STUDIES ON THE BEHAVIOUR OF UNFED BLUE TICK LARVAE (*Boophilus decoloratus* (Koch)

by

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being a thesis submitted for the degree of Master of Science, Rhodes University.

JANUARY, 1963.

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ACKNOWLEDGEMENTS

I should like to express my thanks to Professor D.W. Ewer for all his help and encouragement during the course of this work. My thanks are also due to members of the Departments of Zoology at Rhodes University and the University College of Rhodesia & Nyasaland for much helpful advice.

Finally, I should like to thank Messrs. African Explosives and Chemical Industries Limited for financial support which made the investigation possible.

I. INTRODUCTION

Ticks "are not merely annoying pests but surpass all other anthropods in the number and variety of disease agents for which they are carriers" (Chandler 1955). This statement is certainly true of Boophilus decoloratus (Koch), the blue tick, which is probably one of the most important ticks in South Africa.

Various studies have been made on the blue tick in Africa, among which are investigations on taxonomy (Hoogstraal 1956); distribution (Theiler 1949 and 1962); ecology (Kraft 1961); disease transmission (Neitz and du Toit 1938; du Toit 1947; Neitz 1956 a and b); control and resistance to insecticides (du Toit, Graf and Bekker 1941; Whitnall and Bradford 1947; Whitehead 1958 and 1959) To date, the behaviour of Boophilus decoloratus has not been studied to any extent. The aim of the present work is to analyse the behaviour of the unfed larvae into its constituent patterns in order to determine what are the significant environmental factors which affect this behaviour. It is also hoped that the present laboratory studies might help in understanding their behaviour in the field.

Laboratory studies have been carried out on the behaviour of B. annulatus (Say) and Rhipicephalus sanguineus Latrielle by Krijgsman (1937); Ixodes ricinus L. by Lees (1948 a) B. microplus (Canestrini) by Wilkinson (1953) and on Ornithodorus erraticus (Lucas) by El Ziady (1958). In addition field studies have been carried out my Milne (1950) and Lees and Milne (1951) on I. ricinus; Hitchcock (1955 a and b), Snowball (1957) and Wilkinson and Wilson (1959) on B. microplus.

B. decoloratus is a one host tick and its

life cycle has been described by Hoogstraal (1956) and Arthur (1960). After mating, the engorged female drops off the host and lays about 2000 eggs. The eggs hatch after five to six weeks and the newly emerged larvae remain at the base of the vegetation for about seven days before ascending the vegetation. Once at the top of the vegetation, the larvae wait for a suitable host. When a potential host brushes the support on which the larvae are waiting, they attach themselves to the host, feed, moult to a nymph, feed, then moult into an adult. The life cycle on the host takes about three to four weeks.

The present investigation includes studies on the reaction of the larvae to light, humidity, gravity and temperature. It was considered that such studies might help to explain (a) which factors cause the newly hatched larvae to remain at the base of the vegetation for the first week and (b) which factors cause them to ascend the vegetation when over one week old. In addition, a study was made of their clumping behaviour and also on the stimuli which cause them to "quest" on the approach of a potential host.

The possible ecological significance of the various environmental factors studied is considered in the general discussion at the end.

Collecting and Rearing Methods

Engorged adult female blue ticks were collected from cattle at the Grahamstown Dip. These ticks were then placed in individual glass tubes plugged with cotton wool and stored in a constant temperature oven at 26° C and 71% RH until the larvae

had hatched. The larvae were then kept under these constant conditions of temperature and humidity and in the dark until ready for use in the experiments. The method used is essentially similar to that described for Dermacentor andersoni Stiles by Kohls (1959).

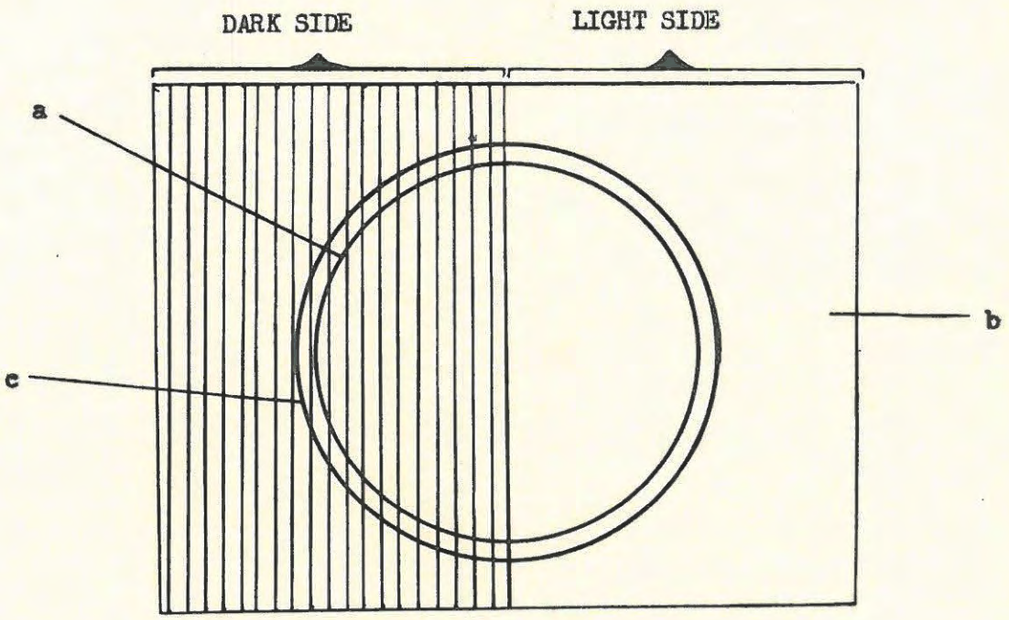
All experiments were carried out at 25 - 26°C and at 71% RH in a constant temperature room unless otherwise mentioned. Light, dark and red light were used as described in the text.

II. REACTIONS TO LIGHT

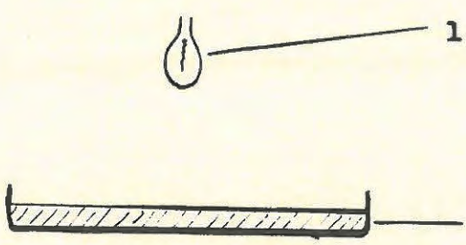
In the study of the phototaxis of the larvae, the first step was to find out whether there was a reaction to non-directional light. The apparatus used consisted of petri-dish choice chambers 62 mm in internal diameter. They were covered by a thin glass plate, "the lid". To create the light gradient, a further sheet of glass, the "gradient glass", which was half covered with black paper was placed over it. The light source was a 40 watt bulb 32 cm. from the apparatus. A water heat filter was placed between the light source and the apparatus. The lid was sealed to the petri-dish with low melting point wax. The technique used in sealing the apparatus with wax was to warm the wax and place a ring of wax around the upper edge of the dish. When the ticks had been placed in the dish, the lid was sealed on. When the wax cooled, it provided a very efficient seal which prevented the escape of the larvae. As the wax was of a very low melting point, it did not harm the ticks inside the apparatus at sealing. To prevent the entry of light from any direction except from above, the apparatus was placed on a sheet of black paper and the sides of the dish were covered with strips of black paper. (See Figure 1).

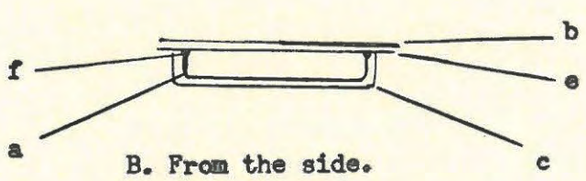
To assess the reactions of the larvae, the gradient glass was removed and the numbers of ticks on each side counted. A line ruled down the black paper on which the apparatus stood corresponded in position with the division of the light-dark margin on the gradient glass. Readings were taken at five minute intervals and the position of the apparatus was reversed after each reading to compensate for any uneveness that there might be in the levelness of the bench. Sometimes, however, only the gradient glass was reversed as a precaution against other factors (e.g. smell due to handling the apparatus being stronger on one side than the other). The results are tabulated below as intensities of reaction expressed as excess percent.

/Table 1.....



A. From above.





B. From the side.

FIGURE 1. Diagram of apparatus used in light choice experiments.

a, petri dish; b, "gradient glass"; c, black paper; d, heat filter; e, glass "lid"; f, wax.

Table 1. Intensity of reaction of larvae given different light choices

Gradient	Condition of Ticks	Age	Excess percent in light	Total No. of ticks
Light - Dark	Normal	7 - 8 weeks	+ 42 %	533
Light - Light	Normal	7 - 8 weeks	+ 1.5 %	272
Light - Dark	Desiccated (1)	7 - 8 weeks	+ 36 %	112
Light - Dark	Desiccated (2)	7 - 8 weeks	+ 45 %	200
(1) Desiccated for 11 hours at 50% R.H. and 27°C.				
(2) Desiccated for 34 hours at 50% R.H. and 27°C.				
Dark - Red light	Normal	7 - 8 weeks	+ 0.1 %	693
Light-Red light	Normal	7 - 8 weeks	+ 36 %	769
Light - Dark	Normal	1 - 2 days	- 42 %	304
Light - Dark	Normal	8 days	+ 29.6 %	628

From the above table, it can be seen that seven to eight week old larvae reared under normal conditions of humidity show a positive phototaxis while no significant response was obtained in a control series in which the gradient glass was omitted. Edney (1954, a & b) has shown that the reactions of isopods to light could be changed by desiccation but no such reaction was observed in tick larvae. It was further found that the tick larvae did not seem able to distinguish between red light and dark. Finally it appears that newly hatched larvae are photo-negative but that this response changes when they are about eight days old. It is interesting to note that the phototaxis becomes more positive with age, i.e., at about eight days of age the excess percent is + 29.6 % while at seven to eight weeks it is + 42%. Tested by X^2 there is a significant difference at the 0.01 level showing that there is a difference of behaviour between the two age groups.

The photonegative response in the newly hatched larvae may be overriden by their tendency to clump. An interesting point which emerged from the experiments was that a newly formed clump would break up on being exposed to light while a mature clump would not. (See Table 2 below). Another fact noted was that, once a clump had formed properly and was not disturbed by light, the numbers in the clump would

sometimes/.....

sometimes increase despite the fact that it was in the light, i.e., the clump seems to exert some influence to attract newly hatched larvae to it despite their preference for the dark. As seen in Table 2, when a clump is mechanically broken up (e.g. using a soft brush) the larvae, now active again, once more show a photonegative reaction.

Table 2. Experiment to determine the interaction of the photo response and clumping in 1 - 2 day old larvae

Reading	Time		No. in Light	No. in Dark	Total
	hrs.	mins.			
1	-	15	6	19	25
Gradient glass reversed					
2	-	30	3	22 (21 inactive starting to clump)	25
Gradient glass reversed - clump breaks up on exposure to light and 19 became active.					
3	-	45	5 (no clumping)	20 (14 clumped (1 active)	25
Gradient glass reversed - clump starts to break up and larvae become active.					
4	1	-	17 (9 active) (7 clumped)	8	25
Most larvae can be seen to still be in the light and the clump has not broken up completely. They were therefore left as above for another 15 minutes to see if perhaps they hadn't had time to react.					
5	1	15	16 (7 active) (9 clumped)	9	25
Still clumped and, in fact, the number in the clump had increased. They were therefore left for another 15 minutes.					
6	2	15	19 (2 active) (17 clumped)	6	25
Clump still not breaking up and is again increasing in size. Therefore disturbed them with a brush.					
7	2	30	6 (5 active)	19	25

This same story emerged from other experiments of this nature, namely that whether the clump breaks up or not on exposure to light is a function of how long it has been formed.

Nature of the phototaxis

In order to find out whether the preference for light shown by the older larvae involved a taxis or a kinesis, individual larvae were tracked in a choice chamber. The method used was to place the choice chamber on ruled black paper and then to plot the animal's path on an

identical/.....

identical piece of graph paper alongside the apparatus. A white light-red light gradient was used as it allowed the animal to be tracked both in the light side and in the dark side.

In these experiments, the larvae spent most of their time on the light side of the apparatus, but in many cases showed a marked "avoiding reaction" at the boundary between the white light and the red. This avoiding reaction consisted of the larva stopping at the boundary or a little way into the red, in many cases feeling around with its forelegs and then turning about and retreating once more into the light. In some cases, however, the ticks did go into the red side, but the larvae mostly soon turned round and once more went into the light. Where the track was started in the red light, the animal went into the light from the red with no noticeable hesitation (see Track 1 a - i).

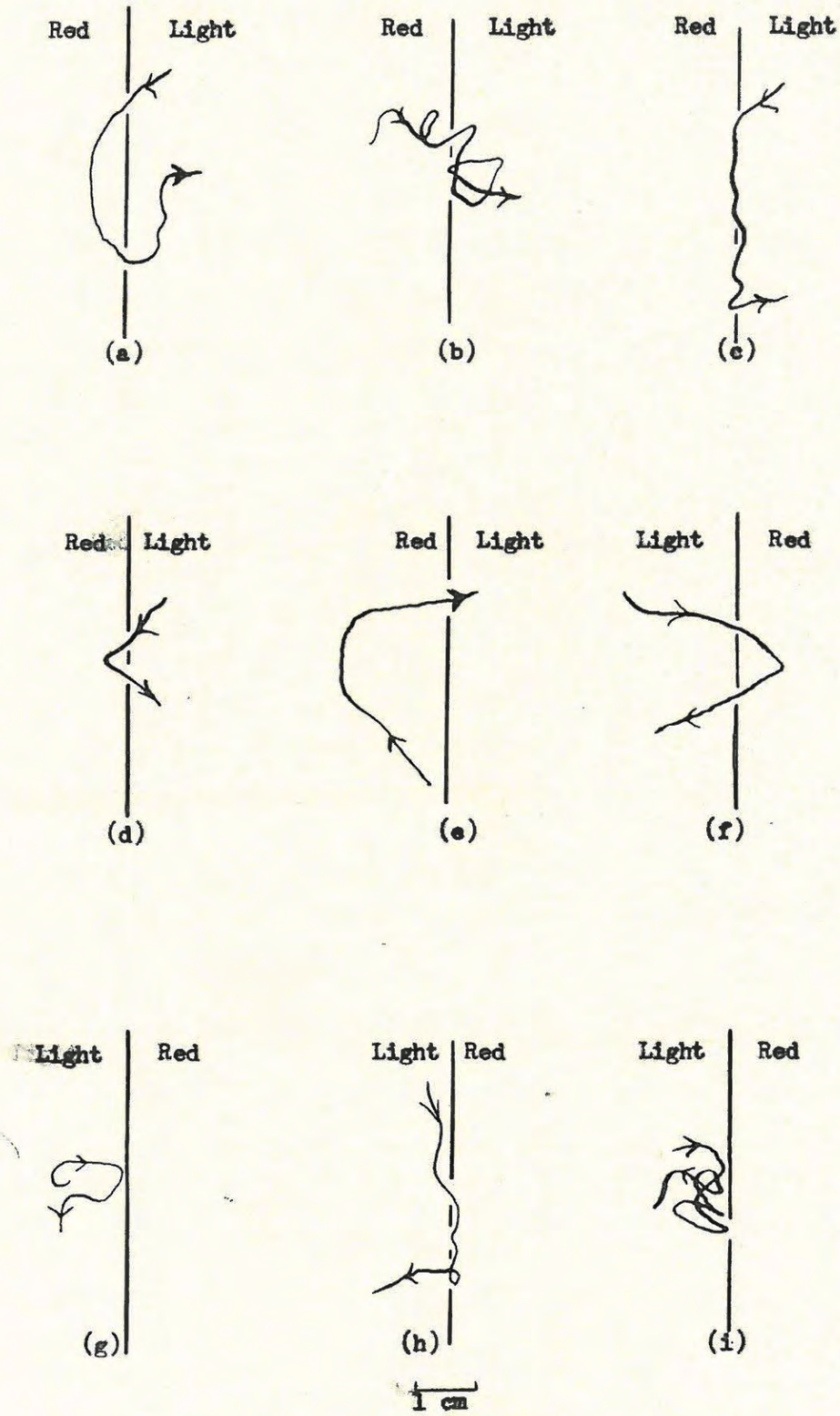
A further series of experiments was carried out to see whether any speed orthokinesis was involved in the photoresponse, i.e., did the larvae move faster in the dark than in the light and thus spend more time in the light? The method used was to track a single tick in both the red and the white light for a set time and then to measure the distance it had travelled with an opisometer (see Table 3 below). From these observations, it seems that there is no difference in speed whether in white light or in red light - that is, no speed orthokinesis is involved in the light reaction.

