbf was found to be irregular attaining a maximum frequency on the 11th day as in the case of males before stabilization at 140/min after 25 days, figure 1.

In vitro studies on Hbf of cockroaches acclimatized at 15°C

The Hbf of male and female insects showed a dramatic decline to 35/min and 50/min respectively 24 hr after acclimatization at 15°C. As acclimatization proceeded the Hbf became irregular through a period of more than 30 days after which an almost regular beat of 38–40/min was established in both the sexes, figure 2.

In vitro studies of Hbf at 28°C of cockroaches acclimatized at 33°C and 15°C.

As shown in figure 3, considerable variations in Hbf occurred in insects acclimatized at 33°C and tested at 28°C. After 30 days the Hbf of both male and female insect was almost stabilized, the Hbf of male insects exceeded that of the female insects by 5 or 6 beats/min.

The Hbf decreased in both the sexes 24 hr after acclimatization at 15°C to 103/min in males and 99/min in females, figure 4 on continuous acclimatization Hbf stabilized after 35 days.

Ach content of normal cockroaches maintained at ambient temperature 28°C

The Ach content in males (0.1307 mg/ml) is more than in females (0.1118 mg/ml).

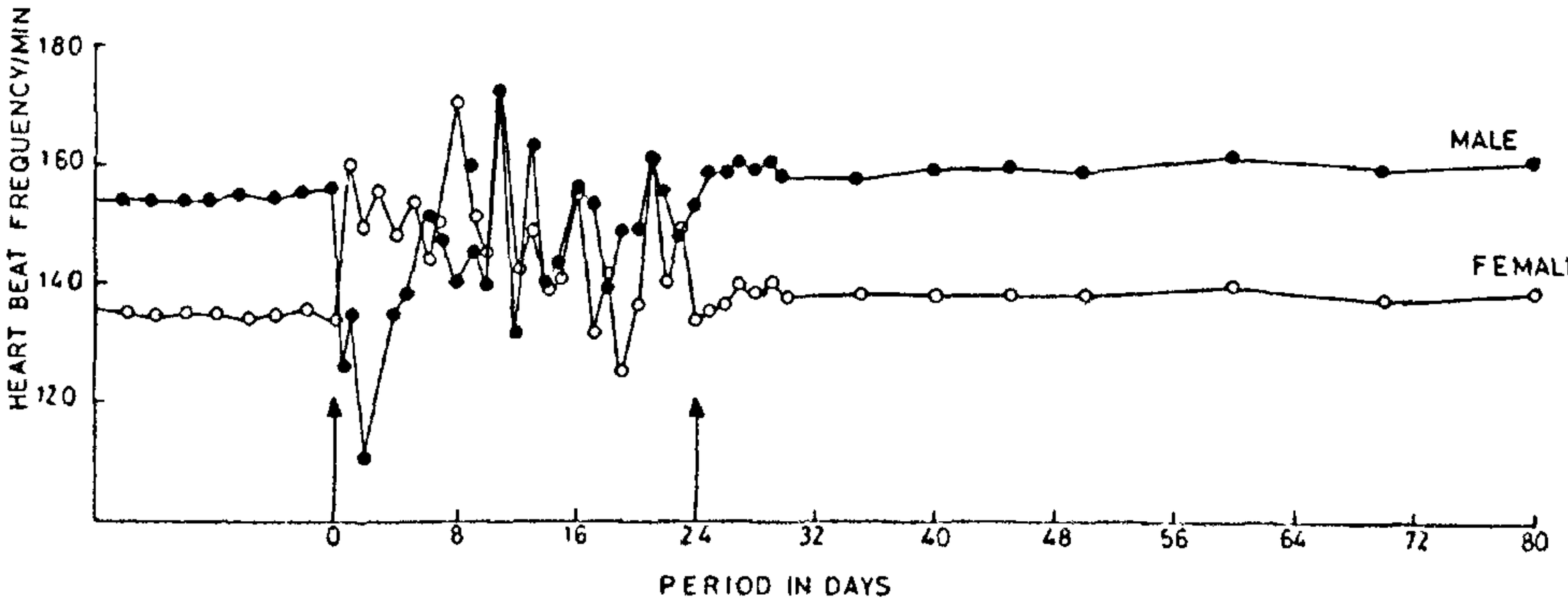

Figure 1. Heartbeat frequency of male & female cockroaches acclimatized and tested at 33°C.

