

well-poised negative O-R potential in combination with a reducing agent.

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1. Chang, S. L., *Parasitology*, 1946, 37, 101.

2. Diamond, L. S., *Science*, 1961, 134, 336.
3. — and Bartgis, I. L., In *Progress in Protozoology*, Excerpta Med., Int. Congr. Ser., No. 91, 1965, p. 102.
4. Wittner, M., *J. Protozool.*, 1968, 15, 403.
5. Montalvo, F. E., Reeves, R. E. and Warren, L. G., *Exp. Parasit.*, 1971, 30, 249.
6. Diamond, L. S., *J. Parasitology*, 1968, 54, 1047.
7. Reed, G. B. and Orr, J. H., *J. Bact.*, 1943, 45, 309.

MECHANISM OF VENTILATORY MOVEMENTS IN *TESSARATOMIA JAVANICA* AND THEIR RECORDING TECHNIQUES

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ABSTRACT

The mechanism of ventilation has been studied in *Tessaratomia javanica* (Thunberg). The expiration is due to contraction of the tergosternal muscles and inspiration is due to elasticity of the pleuron and dorsal membrane. For recording and analysing these movements, optical device is the most suitable one as compared to mechanical or oscilloscopic devices, used. The expiration time is greater than the inspiration time and the tetanic fusion frequency of the tergosternal muscles is in the range of 100-120 c/s.

INTRODUCTION

THE respiratory movements in insects are being studied in order to understand the relevant physiological factors. For recording these movements, different methods such as those of mechanical levers, strain-gauge transducers and the pressure changes in the enclosure surrounding the abdomen have been used (Miller, 1966). In the present investigations the ventilatory mechanism of *Tessaratomia javanica* (Thunberg), a pentatomid bug, has been described and the ventilatory movements have been studied by using different techniques. *T. javanica* being a large terrestrial bug appears to be a suitable model for the analysis of various ventilatory parameters, such as expiration, inspiration, twitch interval and the different factors that effect the ventilation.

MATERIALS AND METHODS

Adult males and females of *T. javanica*, the soapnut bugs were collected from the soapnut trees in and around Hyderabad. Insects were maintained in the rearing cages at room temperature (27°C) and they survive for several weeks. For morphological studies, a piece of abdominal

sternum with segmental mixed nerve, spiracular muscle and tergosternal muscle was dissected and fixed. These preparations were stained with borax carmine or haematoxylin, dehydrated as usual, cleared and mounted in Canada balsam.

In the present study the ventilatory movements have been recorded by using mechanical, oscillographic and optical devices. Although each of the three recording techniques mentioned suits a particular experimental set up it has its inherent limitations which are likely to impair the accuracy of the experimental results. Therefore in the present studies, the results obtained by the three techniques are analysed and compared with a view to fix the most accurate technique for recording the ventilatory movements.

PROPOSED MECHANISM FOR ABDOMINAL MOVEMENTS

In *T. javanica* the ventilatory movements are due to the down and up movements of the abdominal tergum. There are six pairs of expiratory tergosternal muscles in the abdomen and inspiratory muscles are absent. The transverse section of the abdomen (Fig. 1) shows that each bundle

of the muscle occupies a position between the abdominal spiracle and trichobothria. However, the trichobothria are absent below the first and the last abdominal spiracle. Each bundle consists of short and long (750–1,200 μ) but thick (60–200 μ) parallel fibres with distinct striations and the muscle has its origin on the sternum and insertion on the tergum. The tergum and the sternum are articulated by the elastic pleuron. The segmental mixed nerve which arises from the abdominal nerve cord or the thoracic ganglion splits into two main branches. The thick motor branch gives small branches to the spiracular