Table 3. Speed of larvae tracked in white light and red light.

Experiment	Speed in White Light	Speed in Red Light	Difference
1	73.66 mm/min.	72.39 mm/min.	1.27 mm/min. faster in light.
2	77.72 mm/min.	80.01 mm/min.	2.29 mm/min. faster in red.
3	76.96 mm/min.	75.95 mm/min.	1.01 mm/min. faster in light.
4	85.34 mm/min.	83.31 mm/min.	2.03 mm/min. faster in light.
5	89.91 mm/min.	90.42 mm/min.	0.51 mm/min. faster in red.

Experiments were then carried out to determine whether there was any activity orthokinesis involved, i.e., whether the older larvae

move/.....



TRACK 1. Tracks of 6 - 8 week old larvae at light - red light border.

move in the dark and remain motionless in the light, a type of behaviour which would result in their spending more time in the light. For these experiments, a number of larvae were placed in petri dishes covered with a plain sheet of glass sealed on with low melting point wax. The larvae were exposed either to white light or to red light and the number of larvae active and inactive were observed every 15 minutes for a period of 255 minutes. The results obtained are shown in Table 4.

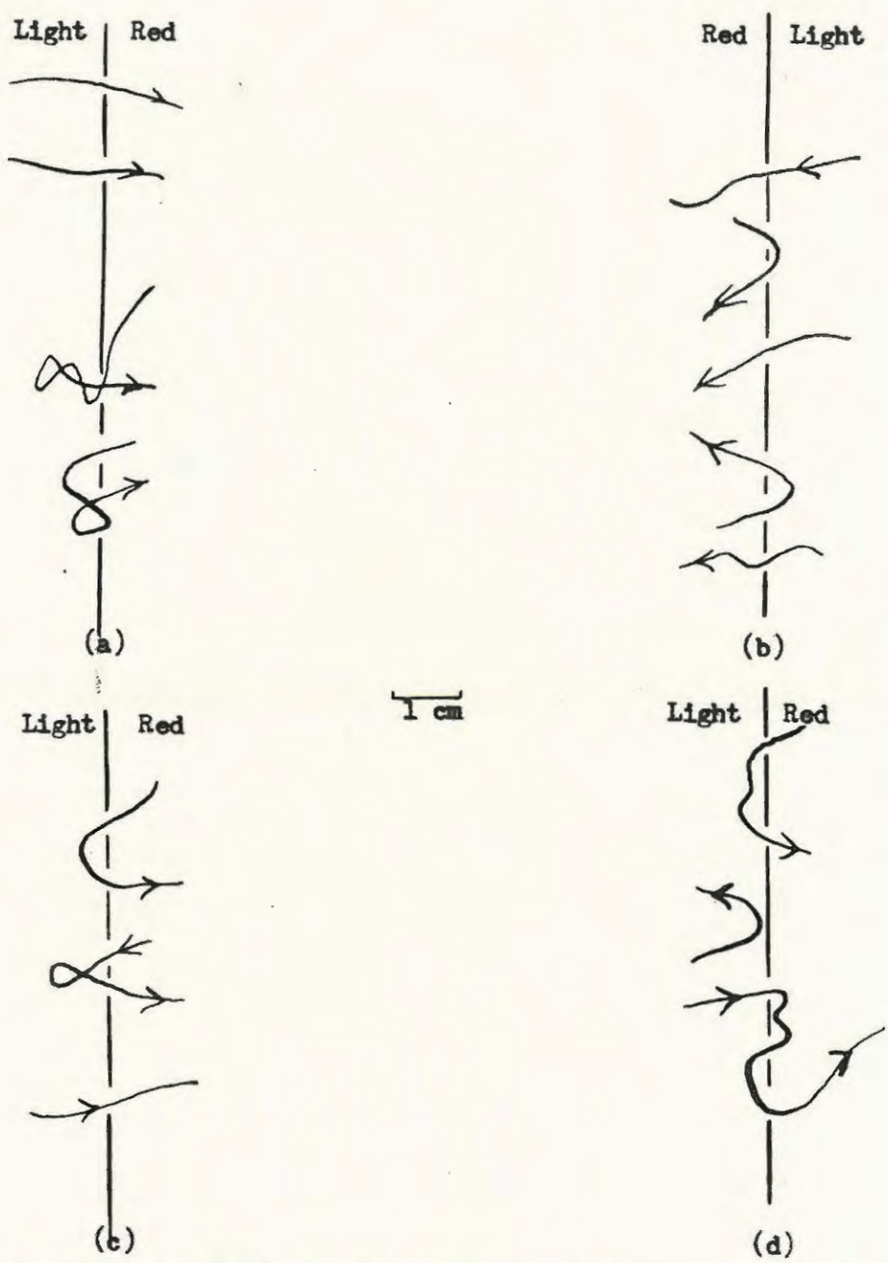
Table 4. Activity of larvae in white light or red light

Experiment	Conditions	Mean Percent Active	Total No. of.Ticks
1	White light	98%	234
2	White light	85%	243
3	White light	93%	198
4	White light	93%	306
5	Red light	95%	198
6	Red light	94%	234
7	Red light	85%	270
8	Red light	91%	207

In all these experiments except one in each series, the larvae do not seem to be inclined to settle any more rapidly in the white light than in the red light. It does not seem therefore that an activity kinesis is involved.

It was next decided to examine the mechanism which keeps the newly hatched larvae in the dark. From the choice chamber experiments, it had seemed that the main mechanism keeping the young larvae in the dark was a preference for the dark coupled with a tendency to clump once there. The young larave were tested in the same manner as the older ones - firstly to see if they showed any boundary avoiding reaction and then to see whether they settled faster in the red light than in the white light. Tracking experiments in a red light - white light choice chamber showed that in most cases the larvae go from white light to red light without any avoiding reaction but that when they move from red light to white light most showed an avoiding reaction to the light at the boundary and that in nearly every case they showed some hesitation before going from red light to white light (see Track 2 a - d).

The/.....



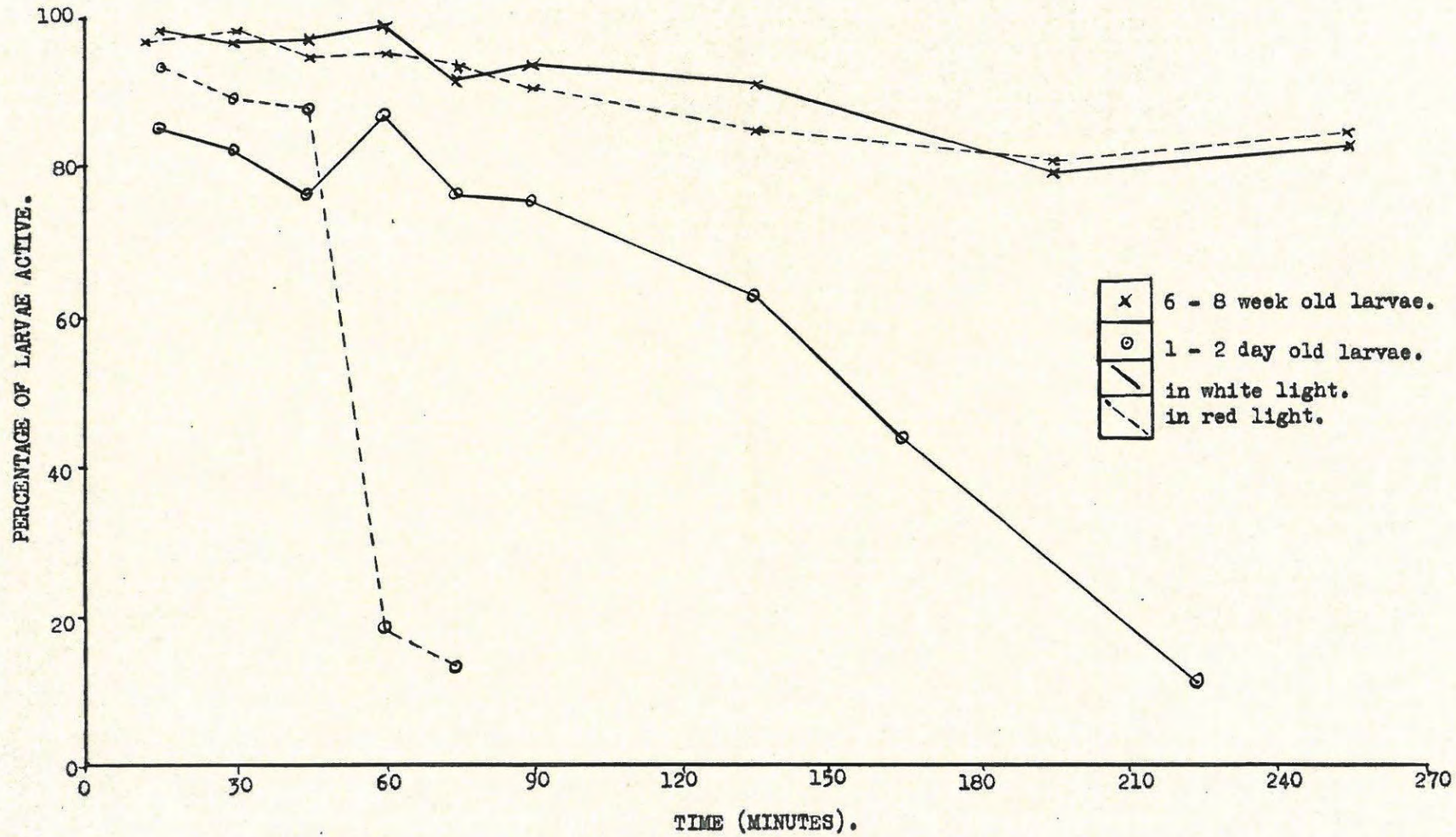
TRACK 2. Tracks of 1 - 2 day old larvae at light - red light border.

The development of a settling reaction was studied in chambers which were uniformly lit with white or red light, observations being made every 15 minutes as in the experiments on mature larvae described earlier. The results are shown in Graph 1 where the settling behaviour of young larvae is compared with that of mature larvae. The data on which this graph is based may be found in Appendix 1. It can be seen that the young larvae become inactive more rapidly than do the old larvae even in white light. The sharp fall in activity of the young ticks in red light is possibly connected with the development of the clumping response which commenced after about 60 minutes. In comparison with this, clumping in the white light was only commenced after 75, 225 and 285 minutes in the three replications.

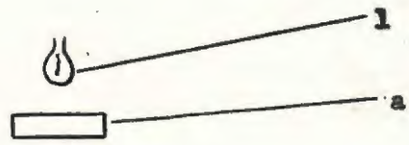
So far, only the responses of the animals to non-directional light has been studied. The responses of the larvae to directional illumination was also investigated below. The source of light used was a microscope lamp, the beam from which was passed through a museum jar full of water which acted as a heat filter. The animals were imprisoned by means of a glass dish sealed to a glass plate floor with vaseline. The walls of the dish were lined inside with black paper - one side of which had a "window" cut out of it to allow the entrance of light from the lamp. A large lens was placed over the apparatus to facilitate the tracking of the ticks. The apparatus was placed on a piece of black paper ruled in an identical manner to a piece of graph paper which was kept alongside the apparatus to plot the path of the tick. One tick at a time was placed in the apparatus and its path in relation to the light plotted. General background illumination was provided by a light set in the roof. Temperature and humidity were, as usual, constant throughout the experiment. The intensity of the beam from the lamp was measured using a wax block photometer and a tungsten standard and was found to be 600 foot-candles (or 700 lumens) at 25.4 cm. See Figure 2 for a diagram of the apparatus.

In these experiments, a single tick was placed in the apparatus and allowed to wander about with only the background illumination.

Then/.....



GRAPH 1 Settling time of newly hatched and old larvae in white and red light.



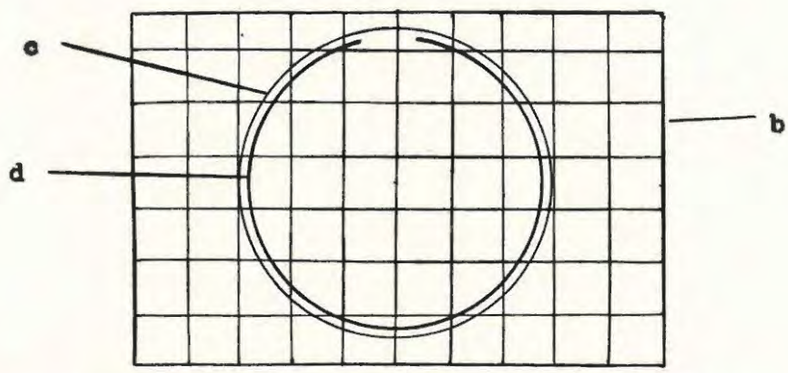


FIGURE 2. Apparatus used to study the reactions of ticks to directional light. One light.