Note: In all the graphs the values represented before the first arrow indicate the Hbf before acclimatization. The points between arrows indicate the Hbf during the period of acclimatization, and the values after the second arrow indicate the Hbf after acclimatization.

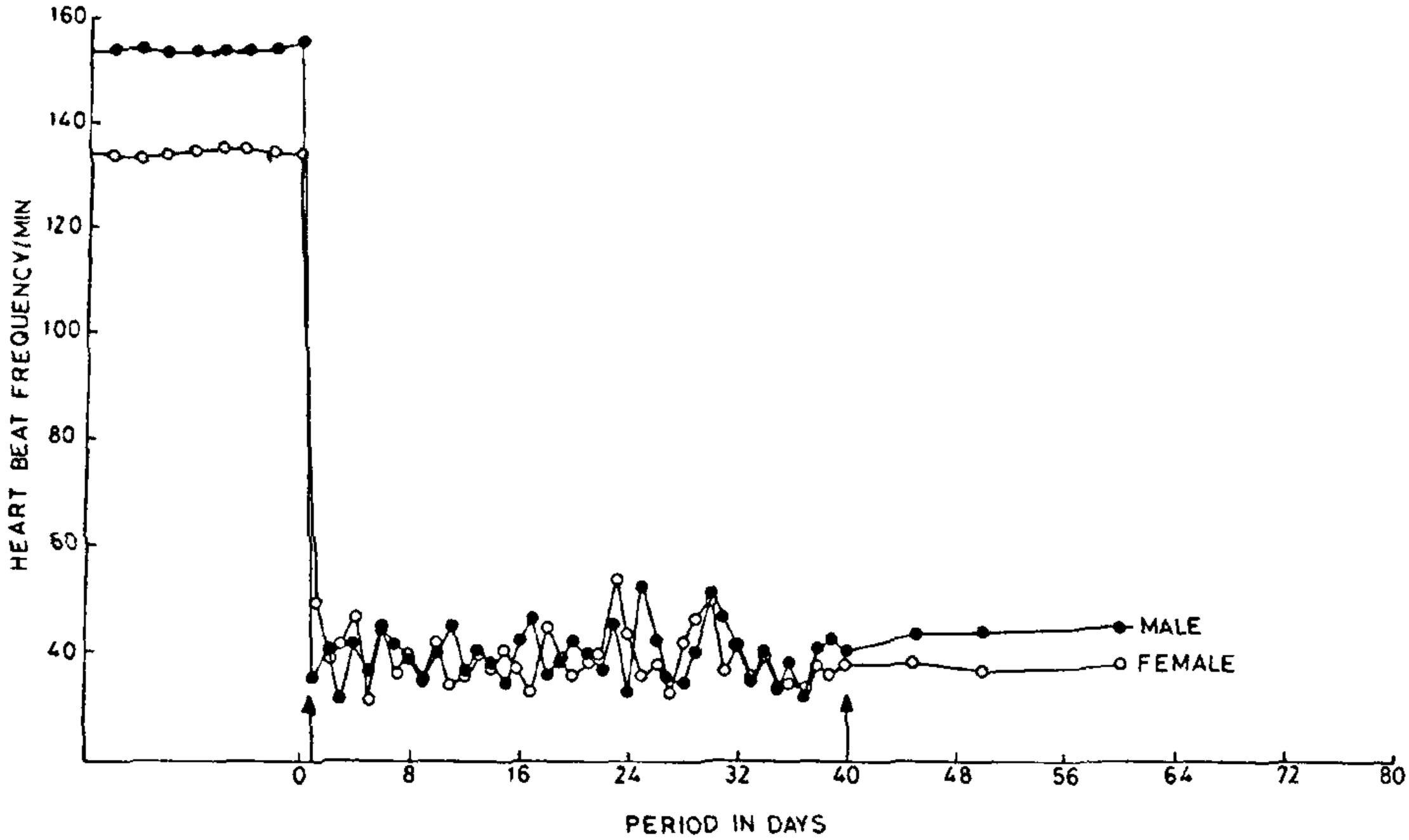

Figure 2. Heartbeat frequency of male and female cockroaches acclimatized and tested at 15°C.