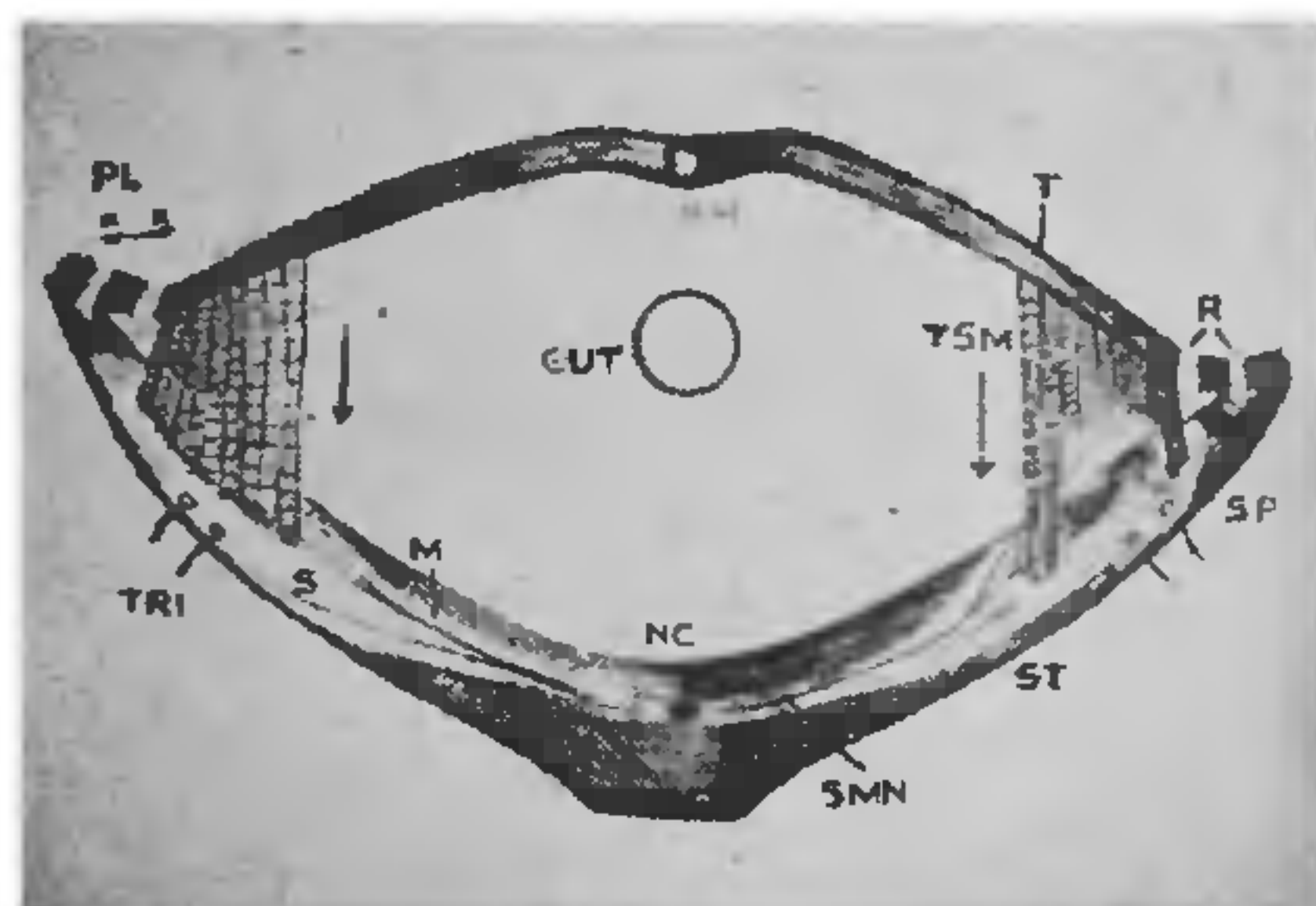


FIG. 1. T.S. of Abdomen *T. javanica* (Schematic). (H, Heart; M, Motor nerve; NC, Nerve cord; PL, Pleuron; R, Resilin; S, Sensory nerve; SMN, Segmental mixed nerve; SP, Spiracle; ST, Sternum; T, Tergum; TSM, Tergosternal muscle; TRI, Trichobothria.)