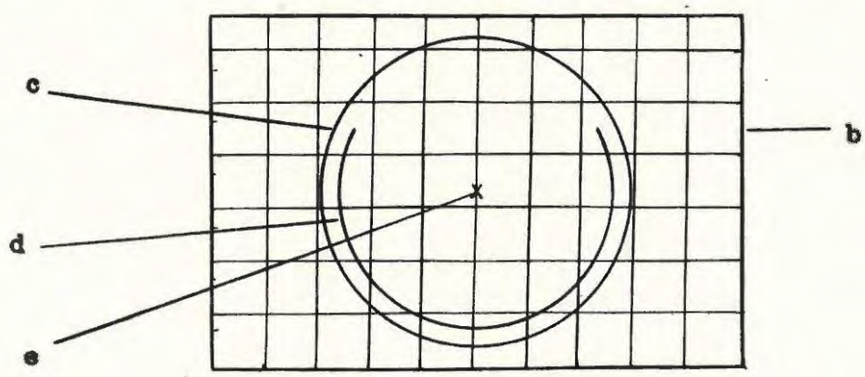


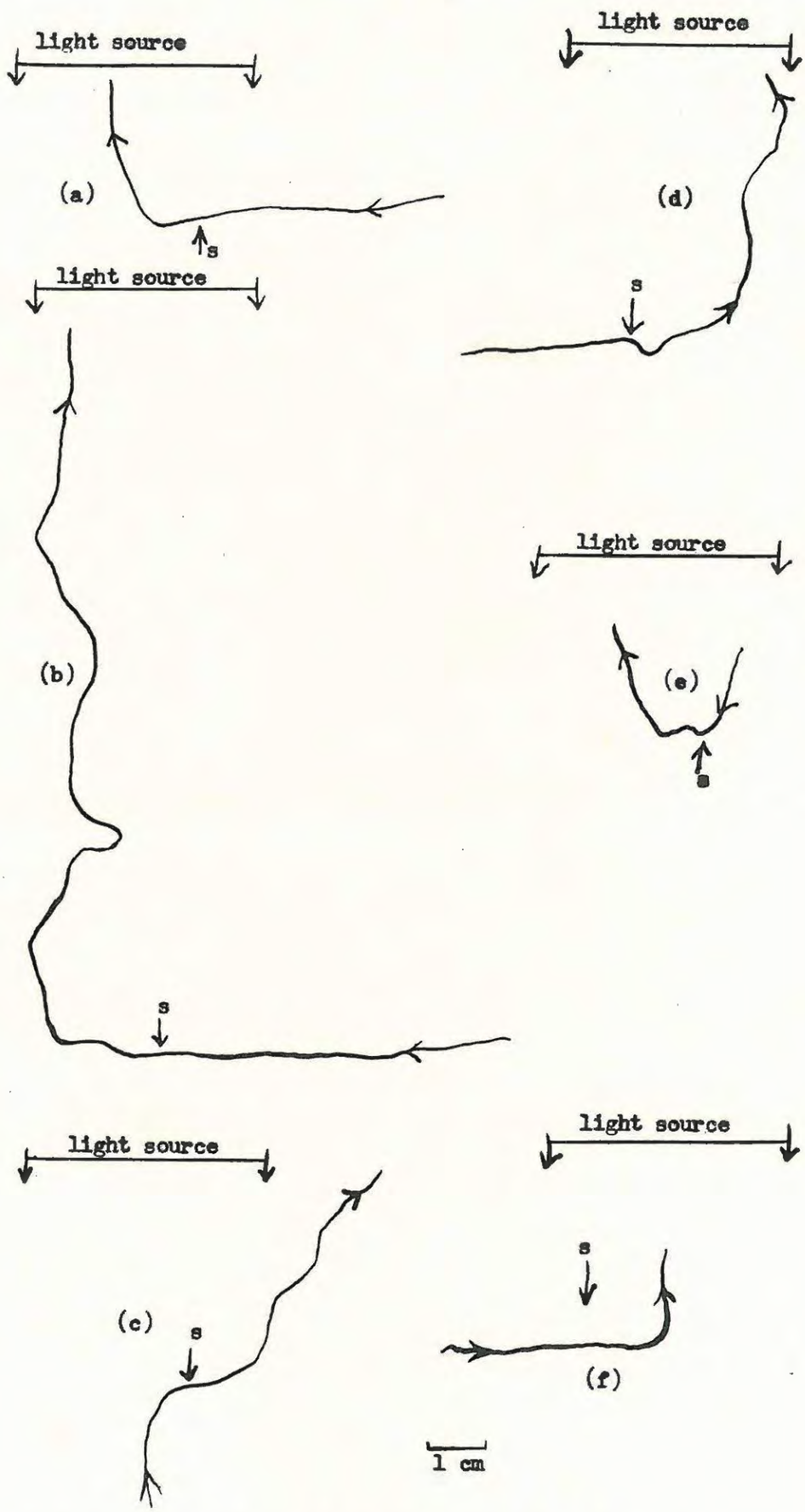
FIGURE 3. APPARATUS used to study the reactions of ticks to directional light. Two lights.

a, heat filter; b, graph paper ; c, glass dish; d, black paper; e, centre spot; l, light source.

Then, after a while, the lamp was switched on to see the reaction of the animal to the beam of directional light. It can be seen from the tracks (Track 3 a - f) that in most cases, irrespective of the direction in which it was moving, the larva turned and moved towards the light source when the light was switched on - i.e., a taxis. In order further to analyse their light behaviour, another series of experiments was carried out using two light sources at right angles to each other, the aim being to see if the taxis was a tropotaxis or a telotaxis (Fraenkel and Gunn 1940). If the reaction is due to a tropotaxis, the larvae will go between the lights while if it is due to a telotaxis, they will go to one or other of the lights. The experiments were carried out as before - tracking the path of a single tick. In the tracks illustrated (Track 4a - f) "x" marks the spot from which each larva started. When the larva reached the edge of the apparatus, its "angle of orientation" was measured, i.e., the angle between line ax and a line drawn from the tick's final position to x (see Figure 3). From Figure 3 it can be seen that at a point vertical to line ab, the angle would be 90° if the animal ended at point y while it would be $+ 45^{\circ}$ if the tick's final position was at l^1 (the left light) or $+ 135^{\circ}$ if its final position was at l^2 (the right hand light). (See Figure 3).

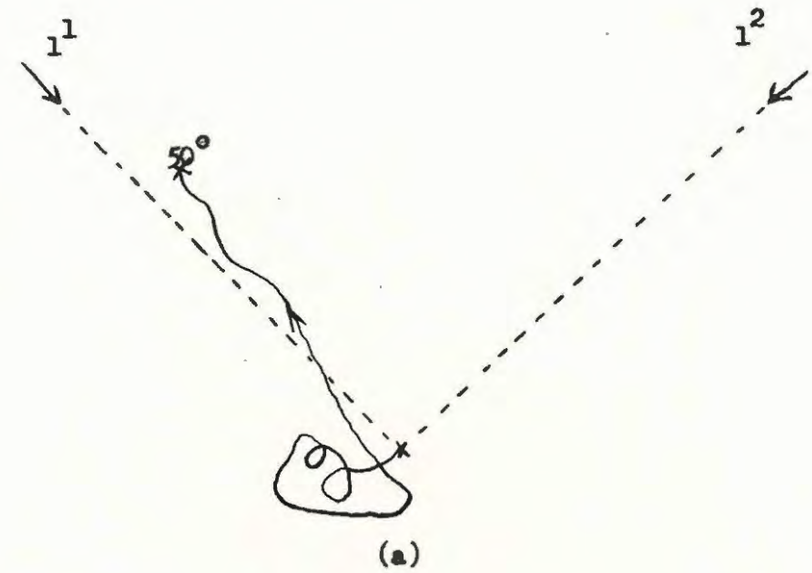
From the histograms provided (Graphs 2 & 3 compiled from Appendix 2), it can be observed that most of the larvae go to one or other of the two lights - the number going to the left hand light being somewhat larger than the number going to the right hand light. The data for Graphs 2 & 3 may be found in Appendix 2. Observations showed that some larvae went directly to the light source with very little or no hesitation while others, although going to the source, did much meandering and turning before finally moving to the light source. Other ticks ended up at different places between the lights and, in some cases, the ticks even went away from the lights before finally turning around and moving to the light source. These tracks can all be seen in Track 4a - f. From these results, the reaction appears to be a telotaxis.

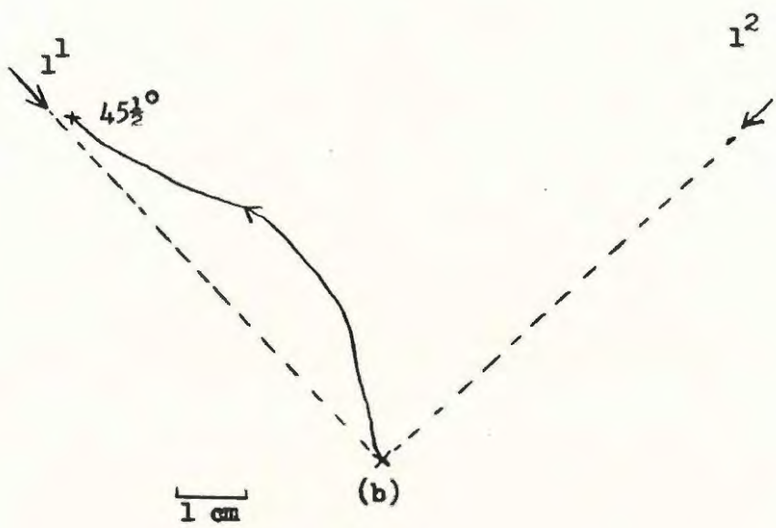
Conclusions/.....

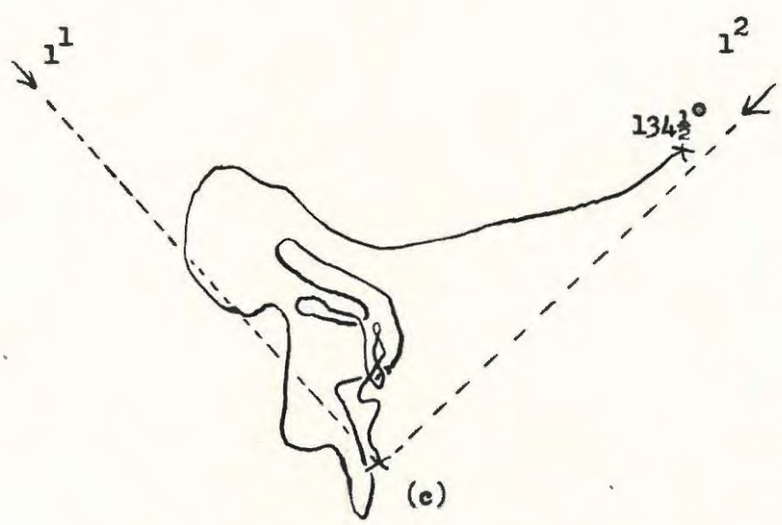


TRACK 3. Orientation of tick larvae to a single light source.

s, position of tick when light switched on.

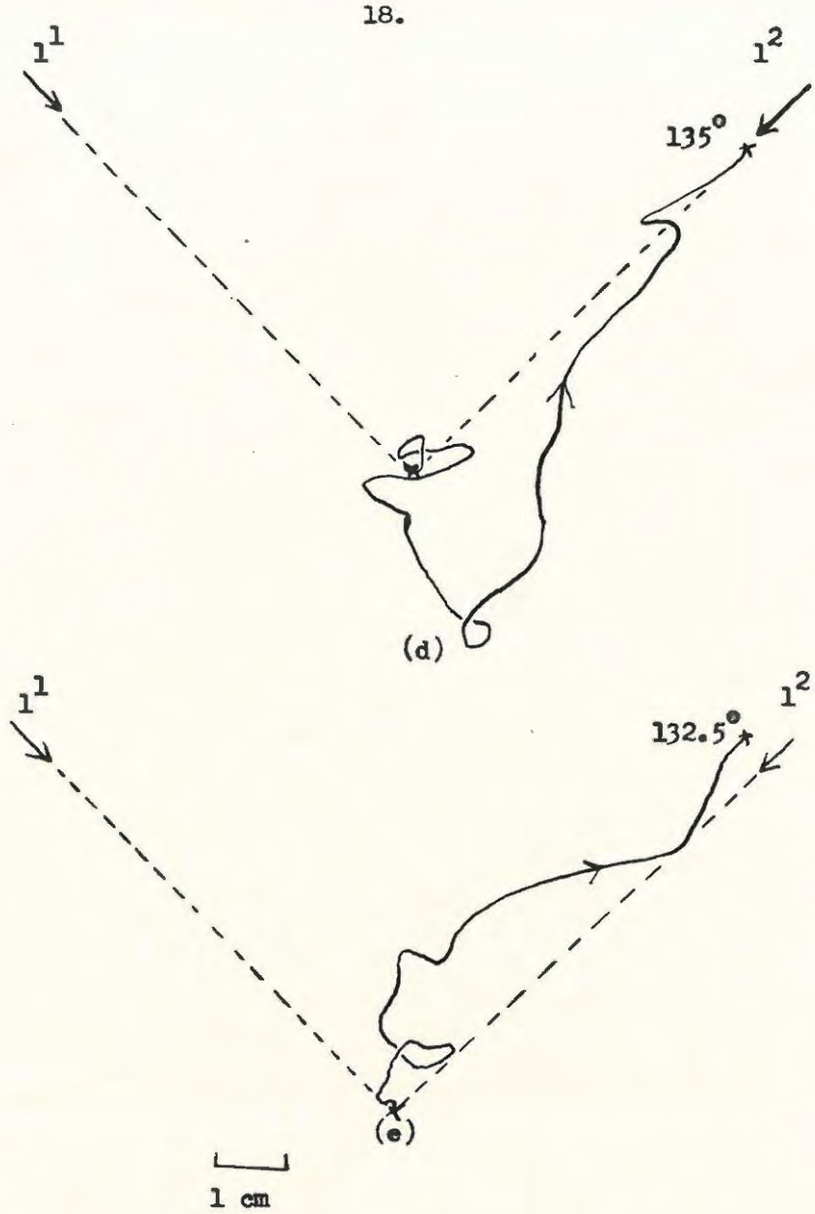


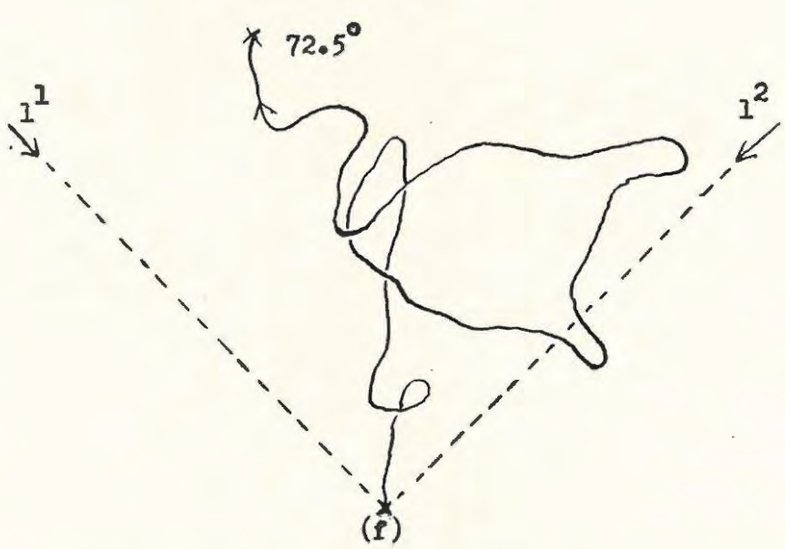




TRACK 4 (a–c). Orientation of tick larvae to two light sources at 90° to each other.

l^1 and l^2, light sources; x, start and finish.