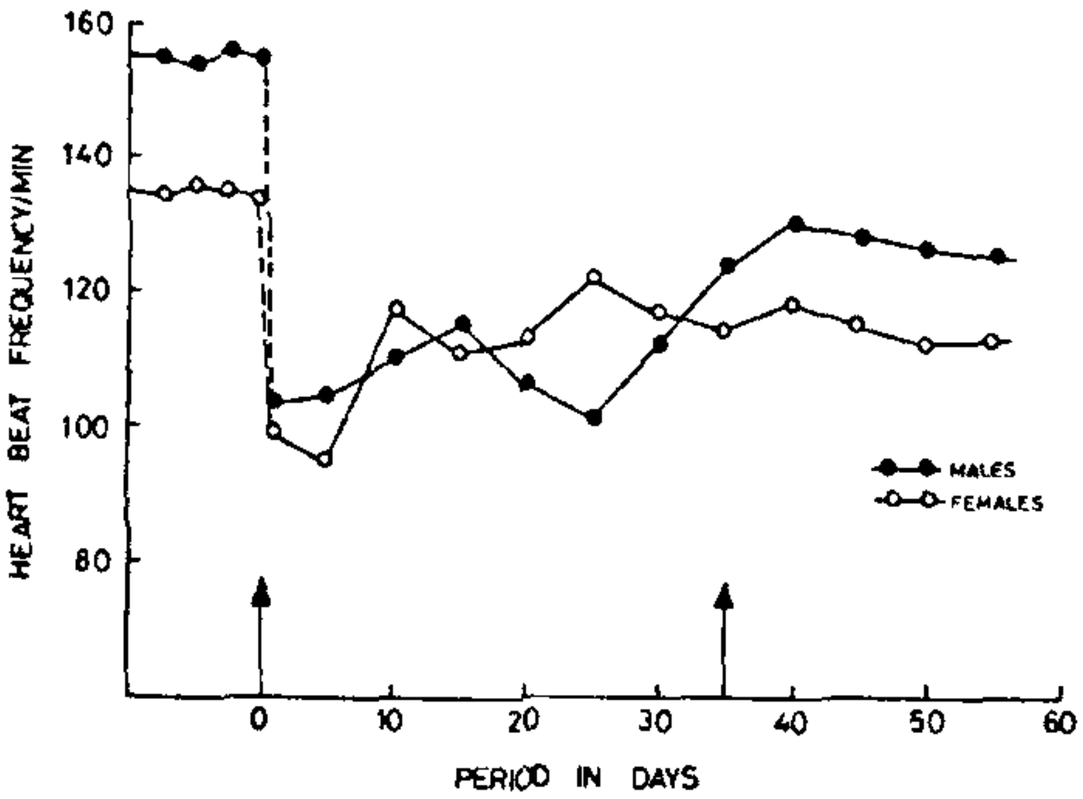

Figure 3. Heartbeat frequency of male and female cockroaches acclimatized at 33°C and tested at 28°C.