occluser muscle and then branches into the tergo-sternal muscle. The thin sensory branch innervates the trichobothria. The mechanism of the abdominal movements appears to be as follows:

During expiration, as a result of contraction of the tergo-sternal muscles and the stationary position of the abdominal sternum, the tergum moves down, the pleuron and the dorsal membrane—connecting the abdomen to the thorax—undergo visible elastic deformations and thus store energy. During inspiration the tergo-sternal muscles relax and the potential energy stored in the elastic pleuron and the dorsal membrane is converted into kinetic energy and the relatively rigid abdominal tergum goes back to its original position. The gaseous exchange takes place through the two pairs of thoracic spiracles having lip-type of closing mechanism and the seven pairs of abdominal spiracles having internal closing mechanism. By cutting the pleuron and the dorsal membrane or tergo-sternal muscles, the tergum falls down and the insect is unable to perform ventilatory movements but can fly for a little while and then hovers back

to the base. Large areas of the pleuron and the dorsal membrane give positive test for resilin (Andersen and Weis-Fogh, 1964).

RECORDING TECHNIQUES

The ventilatory movements are recorded by (i) Mechanical, (ii) Oscillographic and (iii) Optical method.

(i) In the mechanical method the bug is mounted on a 'V'-shaped stand and a delicate mechanical lever rests in the groove of a small copper plate mounted on the abdominal tergum. The other end of the lever writes on a smoked paper fixed to rotating kymograph drum.

(ii) In the oscillographic method the mechanical movements of the delicate lever are converted into electric potentials by interposing a liquid rheostat column between the lever and the oscilloscope (Bureš *et al.*, 1967) and the recorded ventilatory movements are photographed.

(iii) In the optical method a beam of light coming from a point source is focussed on to a small mirror fixed to the abdominal tergum of the bug. The reflected light is condensed by passing through a lens and is made to fall on a photographic film wrapped on the rotating drum of the kymograph (Fig. 2). The movements of the