TRACK 4.(d-f). Orientation of tick larvae to two light sources at 90° to each other.

l^1 and l^2, light sources; x, start and finish.

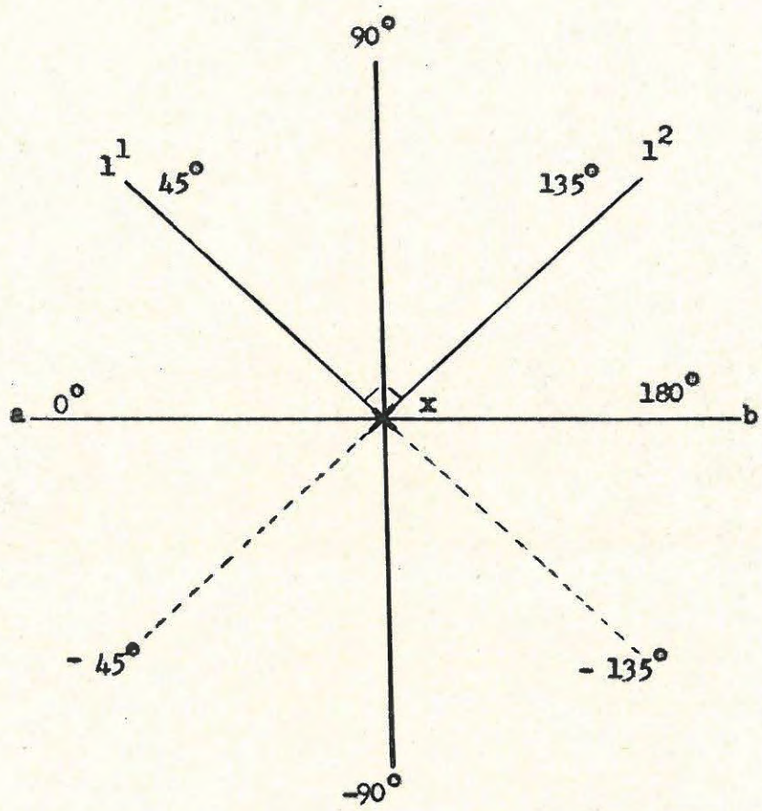
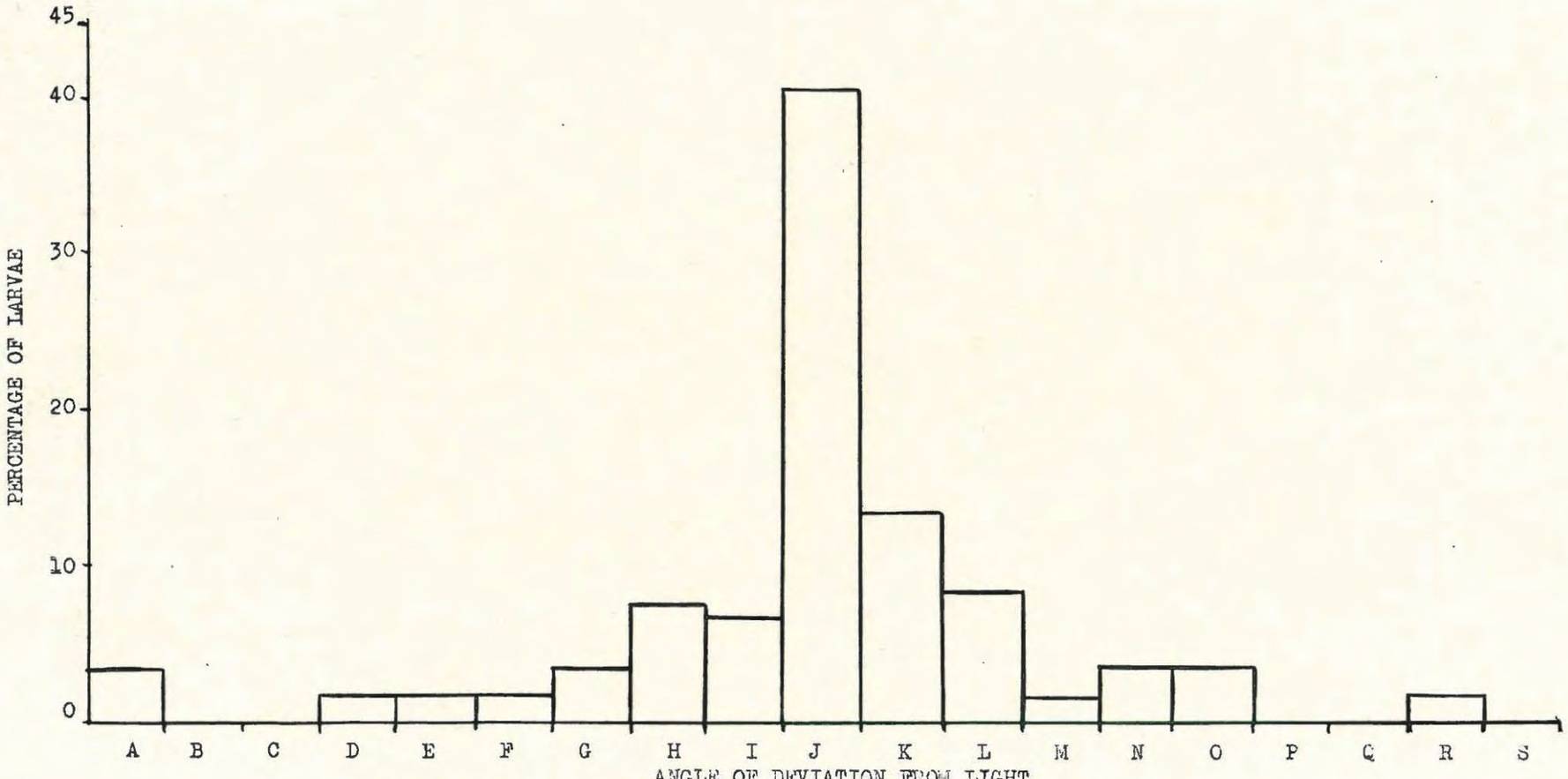
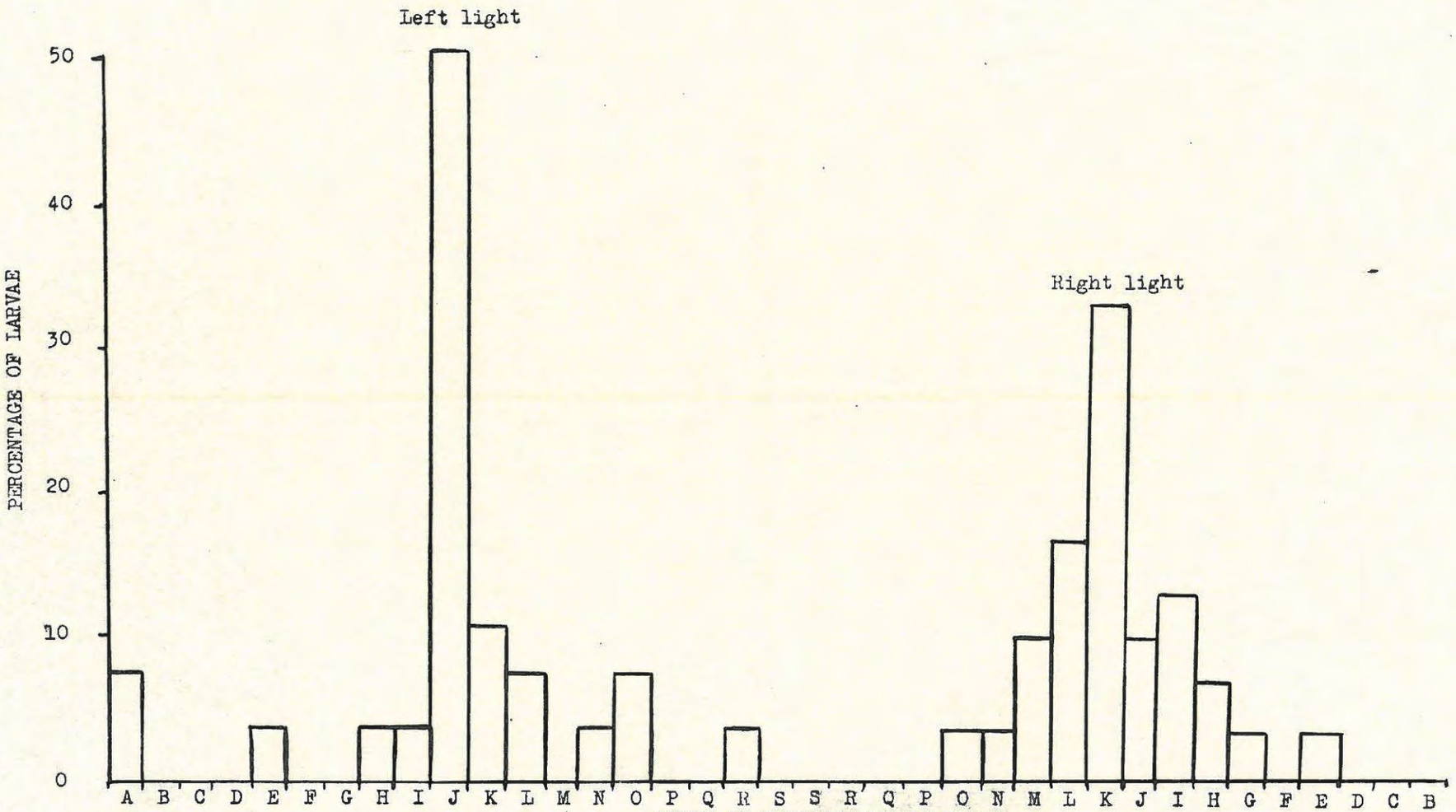


FIGURE 3. Diagram to show method of plotting final position of ticks with respect to light source.



(Each block = 5^{o}; J = $42\frac{1}{2}^{o}$ - $46\frac{1}{2}^{o}$ and $132\frac{1}{2}^{o}$ - $136\frac{1}{2}^{o}$, i.e., directly towards light)

GRAPH 3 : Histogram showing degree of accuracy of orientation to light source (results of Graph 2 "lumped")



(Each block = 5° ; J = $42\frac{1}{2}^{\circ}$ - $46\frac{1}{2}^{\circ}$ and $132\frac{1}{2}^{\circ}$ - $136\frac{1}{2}^{\circ}$ i.e., directly towards light).

GRAPH 2 : Histogram showing degree of accuracy of orientation to light sources.

Conclusions

It thus appears that the light reactions are as follows: The young, newly hatched larvae (i.e., those less than seven days old) are photonegative and tend to remain in the dark (or red light). The mechanism of this behaviour is that, when in the dark, they remain there due to an avoidance to the light at the dark - light border but, if they are initially in the light, they move into the dark with little or no hesitation, become inactive and form clumps. This clumping response is very strong in these young larvae and will, in fact, override the photonegative response once the clump has formed properly.

The older larvae (over one week old) on the other hand, are photopositive and tend to remain in the light and avoid the dark but a telotaxis to directional illumination is also involved. The change in behaviour from a negative phototaxis to a positive one takes place when the larvae are about eight days old and the results obtained suggest that the animals become more photopositive with age.

APPENDIX I

Settling of old (6 - 8 week old) larvae in light.

READING	TIME		ACTIVE	INACTIVE	TOTAL	%ACTIVE	%INACTIVE
	Hrs.	Mins.					
1	-	15	107	2	109	98.2	1.8
2	-	30	105	4	109	96.3	3.7
3	-	45	105	4	109	96.3	3.7
4	1	-	108	1	109	99.1	0.9
5	1	15	100	9	109	91.7	8.3
6	1	30	102	7	109	93.6	6.4
7	2	15	99	10	109	90.8	9.2
8	3	15	87	22	109	79.8	20.2
9	4	15	90	19	109	82.6	17.4

Settling of old (6 - 8 week old) larvae in red light.

READING	TIME		ACTIVE	INACTIVE	TOTAL	%ACTIVE	%INACTIVE
	Hrs.	Mins.					
I	-	15	98	3	101	97.0	3.0
2	-	30	99	2	101	98.0	2.0
3	-	45	95	6	101	94.1	5.9
4	1	-	96	5	101	95.0	5.0
5	1	15	94	7	101	93.1	6.9
6	1	30	91	10	101	90.1	9.9
7	2	15	85	16	101	84.2	15.8
8	3	15	82	19	101	81.2	18.8
9	4	15	85	16	101	84.2	15.8

APPENDIX I (Contd.)

Settling of young (1 - 2 day old) larvae in light

READING	TIME		ACTIVE	INACTIVE	TOTAL	%ACTIVE	%INACTIVE
	Hrs.	Mins.					
1	-	15	64	11	75	85.3	14.7
2	-	30	62	13	75	82.7	17.3
3	-	45	57	18	75	76.0	24.0
4	1	-	65	10	75	86.7	13.3
5	1	15	57	18	75	76.0	24.0
6	1	30	56	19	75	74.7	25.3
7	2	15	47	28	75	62.7	27.3
8	2	45	33	42	75	44.0	56.0
9	3	45	8	67	75	10.7	89.3

Settling of young (1 - 2 day old) larvae in red light.