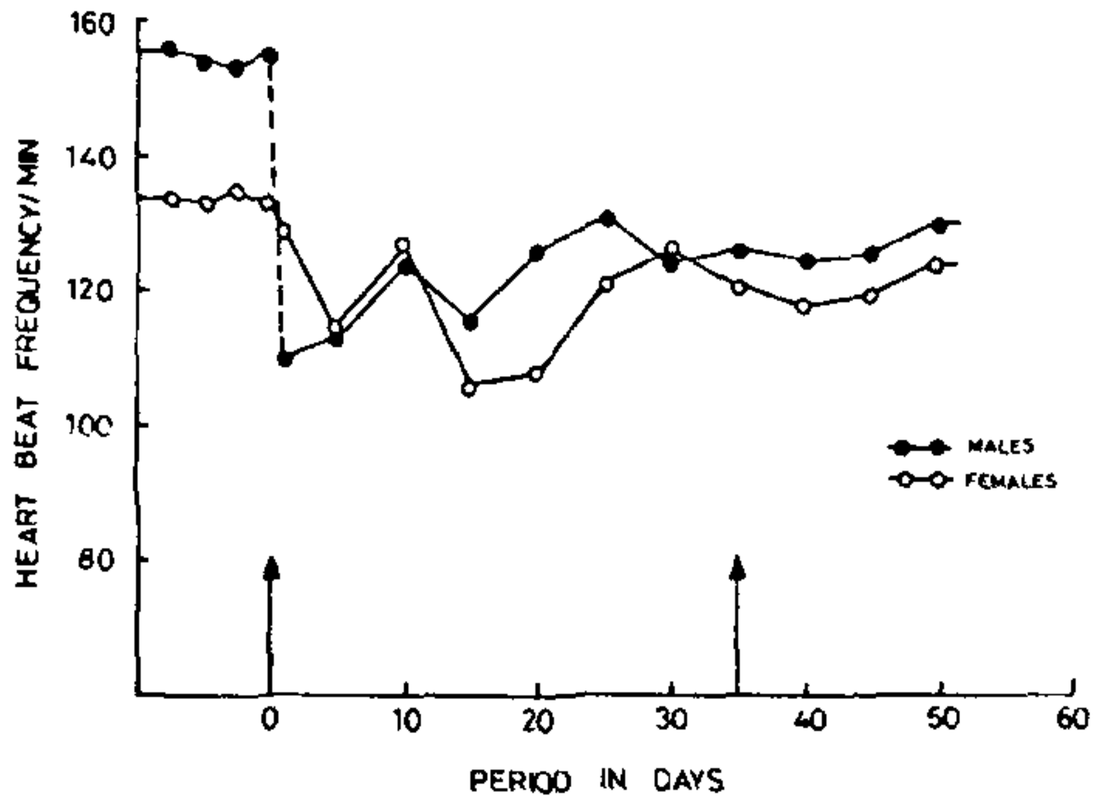

Figure 4. Heartbeat frequency of male and female cockroaches acclimatized at 15°C and tested at 28°C.

Ach content in cockroaches at different periods of acclimatization at 33°C and 15°C

An increase in the Ach content of females was observed on day one of acclimatization to 33°C, in contrast to the decline in males as compared to normal insects. On the 25th day, the Ach content was 0.1118 mg/ml in normal and 0.1344 mg/ml in females acclimatized at 33°C. The amount of Ach remained almost at this level thereafter. However, in males acclimatized to 33°C an increase in Ach was found on the 10th day of acclimatization. After the 25th day the Ach content stabilized to about 0.1362 mg/ml (table 1).

Table 1 *Amount of Ach estimated in the haemolymph of male and female cockroaches acclimatized at 33°C.*

Period of acclimatization in days	Ach mg/ml male insects	Relative sensitivity	Ach mg/ml female insects	Relative sensitivity
1	0.1162 ±.00173	1.00	0.1344 ±.00707	1.158
10	0.1562 ±.00122	1.34	0.1217 ±.00820	1.049
20	0.1416 ±.00581	1.21	0.1307 ±.00992	1.126
25	0.1344 ±.00423	1.15	0.1162 ±.00145	1.00
30	0.13621 ±.00900	1.17	0.1160 ±.00628	1.00
40	0.1360 ±.00269	1.17	0.1162 ±.00447	1.00
50	0.1364 ±.00166	1.17	0.1162 ±.00815	1.00

The Ach content of cockroaches increased considerably on day one of acclimatization at 15°C, more so in females (table 2). As acclimatization proceeded a decrease in Ach content was observed until the 20th day. However, after 20 days the Ach content again tended to increase, and on the 40th day it was found to be 0.1326 mg/ml. After this period, the amount of Ach stabilized in both sexes.