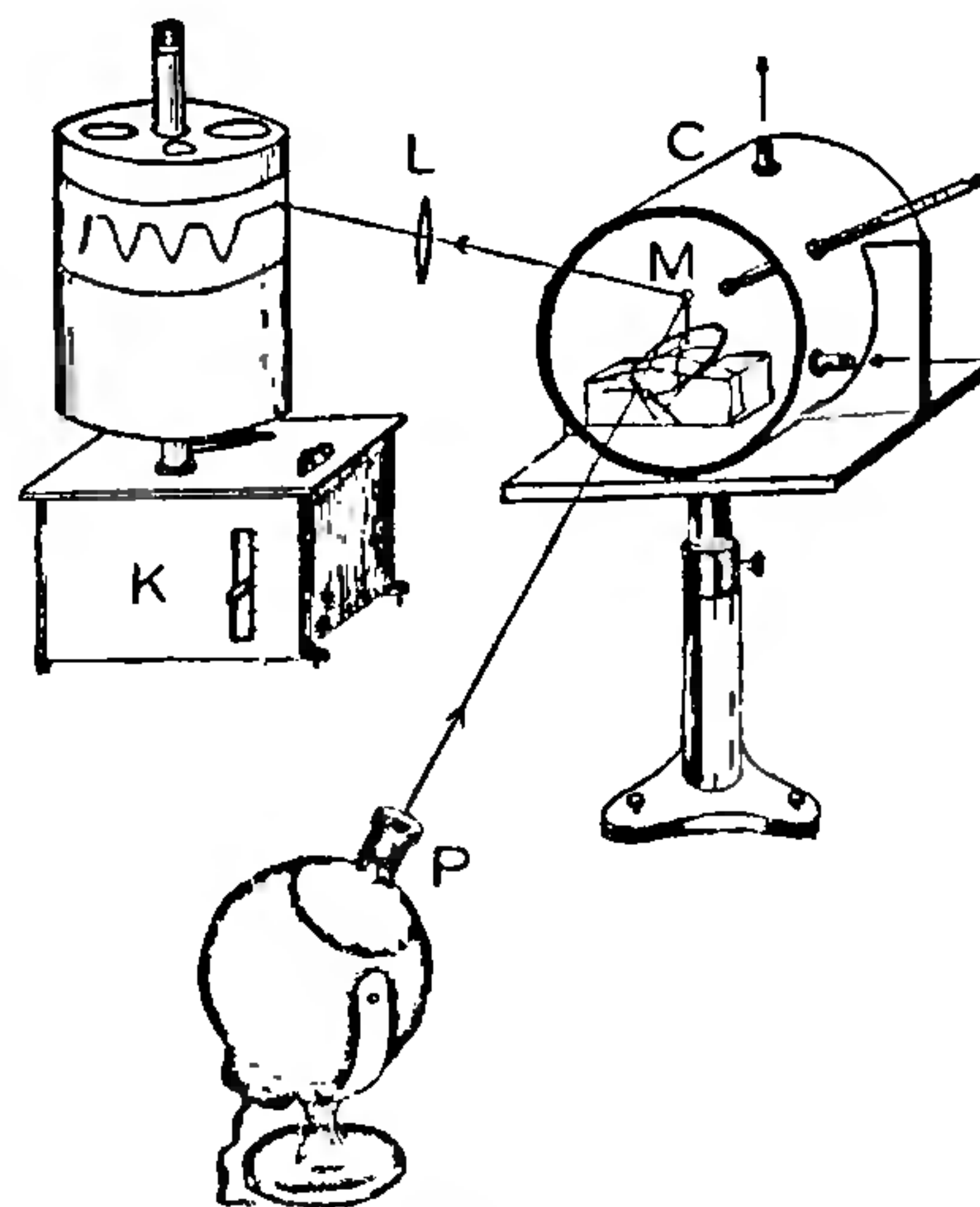


FIG. 2. Optical arrangement for recording ventilatory movements of *T. javanica*. (P, Point source of light; L, Lens; M, Mirror on the insect tergum; K, Kymograph; C, Double-walled chamber.)

TABLE I
Analysis of ventilatory movements of *T. javanica*

Techniques	Expiration time in m. sec. (E.T.)	Inspiration time in m. sec. (I.T.)	Total twitch time in m. sec. (T.T.)	Twitch intervals in m. sec. (I.T.)	E.T./I.T.	Students 't' test for pairs of E.T. and I.T.	Table value 't' at 5% level	Remarks
Mechanical ..	115 \pm 6	80 \pm 5	195	338	1.4	1.8	2.145	Not significant
Oscilloscopic ..	145 \pm 8	109 \pm 20	254	240	1.3	3.6	2.228	Significant
Optical ..	165 \pm 17	93 \pm 4	258	326	1.8	5.2	2.447	Significant

light spot is recorded as a reference line on the stationary drum and this reference line helps in eliminating the errors from the twitch curves. Similar precautions were taken in mechanical and oscillographic methods. The ventilatory parameters were calculated from the photograph.

The contraction properties of the tergosternal muscles were studied in the intact abdomen-semivivo preparation (decapitated, thoracic muscles and alimentary canal removed) by using the optical device and an INCO stimulator.

RESULTS AND DISCUSSIONS

For recording ventilatory movements fifty insects were used. As a representative case only the recordings from one experiment (Fig. 3) and the data showing the ventilatory parameters such as expiration time (E.T.), inspiration time (I.T.), total twitch time (T.T.) and twitch interval (T.I.) as analysed from the successive twitch curves has been shown in Table I.