READING	TIME		ACTIVE	INACTIVE	TOTAL	%ACTIVE	%INACTIVE
	Hrs.	Mins.					
1	-	15	70	5	75	93.3	6.7
2	-	30	67	8	75	89.3	10.7
3	-	45	64	11	75	88.0	12.0
4	1	-	25	50	75	18.8	81.2
5	1	15	10	65	75	13.3	86.7

APPENDIX II

KEY LETTER OF ANGLE GROUPS (as on Histogram)	ANGLE RANGE (Degrees)	NUMBER OF TICKS AT LEFT LIGHT	NUMBER OF TICKS AT RIGHT LIGHT	TOTAL FOR RANGE
A	$-7\frac{1}{2}^{o}$ to $+2\frac{1}{2}^{o}$ $+177\frac{1}{2}$ to $+182\frac{1}{2}$	2(7.1%)	-	2(3.4%)
B	$+2\frac{1}{2}^{o}$ to $+7\frac{1}{2}^{o}$ $+172\frac{1}{2}^{o}$ to $+177\frac{1}{2}^{o}$	-	-	0
C	$+7\frac{1}{2}^{o}$ to $+12\frac{1}{2}^{o}$ $+167\frac{1}{2}^{o}$ to $+172\frac{1}{2}^{o}$	-	-	0
D	$+12\frac{1}{2}^{o}$ to $+17\frac{1}{2}^{o}$ $+162\frac{1}{2}^{o}$ to $+167\frac{1}{2}^{o}$	-	1(3.2%)	1(1.7%)
E	$+17\frac{1}{2}^{o}$ to $+22\frac{1}{2}^{o}$ $+157\frac{1}{2}^{o}$ to $+162\frac{1}{2}^{o}$	1(3.6%)	-	1(1.7%)
F	$+22\frac{1}{2}^{o}$ to $+27\frac{1}{2}^{o}$ $+152\frac{1}{2}^{o}$ to $+157\frac{1}{2}^{o}$	-	1(3.2%)	1(1.7%)
G	$+27\frac{1}{2}^{o}$ to $+32\frac{1}{2}^{o}$ $+147\frac{1}{2}^{o}$ to $+152\frac{1}{2}^{o}$	-	2(6.4%)	2(3.4%)
H	$+32\frac{1}{2}^{o}$ to $+37\frac{1}{2}^{o}$ $+142\frac{1}{2}^{o}$ to $+147\frac{1}{2}^{o}$	1(3.6%)	4(12.9%)	5(8.5%)
I	$+37\frac{1}{2}^{o}$ to $+42\frac{1}{2}^{o}$ $+137\frac{1}{2}^{o}$ to $+142\frac{1}{2}^{o}$	1(3.6%)	3(9.6%)	4(6.8%)
J	$+42\frac{1}{2}^{o}$ to $+47\frac{1}{2}^{o}$ $+132\frac{1}{2}^{o}$ to $+137\frac{1}{2}^{o}$	14(50%)	10(32.6%)	24(40.5%)
K	$+47\frac{1}{2}^{o}$ to $+52\frac{1}{2}^{o}$ $+127\frac{1}{2}^{o}$ to $+132\frac{1}{2}^{o}$	3(10.7%)	5(16.1%)	8(13.6%)
L	$+52\frac{1}{2}^{o}$ to $+57\frac{1}{2}^{o}$ $+122\frac{1}{2}^{o}$ to $+127\frac{1}{2}^{o}$	2(7.1%)	3(9.6%)	5(8.5%)
M	$+57\frac{1}{2}^{o}$ to $+62\frac{1}{2}^{o}$ $+117\frac{1}{2}^{o}$ to $+122\frac{1}{2}^{o}$	-	1(3.2%)	1(1.7%)
N	$+62\frac{1}{2}^{o}$ to $+67\frac{1}{2}^{o}$ $+112\frac{1}{2}^{o}$ to $+117\frac{1}{2}^{o}$	1(3.6%)	1(3.2%)	2(3.4%)
O	$+67\frac{1}{2}^{o}$ to $+72\frac{1}{2}^{o}$ $+107\frac{1}{2}^{o}$ to $+112\frac{1}{2}^{o}$	2(7.1%)	-	2(3.4%)
P	$+72\frac{1}{2}^{o}$ to $+77\frac{1}{2}^{o}$ $+102\frac{1}{2}^{o}$ to $+107\frac{1}{2}^{o}$	-	-	0
Q	$+77\frac{1}{2}^{o}$ to $+82\frac{1}{2}^{o}$ $+97\frac{1}{2}^{o}$ to $+102\frac{1}{2}^{o}$	-	-	0
R	$+82\frac{1}{2}^{o}$ to $+87\frac{1}{2}^{o}$ $+92\frac{1}{2}^{o}$ to $+97\frac{1}{2}^{o}$	1(3.6%)	-	1(1.7%)
S	$+87\frac{1}{2}^{o}$ to $+92\frac{1}{2}^{o}$	-		0
TOTAL		28	31	59

III. REACTIONS TO HUMIDITY

In order to find the humidity preference of unfed blue tick larvae, petri dish choice chambers were constructed similar to those used by Wiggleswcrth (1941) and Lees (1948a). The choice chambers consisted of two petri dishes of 62mm internal diameter which fitted exactly over one another. The lower dish was divided into two halves by means of a waterproof celluloid partition. The halves were used to hold the chemicals that created the various relative humidities (RH) in the upper chamber. Between the two dishes was a "false floor" consisting of a fine nylon cloth tightly stretched over a wire ring. The false floor was at first merely placed over a circular piece of perforated zinc of the same size, but later it was fastened to the zinc by means of paper clips to prevent buckling of the "false floor" during the course of the experiments. The upper petri dish was fixed to the false floor by means of low melting point wax as explained previously in the light experiments. The petri dishes used in the construction of the choice chambers had rounded corners in order to eliminate as far as possible, any angled corners in which the larvae might tend to settle (See Figure 4).

The apparatus was tested using cobalt chloride paper and was found to give very satisfactory humidity gradients. The humidity gradients were established using saturated solutions of various chemicals, distilled water and phosphorous pentoxide. (Buxton 1931; Buxton and Mellanby 1934; O'Brein 1948; Shulov 1952) The chemicals used were:

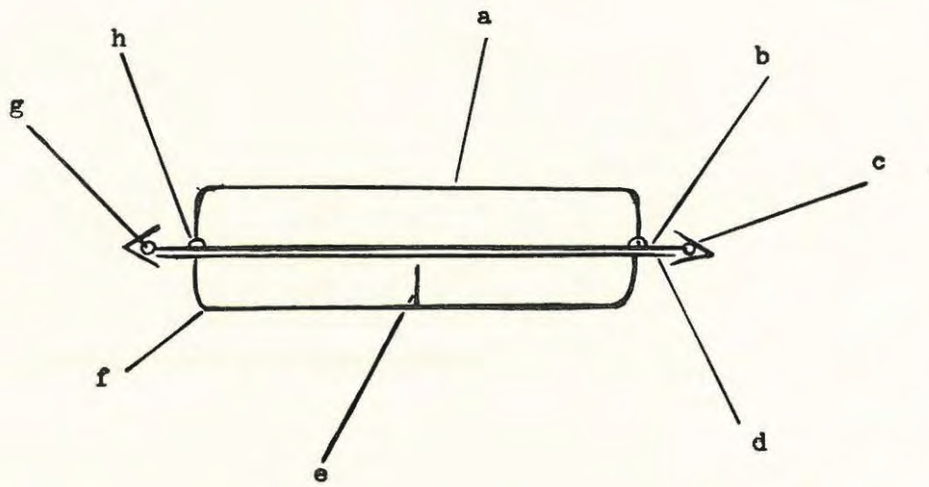


FIGURE 4. Diagram of apparatus used in humidity choice experiments.

a, upper petri dish; b, false floor; c, paper clips;
d, perforated zinc; e, celluloid partition;
f, lower petri dish; g, copper ring; h, wax.

Distilled water (100% R.H.)

Potassium nitrate (93% R.H.)

Sodium potassium tartrate (87% R.H.)

Sodium chloride (75% R.H.)

Potassium nitrite (65% R.H.)

Calcium nitrate (50% R.H.)

Calcium chloride (31% R.H.)

Phosphorus pentoxide (0% R.H.)

When phosphorous pentoxide was used to create 0% R.H. in the chamber, it was found that the chemical tended to become soggy and useless in the damp atmosphere of the constant temperature room. This problem was overcome by placing the choice chamber in a dessicator containing calcium chloride. The normal dessicator lid was replaced with a plain sheet of glass and the larvae in the choice chamber inside the dessicator could still be counted at intervals during the course of the experiment without disturbing the apparatus. This modification was used in all the humidity experiments

The experiments were carried out by giving the animals a choice between various humidities. Every possible effort was made to eliminate all factors which might influence the behaviour of the larvae except the factor being tested. For this reason, the experiments were carried out in a dark constant temperature room, the light only being switched on at fifteen minute intervals for readings to be taken. The apparatus was reversed at intervals as in the light experiments but sometimes the upper chamber alone was reversed.

The first series of experiments was carried out to test the reactions of the ticks to various relative humidities against 0% R.H.

TABLE 5. HUMIDITY REACTIONS OF BOOPHILUS DECOLORATUS LARVAE

EXPERIMENT	CHOICE	EXCESS % IN WET (or side A)	TOTAL TICKS USED N
1	0 - 100% RH	+ 48.2%	224
2	0 - 93% RH	+ 45.4%	220
3	0 - 75% RH	+ 4.2%	320
4	0 - 31% RH	- 5.6%	390
5	0 - 0% RH	- 1.0%	295
6	100- 100% RH	+ 7.2%	259
The experiment with the 0 - 100% RH choice was then repeated.			
7	0 - 100% RH	+ 17.8%	329

From Table 5 it appeared that the larvae were positively hygrotactic when the upper humidity was high (see readings 1 and 2 in Table 5) but when offered a choice of 0% and 75% R.H. or less, there was no significant response (See readings 3 and 4 in above table) i.e. a similar effect to that obtained for Tenebrio molitor L. by Pielou and Gunn (1940)

When, however, the 0-100% RH choice was tried again, the number in the wet was not significantly greater (at more than 0.1 level using standard error of proportion)
than the number in the dry (see reading 7). The question which then arose was why had this change in behaviour occurred? There appeared to be a number of possible reasons for this change in behaviour:

(a) The different behaviour was shown by the offspring of one female only (as those used in reading 7 were a different batch to those used in readings 1 to 6).

(b) It could be that the larvae of different ages react differently to humidity as the ticks which were positively hygrotactic were about eight weeks old while those that were indifferent were only about four and a half weeks old.

(c) The difference in behaviour could be due to the water balance of the eggs from which the larvae had hatched, i.e. desiccated eggs would result in the hatching of desiccated larvae and this might result in a positive hygrotaxis.

(d) The humidity behaviour of the larvae could depend on the water balance of the larvae rather than the eggs prior to the experiment.

(e) The behaviour of the larvae with regard to humidity could perhaps be connected in some way with the physiological state of the females.

(f) The difference in behaviour could be seasonal.

These various possibilities were tested experimentally and are discussed further below:

(a) As the experiments shown in readings 1 & 2 and that shown in reading 7 of Table 5 were carried out on the offspring of different females, it was thought that perhaps the difference might be due to the fact that the larvae from the one mother were somehow abnormal in their behaviour. To test this theory, more experiments were carried out using the larvae from a new female (of a different batch to those used in Table 5 readings 1 & 2) with a batch of the indifferent larvae as controls.

TABLE 6. EXPERIMENT TO SEE IF THE CHANGE IN HUMIDITY BEHAVIOUR IS DUE TO A DIFFERENCE BETWEEN LARVAE FROM DIFFERENT FEMALES.

EXPERIMENT	CHOICE	EXCESS % IN WET	TOTAL
I (new batch)	0 - 100% RH	- 7.8%	232
2 (old batch)	0 - 100% RH	- 12.4%	304

From these results it can be seen that the larvae of both batches are indifferent to humidity (using standard error of proportion) and when when tested statistically (X^2) it was found that there was no significant difference between the behaviour of the two groups of larvae (p= more than 0.99).

(b) It was next decided to see whether age had any effect on the humidity behaviour of the animals as the positively hygrotactic animals and the negatively hygrotactic ones differed in age by about four weeks. A further reason for suspecting that age might be involved was that Krijgsman (1937) had showed that age seemed to have some effect on the humidity behaviour of Boophilus annulatus larvae. He found that while larvae over seven days of age were positively hygrotactic, those under seven days old were indifferent to humidity. The hypothesis was tested by carrying out a series of experiments as before but on larvae of different ages. In the experiments, the larvae were given a choice between 0 - 100% RH.

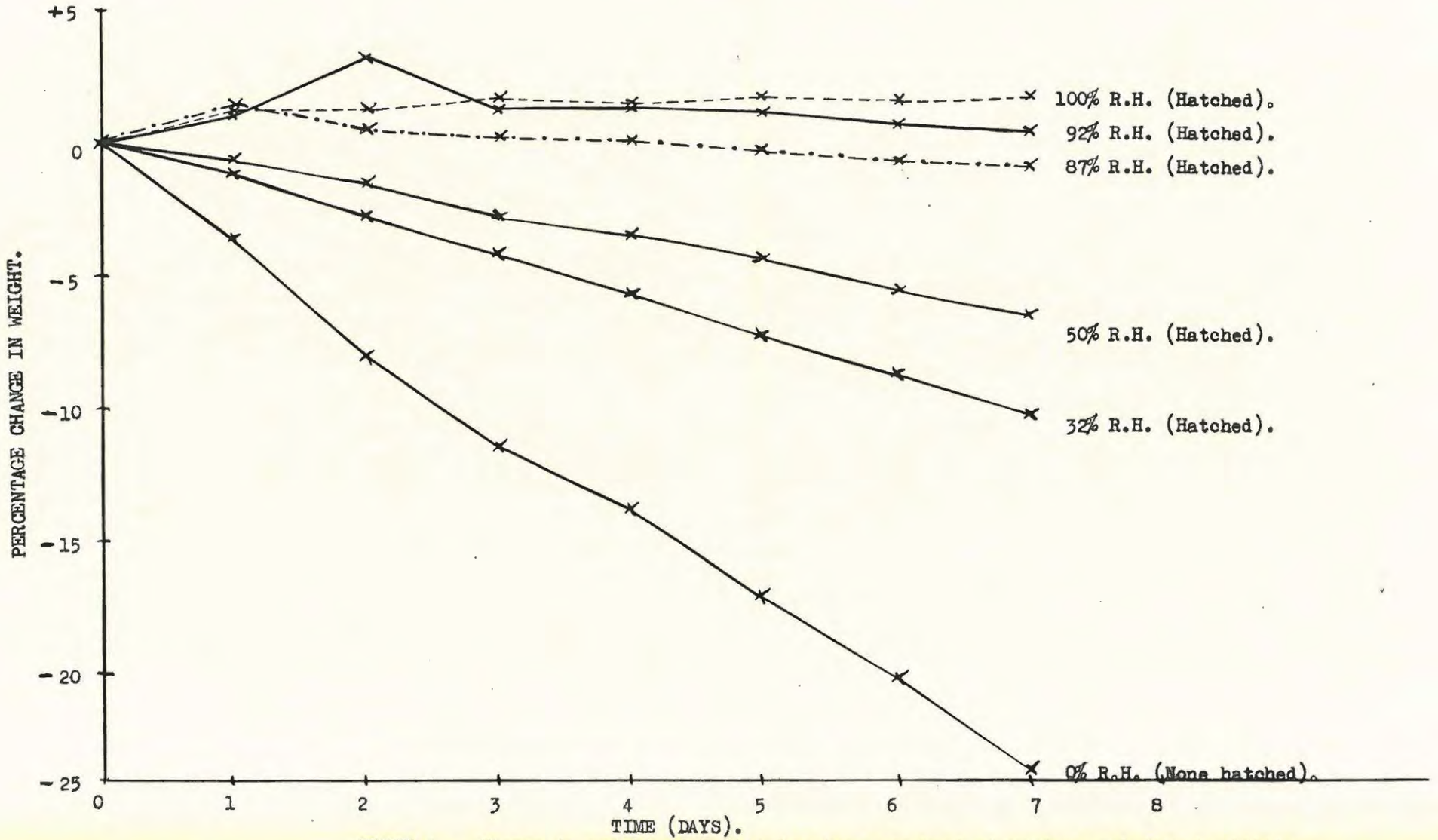
TABLE 7. HUMIDITY BEHAVIOUR OF LARVAE OF DIFFERENT AGES

EXPERIMENT	AGE OF LARVAE	CHOICE	EXCESS % IN WET	TOTAL
1	1 - 4 days	0-100%RH	-0.2%	415
2	10 - 12 days	0-100%RH	-3.8%	578
3	2½ - 3½ weeks	0-100%RH	-1.6%	1864
4	4½-5 weeks	0-100%RH	-5.4%	890
5	6-8 weeks	0-100%RH	+2.2%	1811
6	9 weeks	0-100%RH	-2.4%	252

In all these experiments, no significant difference between the numbers in the wet and the numbers in the dry could be found. It is noteworthy that while it appears from the table above that the one to four day old larvae are indifferent to relative humidity, the experiments on this age group were unsatisfactory because the larvae started to clump about half an hour after their introduction into the apparatus. From direct observations, however, it appeared that they seemed to be indifferent to humidity. This might be because, by their clumping, they created their own microclimate around the clump. Nevertheless, these experiments indicate that age does not affect the humidity behaviour of the larvae, as expressed in these conditions.