DISCUSSION

An important finding which emerges out of this investigation is that the physiological parameters remain in a state of flux during the period of acclimatization at the temperatures studied. But as acclimatization sets in, these parameters get stabilized at their respective levels and frequencies. The sensitivity of temperature changes in insects has been clearly demonstrated in the experiments on Hbf. Irregularities in the Hbf were also seen in cockroaches acclimatized to 33°C and 15°C when examined at 28°C. Hbf has also been reported as an indicator of general metabolism[26]. Although sexual difference in the Hbf of nymphs of *Periplaneta* was not observed earlier[17], the present study shows that the adult males have consistently higher rates of Hbf than adult females. Similar observation has also been reported in newly moulted cockroaches and last instar nymphs of either sex which do not exhibit any sex difference in Hbf[24]. The sex difference in the Hbf of male and female cockroaches sets in six days after final moult which continues thereafter[24]. This is approximately the same age when muscle biochemistry becomes stabilized. This finding is of significance considering the fact that alary muscles play an important role in the heartbeat.

The significance of the difference in the Ach levels at both the acclimatization temperatures and the very little sex difference in the Ach levels when compared to normal insects, could be attributed to the possible role of isozymes of AchE[25] in temperature adaptation. One thing common between these parameters (Hbf and Ach) is that there is a deviation from normal and

Table 2 *Amount of Ach estimated in the haemolymph of male and female cockroaches acclimatized at 15°C.*

Period of acclimatization in days	Ach mg/ml male insects	Relative sensitivity	Ach mg/ml female insects	Relative sensitivity
1	0.1780 ±.00320	1.342	0.1907 ±0.00300	1.75
10	0.1671 ±.00884	1.260	0.1126 ±0.00640	1.03
20	0.1562 ±.00186	1.177	0.1089 ±0.00195	1.00
30	0.1671 ±.00195	1.260	0.1289 ±0.00766	1.18
40	0.1326 ±.00428	1.00	0.1326 ±0.00686	1.21
50	0 1344 ±.000818	1.01	0.1217 ±0.00192	1.11
60	0.1326 ±.00263	1.00	0.1235 ±0 00500	1.13

the extent of deviation is statistically significant. It appears that the levels of Ach does not affect the frequency of heartbeat, their variations are independent of each other. At lower temperatures there is an increase in Ach, and decrease in Hbf, while at higher temperatures there is an increase in Ach as well as Hbf. Hence it can be concluded that the variation of Ach at different temperatures is seen to a lesser extent while there is a dramatic variation in the Hbf. At the lower temperature due to a decreased O_2 demand and a consequent decrease in metabolic activity, there is a lower Hbf seen. However, at higher temperature the increase in metabolic turnover leads to an increase in Hbf. Therefore, it can be concluded that Hbf could be a valuable indicator for acclimatization as it becomes an indicator for general metabolism.

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NEWS

ACTIVISTS RAID ANIMAL RESEARCH LAB

. . . "A group calling itself the Animal Liberation Front [ALF] has broken into a laboratory at U. Pennsylvania, vandalizing equipment worth thousands of dollars and stealing 20 videotapes of experiments on baboons. . . . Thomas Langfitt, principal investigator of the research programme, defended the [research] as humane and vital for the improvement of the treatment for head injuries. The injuries are induced by accelerating the baboons' heads, without impact. Langfitt said the research has already revealed the mechanism by which damage occurs in such accidents; results have led to mathematical and physical models of the axons and blood vessels in the brain during injury and to the design of improved crash helmets. Langfitt said that the baboons are tranquillized and lightly anaesthetized during the procedure. All the animals are eventually destroyed." [(Stephen Budiansky in *Nature* 309(5968):487, 7 Jun. 84) (Reproduced with permission from Press Digest, *Current Contents* ®, No. 42, October 15, 1984, p. 13. Published by the Institute for Scientific Information ®, Philadelphia, PA, USA.)]