The absence of the inspiratory muscles and the presence of elastic pleuron and the dorsal membrane which are positively stained for resilin test are interesting. The inability of the insect to perform the ventilatory movements after cutting the pleuron and the dorsal membrane or the tergosternal muscles leads to the inference that the resilin probably keeps the tergosternal muscles under isometric tension and hence the resilin appears to be *sine qua non* for the ventilatory movements. The complex innervation pattern in the abdomen suggests a feed back mechanism regulating the action of the tergosternal muscles (ventilatory movements) at the ganglionic level. The disappearance of the anisotropic bands and subsequent degeneration of the tergosternal muscles of *T. javanica* after fifteen days of denervation of the segmental mixed nerve as reported by Chari and Sarala Devi (1972) are noteworthy.

Although there are clear differences in the morphology of thoracic and abdominal spiracles of *T. javanica*, which help in gaseous exchange, each spiracle has an occlusor muscle, rigid lever and in

the absence of an antagonistic muscle the inset probably has an elastic opening hinge device.

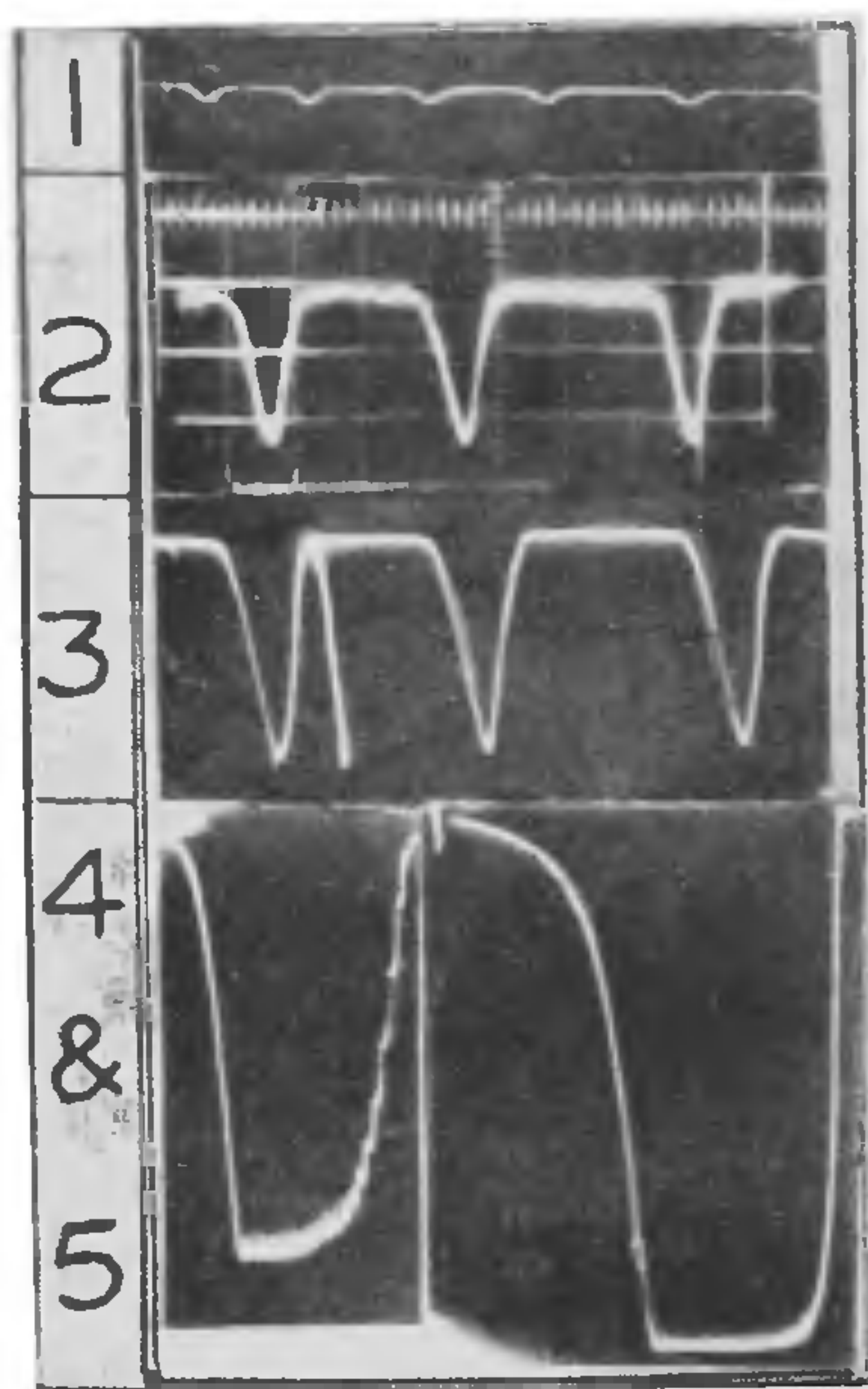


FIG. 3. Ventilatory movements of *T. javanica*. (1. Mechanogram; 2. Oscillographic recording; 3. Optical recording; 4. Tetanus—20 c/s; 5. Tetanus—100 c/s.)