(c) The next point investigated was whether the water balance of the eggs played any part in the humidity behaviour of the larvae. A series of experiments was carried out on the water relationships of B. decoloratus eggs.

The results, plotted as percentage gain or loss of weight, are shown in Graph 4 and the figures are given on Appendix III.



GRAPH 4 . Change in weight of B. decoloratus eggs kept at various relative humidities.

In these experiments, batches of eggs were kept at 26°C in screw top bottles containing various chemicals to give the required humidities. The batches of eggs were weighed at intervals to see what effect the R.H. of the atmosphere had on the water balance of the eggs. A record was kept as to whether or not the eggs hatched to ensure that they had not been killed by the treatment they had received. During the course of the experiments, care was taken to prevent condensation of water on the eggs before weighing and also to ensure that the eggs kept at 100% R.H. did not become covered with fungus which, although it did not appear to harm the eggs, could have affected the weight.

In all batches, except those kept at 0% R.H. most of the eggs hatched but none of those kept at 0% did - this could have been due either to the fact that the dry atmosphere had killed the eggs or to the fact that lack of water had failed to allow hatching. From the above results it appeared that the water balance of the eggs was affected by the atmospheric humidity in which they stood and it was thought that perhaps the humidity behaviour of the larvae was somehow affected by the water balance of the eggs. Lees (1946 & 1948 a and b) has shown that unfed Ixodes ricinus can absorb moisture from the atmosphere at humidities above 90% R.H. and Hitchcock (1955) and Wilkinson and Wilson (1959) showed that partially desiccated B. microplus larvae can take up water from the atmosphere at humidities above 95% R.H. Unfortunately no equivalent figures are available for B. decoloratus but assuming that they behave like I. ricinus and B. microplus, the larvae hatching

from desiccated eggs, i.e. eggs incubated at less than 87%R.H. would remain in a state of desiccation as they were reared at 75% R.H. and had no contact with free water i.e. during incubation the eggs in the atmosphere below 87%R.H. would lose water and the larvae, on hatching would not be able to make up the water deficit.

In the following series of hygrotaxis experiments, the eggs were kept at various humidities for about a week just prior to hatching. When the larvae had hatched, they were kept at the usual rearing humidity and their humidity reactions were tested when they were about four weeks old. A control experiment was carried out with untreated eggs to check that the behaviour of the larvae had not changed since the previous experiments. As usual the larvae were given a 0% - 100% R.H. choice. The results are shown on Table 8.

TABLE 8. HUMIDITY BEHAVIOUR OF LARVAE REARED FROM EGGS KEPT AT DIFFERENT HUMIDITIES

EXPERIMENT	RH OF INCUBATION	EXCESS % IN WET	TOTAL
I	32% RH	- 4.8%	536
2	65% RH	- 4.6%	640
3	100% RH	+ 1.6%	792
4	75% (Control)	+ 9.2%	216

It thus appeared that desiccation of the eggs had no effect on the humidity behaviour of the larvae. Using X^2, no significant difference was found between experiments.

(d) Lees (1948a) has shown, in I. ricinus, that when

the water balance of the numphs is normal, they are negatively hygrotactic but, when desiccated, they lose this negative hygrotaxis and show no humidity response. From Lees' results it was thought that perhaps the sudden change of humidity behaviour in the B. decoloratus larvae might somehow be tied up with the water balance of the larvae. Although this possibility seemed remote, as both positive and indifferent ticks had been reared under the same conditions, it was decided to test the theory by desiccating batches of larvae. For these experiments a batch of larvae was desiccated in 50% R.H. for nine hours and then tested in the normal way. A control experiment was carried out using undesiccated larvae. The results are shown in Table 9.

TABLE 9. HUMIDITY REACTIONS OF LARVAE DESICCATED AT 50% RH FOR NINE HOURS.

EXPERIMENT	EXCESS % IN WET	TOTAL
1 (Experimental)	- 3.8%	154
2 (Control)	- 5.2%	175

As the desiccated larvae were still indifferent to humidity the experiment was repeated but with larvae desiccated at 0% R.H. for 8½ hours. Again a parallel control with undesiccated ticks was used.

TABLE 10. HUMIDITY REACTIONS OF LARVAE DESICCATED AT 0% FOR 8½ HOURS

EXPERIMENT	EXCESS % IN WET	TOTAL
1 (Experimental)	- 7.2%	138
2 (Control)	- 11.4%	138

A similar result was obtained, i.e. there was no difference in the behaviour of desiccated and undesiccated ticks - neither group showing a significant reaction to humidity.

The point which next arose was what happens when the larvae are hydrated at 100% R.H. rather than desiccated? This too was tested experimentally in the usual way on larvae that had been kept in a desiccator at 100% R.H.

TABLE 11. HUMIDITY REACTIONS OF LARVAE KEPT AT 100% RH

EXPERIMENT	TIME IN 100% RH	EXCESS % IN WET	TOTAL
1	48 hours	+ 3.8%	208
2	108 hours	- 5.4%	408

The hydrated larvae like the others were found to be indifferent to humidity.

(e) During the incubation of the eggs and rearing of the larvae one factor was uncontrolled - the humidity conditions in which the female grew up. It was not possible to control the relative humidity during feeding of the females, but an attempt was made to see whether desiccation of the engorged females during the preoviposition period (i.e. between

leaving the host and the commencement of laying) had any effect. For this purpose a number of female ticks were desiccated at 0% RH until just before the commencement of laying, i.e. for a period of about three days. Just before laying was due to begin the females were placed in the standard rearing conditions. When the resulting larvae were 4½ weeks old they were tested with the usual 0% - 100 RH choice.

TABLE 12. HUMIDITY REACTIONS OF LARVAE FROM FEMALES DESICCATED AT 0% RH FOR THREE DAYS PRIOR TO LAYING.

EXCESS % IN WET	TOTAL
- 0.2%	664

The desiccation of the females had no effect on the behaviour of the larvae which were still indifferent to humidity.

(f) It was finally decided to see whether any seasonal variation in humidity behaviour could be detected. To see whether this possibility held the answer, experiments were carried out at intervals over a long period. The results of these and the earlier humidity experiments were tabulated according to the time of year the females were collected.

TABLE 13. HUMIDITY REACTIONS OF LARVAE TABLED ACCORDING TO THE TIME OF YEAR THAT THE FEMALES WERE COLLECTED.

				NORMAL LARVAE		DESICCATED LARVAE		
EXPT.	FEMALE COLLECTED	LARVAL AGE	RH CHOICE	EXCESS % IN WET	TOTAL	EXCESS % IN WET	TOTAL	SEASON
1	9th Feb.	6-7 wks.	0-100%RH	+ 48.2%	224			Summer
2	16th Feb.	7½ wks.	0-93%RH	+ 45.4%	220			
3	10th Aug.	4½-5 wks.	0-100%RH	- 1.0%	547	3.8%	154	
4	24th Aug.	3½-9 wks.	0-100%RH	- 7.2%	564	- 10.2%	138	Winter
5	29th Aug.	5-6 wks.	0-100%RH	+ 0.2%	717			
6	21st Sept.	5 wks.	0-100%RH	- 9.8%	189			
7	12th Oct.	2½ wks.	0-100%RH	- 0.6%	120			Spring
8	2nd Dec.	3 wks.	0-100%RH	+ 12.2%	560			
9	5th Dec.	3-3½ wks.	0-100%RH	- 3.6%	880			Summer
10	9th Jan.	4-12 days	0-100%RH	- 2.6%	723			

From an analysis of all the results as seen tabulated in Table 13 above, there does not seem to be any correlation between the season that the female was collected and the humidity behaviour of the larvae. Conclusions: From these results it appears that for some reason a change in the humidity behaviour of the larvae of B. decoloratus occured - the first larvae tested being positively hygrotactic while the later larvae were indifferent to humidity. To find out why this change in behaviour had occurred, experiments were carried out to see the effect of a number of factors on the behaviour of the larvae. The factors examined were: (a) Larvae from different females; (b) Age of the larvae; (c) Water balance of the eggs; (d) Water balance of the larvae; (e) Water balance of the mother ticks prior to oviposition; (f) A seasonal difference in the behaviour of the larvae. None of these factors was shown to affect the larval humidity behaviour and thus the reason for the change is still unexplained. In conclusion it can only be suggested that it may be that the condition of the host on which the female tick fed has some effect on the humidity behaviour of the larvae, but no evidence for this hypothesis exists - and in fact, it is made with little conviction that it holds the answer.

APPENDIX III

EFFECT OF VARIOUS RELATIVE HUMIDITIES ON THE WEIGHT OF DIFFERENT BATCHES OF B.DECOLORATUS EGGS

BATCH	TIME (HRS) / RH	0	24	48	72	96	120	144	168
A	0%	0.1369g	0.1306g	0.1257g	0.1212g	0.1178g	0.1135g	0.1092g	0.1046g
B	32%	0.0771g	0.0762g	0.0749g	0.0739g	0.0728g	0.0715g	0.0704g	0.0693g
C	50%	0.1436g	0.1426	0.1414g	0.1398g	0.1387g	0.1373g	0.1356g	0.1342g
D	87%	0.1495G	0.1515g	0.1503g	0.1499g	0.1497g	0.1492g	0.1485g	0.1481g
E	92%	0.1475g	0.1490g	0.1521g	0.1493g	0.1494g	0.1492g	0.1485g	0.1482g
F	100%	0.1522g	0.1540g	0.1541g	0.1548g	0.1545g	0.1548g	0.1547g	0.1550g

IV. REACTIONS TO GRAVITY

Initial experiments to test the gravity reactions of the older larvae were carried out in glass tubes sealed at the open end with terylene. This apparatus was, however, found to be unsatisfactory as the larvae always settled at the terylene end irrespective of whether it was at the top or bottom end.

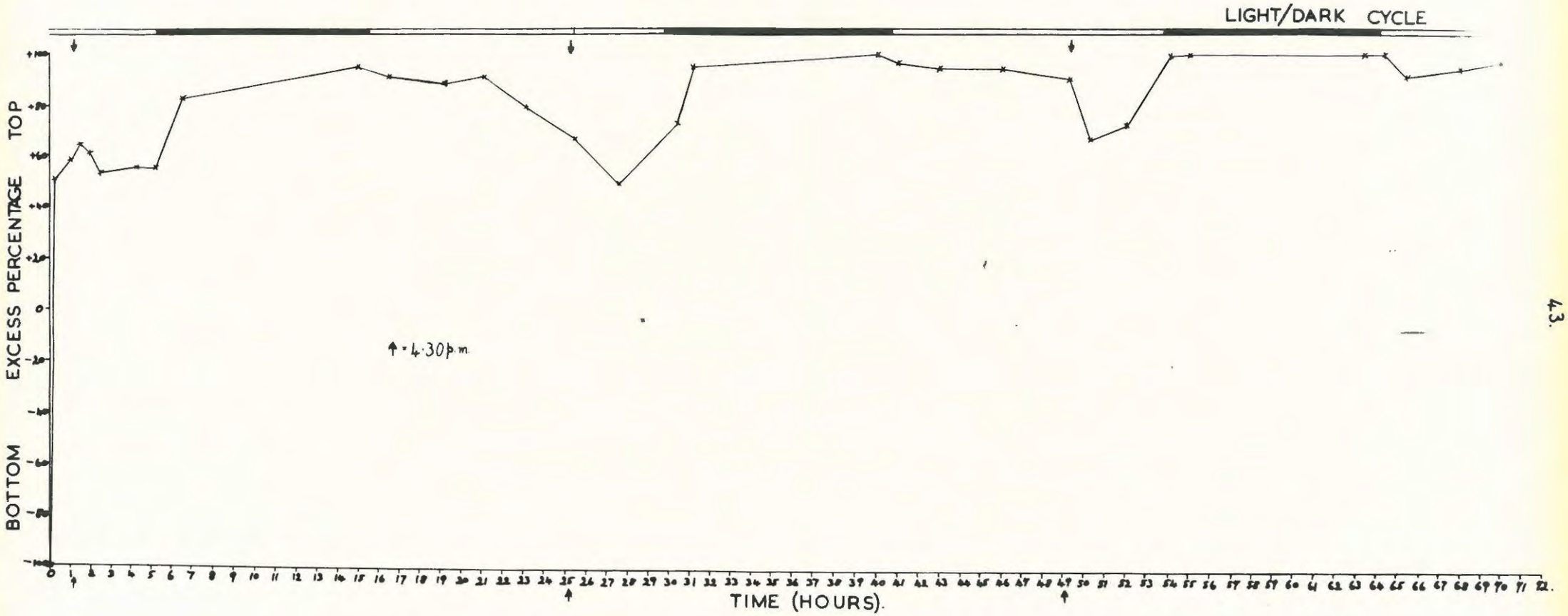
The apparatus devised next was very simple, consisting merely of two identical glass tubes joined together smoothly at their open ends by means of sticky tape after the introduction of the larvae. The tubes were marked off into quarters so that the numbers at various points along the tube could be counted. The four sections were designated "top", "top middle", "bottom middle" and "bottom".

The experiments were carried out over a long period in a constant temperature room with the light more or less synchronised to day and night. The time of each reading was also noted in order to see whether any diurnal rhythm occurred. The larvae were placed in the bottom quarter at the start of the experiments.

The results of this series of experiments are tabulated in the appendix (see Appendix IVa) and plotted in Graph 5a. In the graph, the excess percentage in the top half (given a positive value) or in the bottom half (given a negative value) was plotted for each reading.