Statistical analysis, taking pairs of E.T. and I.T. for successive twitches at room temperature (Table I), shows that the expiration time is greater than the inspiration time which is different as compared to contraction properties of slow and fast skeletal muscles of higher vertebrates and *Astacus* (Gutmann, 1966; Chari, 1970). In our studies the ratio of E.T. and I.T. for *T. javanica* ranges from 1.4 to 1.8. The expiration time and the inspiration time can be correlated to the contrac-

tion and relaxation of the tergosternal muscles. Since these muscles of *T. javanica* indicate an adaptation for showing series of discrete slow twitch movements, the relaxation time appears to be speeded up as suggested by Neville (1965) for the locust flight muscles. Neville and Weis-Fogh (1963) have regarded locust flight muscles as incomplete tetanus muscles. The authors in their studies have observed that the tergosternal muscles of *T. javanica* have tetanic fusion frequency in the range of 100 to 120 c/s (Fig. 3).

tory frequency varies from 1 to 3 c/s, whereas the wing-beat frequency ranges from 70 to 80 c/s during stationary flight and this probably helps in thoracic ventilation. After flight the abdominal ventilatory frequency increases three to four times or even more. It may also be noted that the ventilatory frequency is higher at 38° C than that at 18° C (Fig. 4). Thus the detailed studies on the contraction properties of the tergosternal muscles of *T. javanica* may throw some light on the behaviour and functional role of ventilatory muscles.

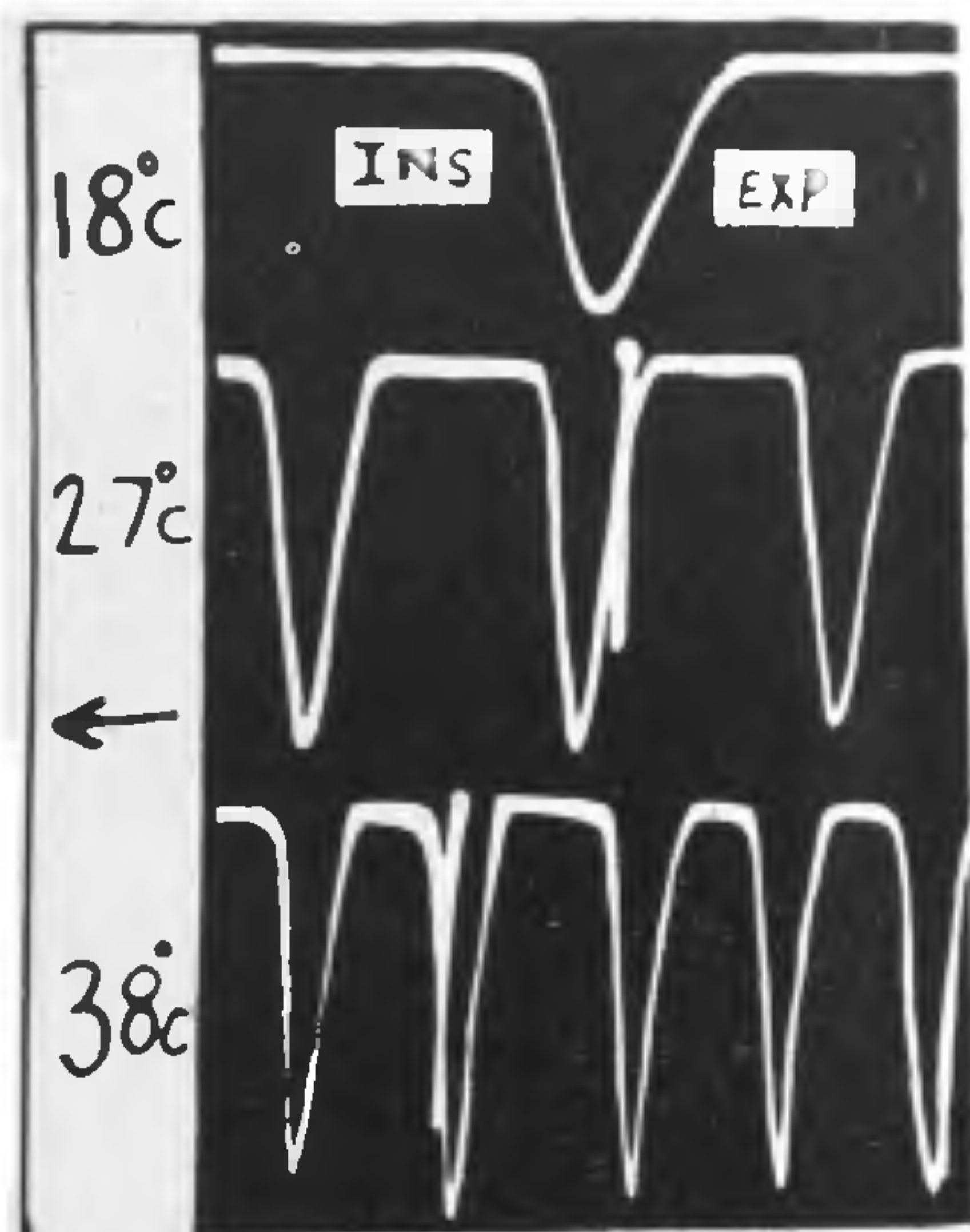


FIG. 4. Ventilatory movements of *T. javanica* at 18° C, 27° C and 38° C.

Thoracic ventilation has been reported in many insects (Miller, 1966). Preliminary studies on *T. javanica* clearly indicate the presence of thoracic ventilation during flight even after cutting the elastic pleuron and the dorsal membrane. We have observed that the normal abdominal ventila-

A comparative study of the results from the three techniques (Fig. 3; Table I) shows that the optical device is more suited for the analysis of the ventilatory parameters. The recondite feature of the optical method is that it is free from inertia, resistance, the tension of the lever as present in the mechanical method and the consequences of electrolytic deposition offered by the liquid rheostat column in the oscillographic method.

1. Andersen, S. O. and Weis-Fogh, T., *Advances in Insect Physiology*, Academic Press, New York, 1965, 2, 1.
2. Bures, J., Petran, M. and Zachar, J., *Electrophysiological Methods in Biological Research*, 1967, p. 392.
3. Chari, N., *CSC Thesis Submitted to Czech. Acad. Sci.*, Praha, 1970.
4. — and Sarala Devi, M., *The Association of Physiologists and Pharmacologists of India, XVIII Annual Conference*, 1972, p. 10.
5. Gutmann, E., *MCV Quarterly*, 1966, 2 (2), 78.
6. Miller, P. L., *Advances in Insect Physiology*, 1966, 5, 279.
7. Neville, A. C., *Sci. Progr.*, 1965, 53, 203.
8. — and Weis-Fogh, T., *J. Expt. Biol.*, 1963, 40, 111.