To see whether the result over the length of the experiments was significant, they were tested using the Standard Error of Proportion. The result turned out to be significant at less than .001 level.

In the graph, it appears that there is some rhythm (or periodicity? Kleitman, 1949 and Cloudsley-Thompson, 1961) in their reaction to gravity

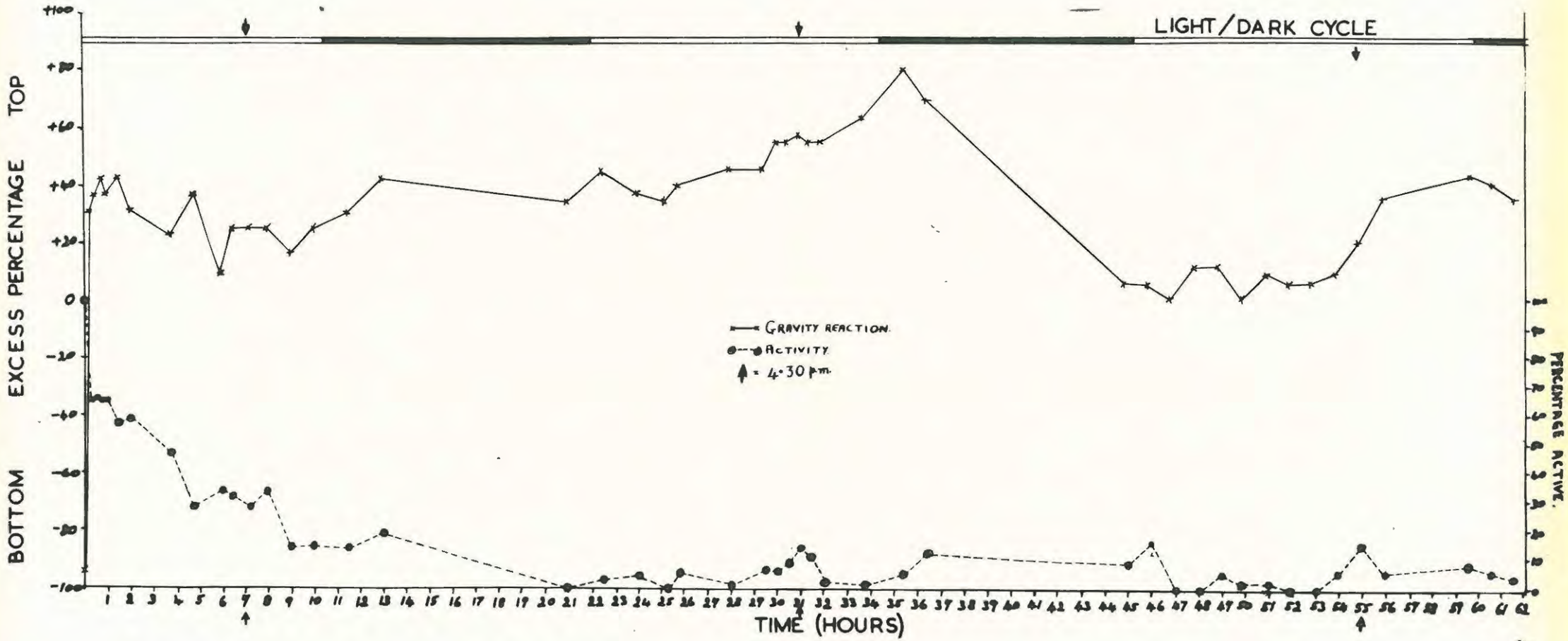


GRAPH 5a. GRAVITY REACTIONS OVER THE 24 HOUR PERIOD.

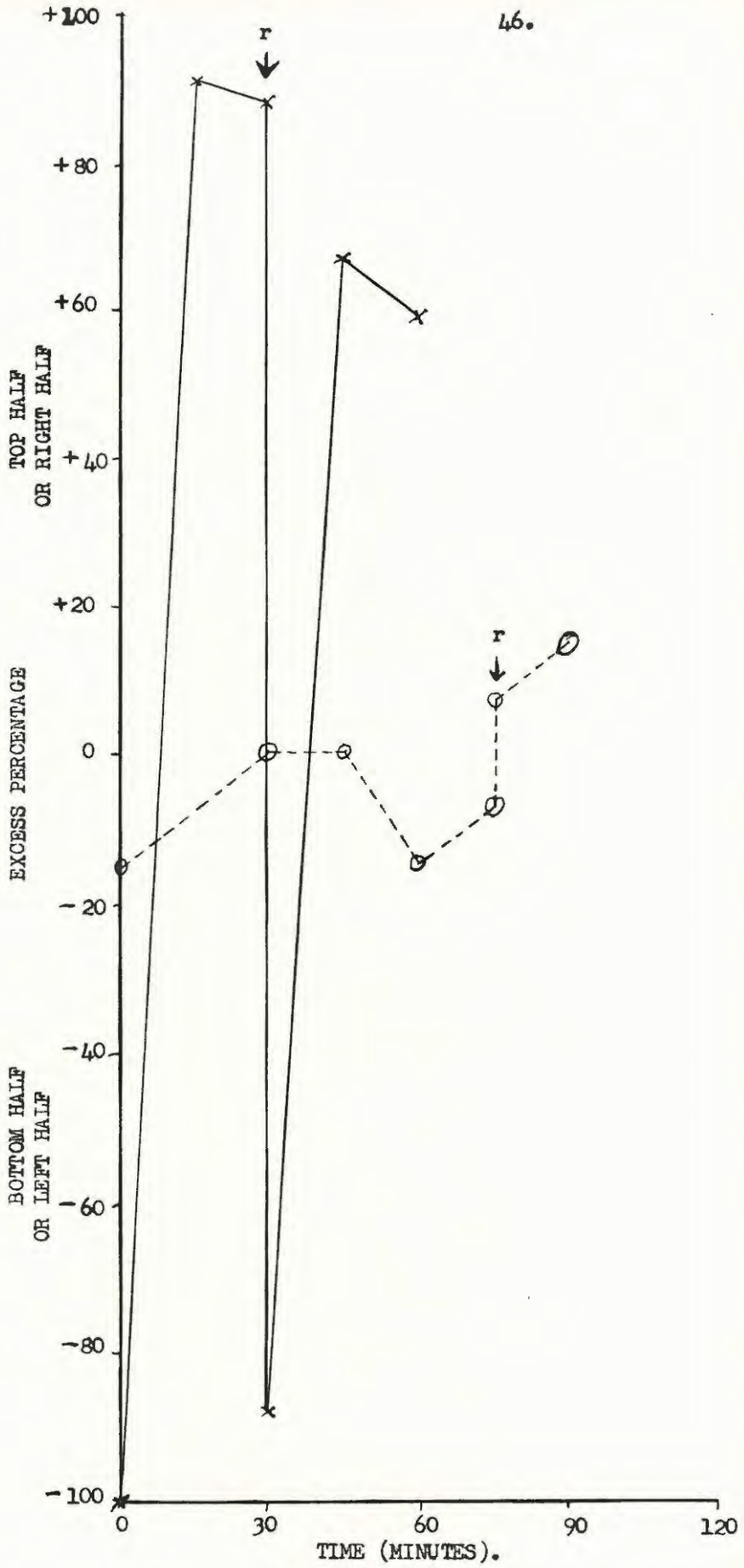
which appears to be associated with the light/dark cycle - i.e. the number of animals at the top increased in the evening and remained high during the dark period. During the light period, however, the number at the top decreased (See Appendix IVa and Graph 5a).

The experiments were then repeated but this time details were noted about the numbers active as well as the numbers at the top. This enables one to measure separately the numbers at the top and the numbers active. It was found that while the animals were still negatively geotactic (result significant at 0.01 level), the apparent gravity cycle was not obtained although there was an increase in the number of ticks active at about 4.30 p.m. each day (See Appendix IVb and Graph 5b which show the excess percent in top half of the tube and the percentage active throughout the experiment), i.e. it appeared that an activity rhythm occurred. Observations indicated that, associated with the increase in activity was a partial breaking up of the clumps. This apparent rhythm of activity is examined later (See Chapter VIII).

In a further series of experiments carried out in red light, a significant geonegative result (at less than 0.01 level) was also obtained (See Appendix V and Graph 6). In conjunction with this latter series, a horizontal control was carried out in which no significant difference was found between the numbers in each half (See Appendix VI and Graph 6). In the control, the animals were spread more or less evenly along the apparatus at the start and remained so throughout the experiment.



GRAPH 5b. GRAVITY REACTIONS AND ACTIVITY OVER 24 HOUR PERIOD.



r, point at which the apparatus was reversed.

×——× Apparatus vertical (Experimental).

o---o Apparatus horizontal (Control).

GRAPH 6. Gravity reactions of 6 - 8 week old larvae.

From these experiments, it appears that there is a negative geotaxis. Using numbers of larvae in tubes, however, there is the complication of the clumping response and the effect of the clump on the individual tick (see clumping section later.)
In order further to study the gravity behaviour of the larvae and to attempt to cut out the possible effect of clumping, a series of experiments was carried out in which the behaviour of indivual larvae on supports was studied.

The apparatus used consisted of a glass road held in a vertical position in a cork. The rod was then placed in a glass tube to prevent the larvae from detecting the observer (see Fig.5). Where it was inserted into the cork, the glass rod was vaselined to prevent the larvae from leaving the rod. The apparatus was divided into sections by means of a scale marked on the outer glass tube. This scale corresponded with measurements on the graph paper on which the track of the tick was plotted. The light source was a 60 watt bulb set in the roof above the apparatus. It can be seen that the above apparatus is modified after that used by Lees (1948a) in his study of the gravity reactions of I. ricinus nymphs.

A typical result of such an experiment is illustrated in Track 5. Every time the larva stopped, the track was marked with an "x". In this track the animal first moved upwards (a negative geotaxis) until it reached the free end of the glass rod when it turned and descended for a short distance before again turning and moving to the top. This behaviour was repeated as can be seen from the track. The larva

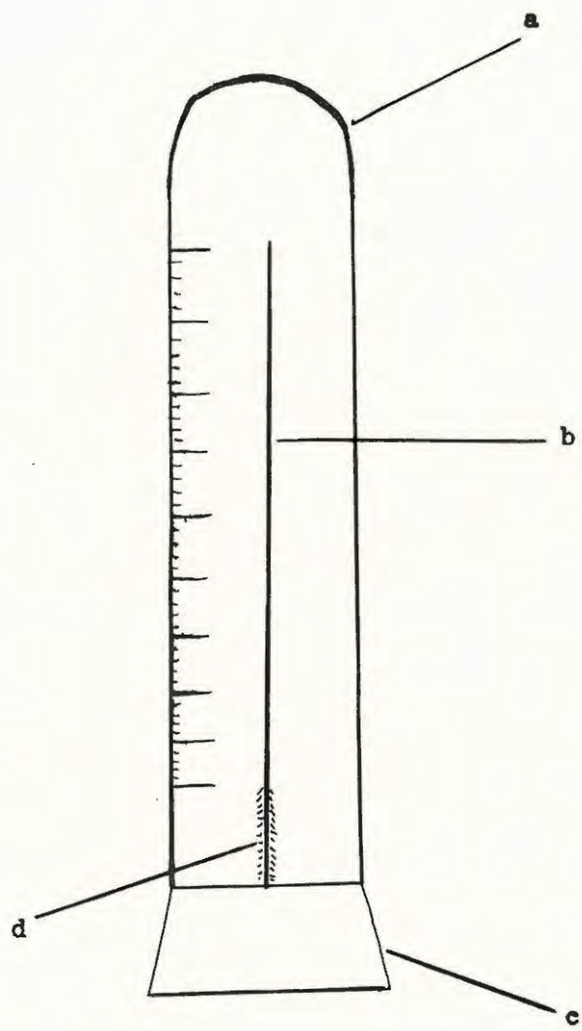
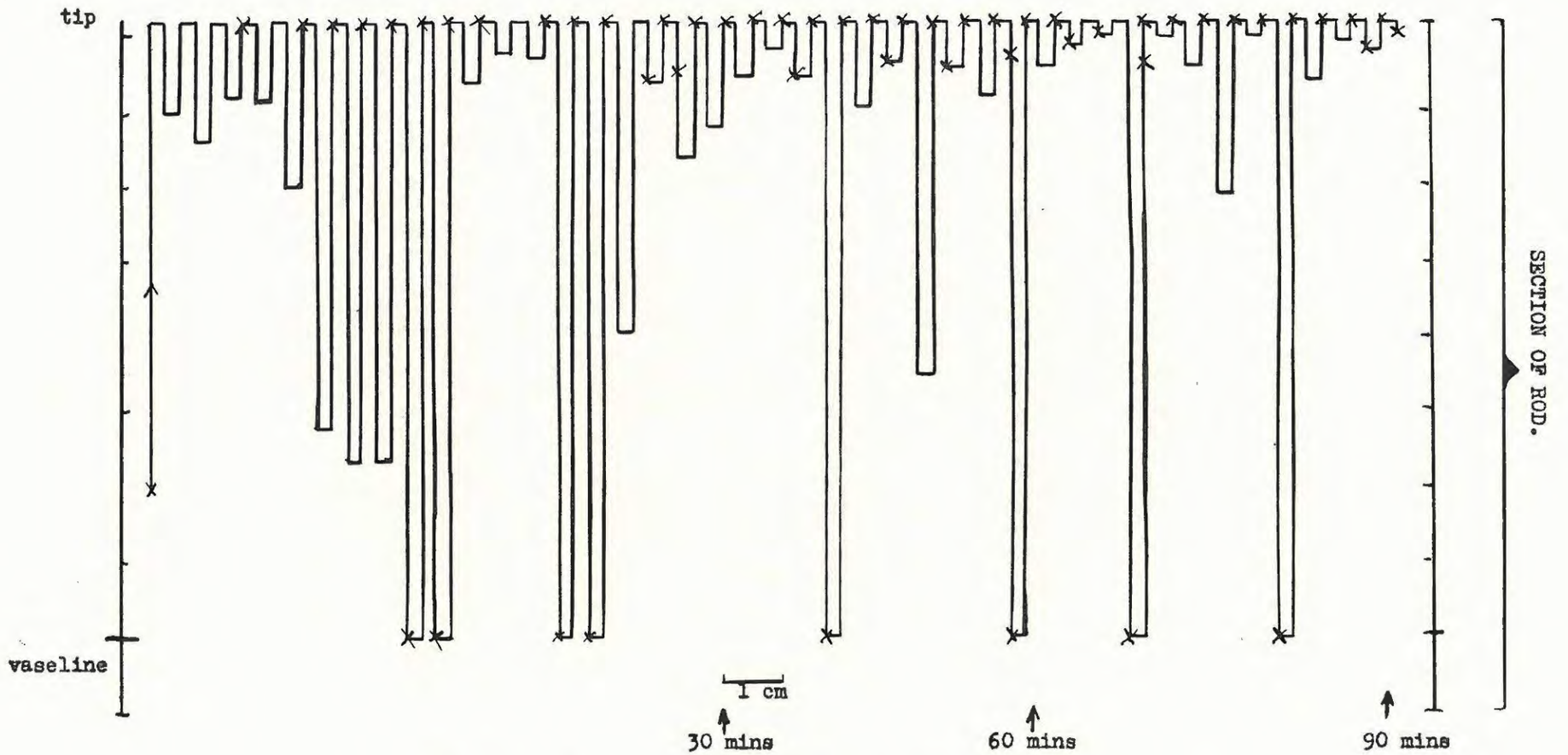


FIGURE 5. Gravity tracking apparatus - straight rod.

a, outer glass tube; b, glass rod;
c, cork; d, vaseline.



TRACK 5. Track made by a 6 - 8 week old tick larva on a vertical rod - tip up.

continued this up and down movement for 90 minutes and then settled 3mm below the tip. The track shows that, although the animal moved up and down during this 90 minutes, it spent far more time at the top than at the bottom. It is interesting to note that the larvae never turned downwards until it had reached the tip (free end) and also that it never gave this "tip reaction" (i.e. the turning back to the tip) at the vaseline end.

More of these experiments were then carried out in the light and the same general pattern of behaviour was observed. However, during the course of one of these experiments the light was switched off and the red light substituted to see what effect it would have on the behaviour of the tick. The picture which emerged (see Track 6) is summarised below:

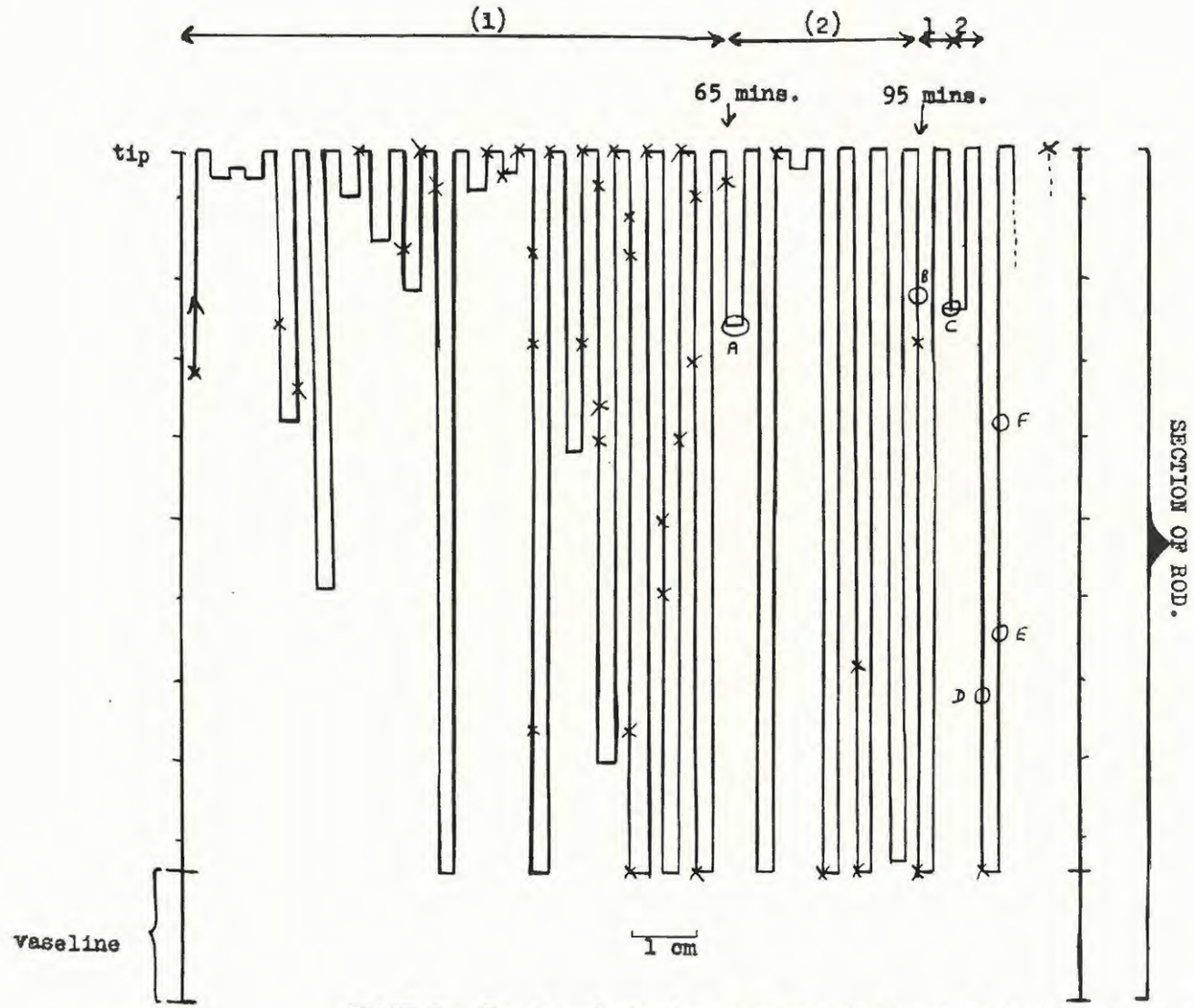
Tick going up: Light on, red light off - no reaction
Light off, red light on - no reaction.

Tick going down: Light on, red light off - no reaction.
Light off, red light on - change of direction.

The significance of this is discussed in the sectional summary.

The experiments raise a number of interesting questions:-

Do the ticks stay near the tip of the rod due to the geonegative reaction or is it a reaction to the free tip or perhaps a reaction to both? To answer these questions the experiment was repeated using the same apparatus but in various positions, i.e. upside down with free end at the bottom, horizontal with the free end at the right or left sides, and finally with no free ends, i.e. with vaseline at both ends.



(1) White light on.
(2) Red light on.

a, point at which the white light was switched off and the red light substituted - tick changed direction.
b, red light off and white light switched on - no change of direction by tick.
c, white light switched off and red light switched on - tick changed direction.
d, red light off and white light switched on - no change of direction by tick.
e, tick going up, white light off and red light switched on - no change of direction.
f, red light switched off and white light substituted - no change of direction.

TRACK 6. Track made by larva on vertical rod with the tip up in red or white light.

These experiments were carried out under red light to rule out the possible influence of light. The picture that appeared is as follows:

Tip down - Most time spent at bottom (tip). Normal tip reaction obtained. An example of this type is shown in Track 7.

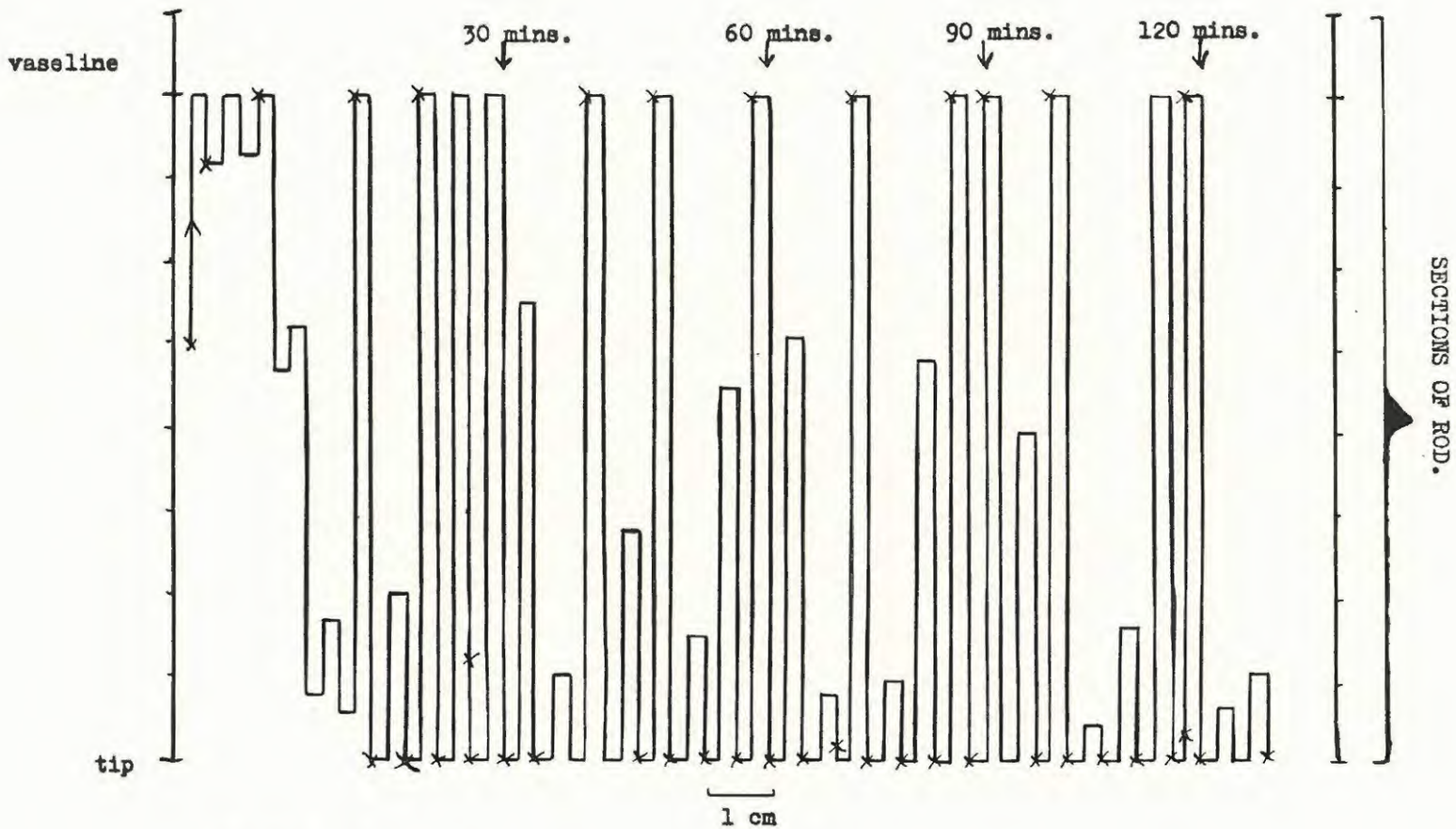
Tip right (Apparatus horizontal) - Most time spent at tip and normal tip reaction obtained. See Track 8 for example of this type.

No tip (Apparatus vertical with vaseline at both ends) Moved from end to end and the larvae settled at various points along the rod, but mostly in the top half. See Track 9 for an illustration of this type.

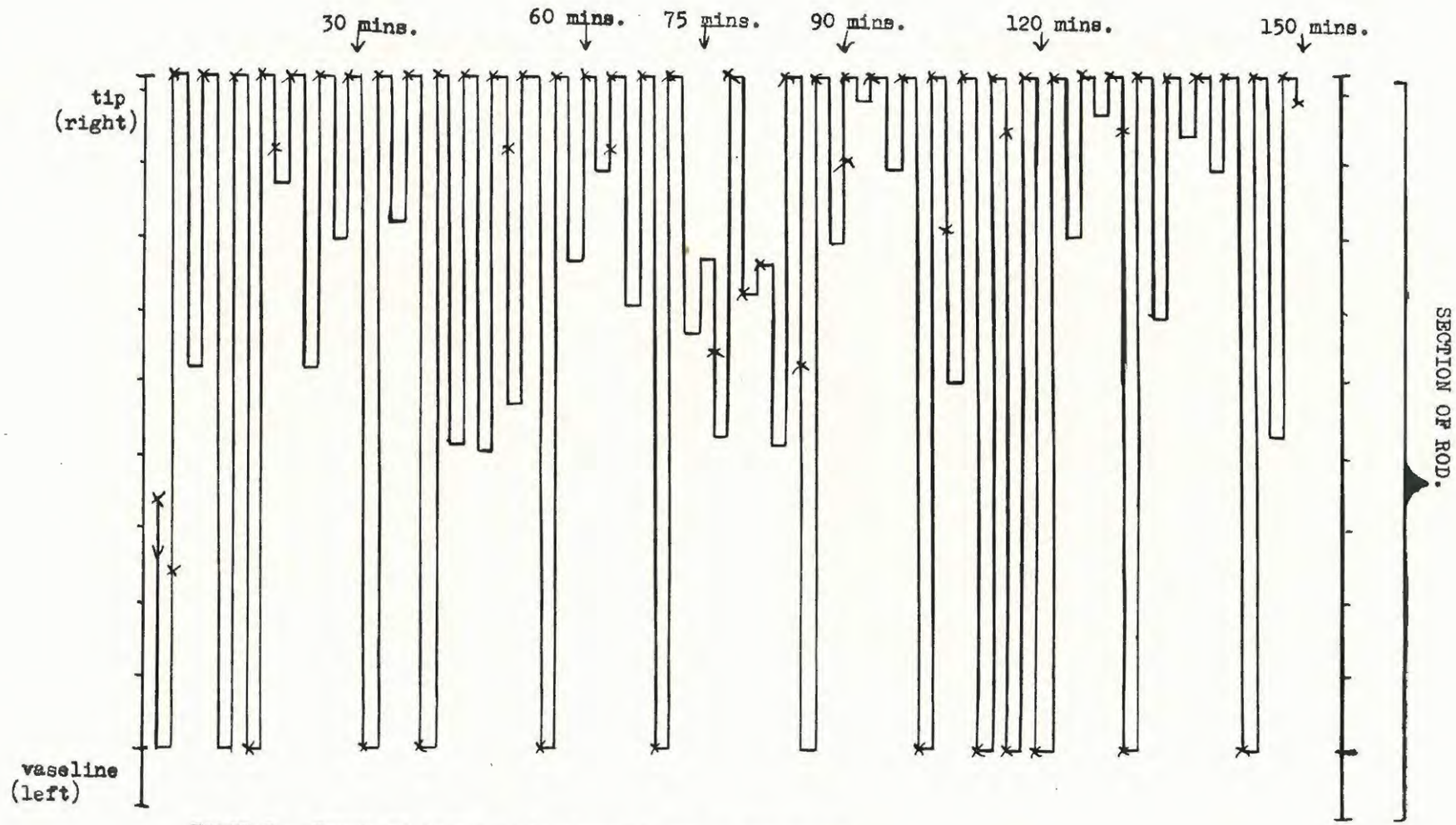
Finally control experiments were carried out with vaseline at both ends and with the rod horizontal (See Track 10 for an example). Here as in the experiments using vertical rods with vaseline at both ends, the larvae tended to move from end to end.

In addition to the above, a number of further experiments were carried out with the apparatus in various positions (as indicated in the table in Appendix VII) but here only the final settling position of the ticks was noted. The results were then plotted in the form of histograms (See Graphs 7 - 11).

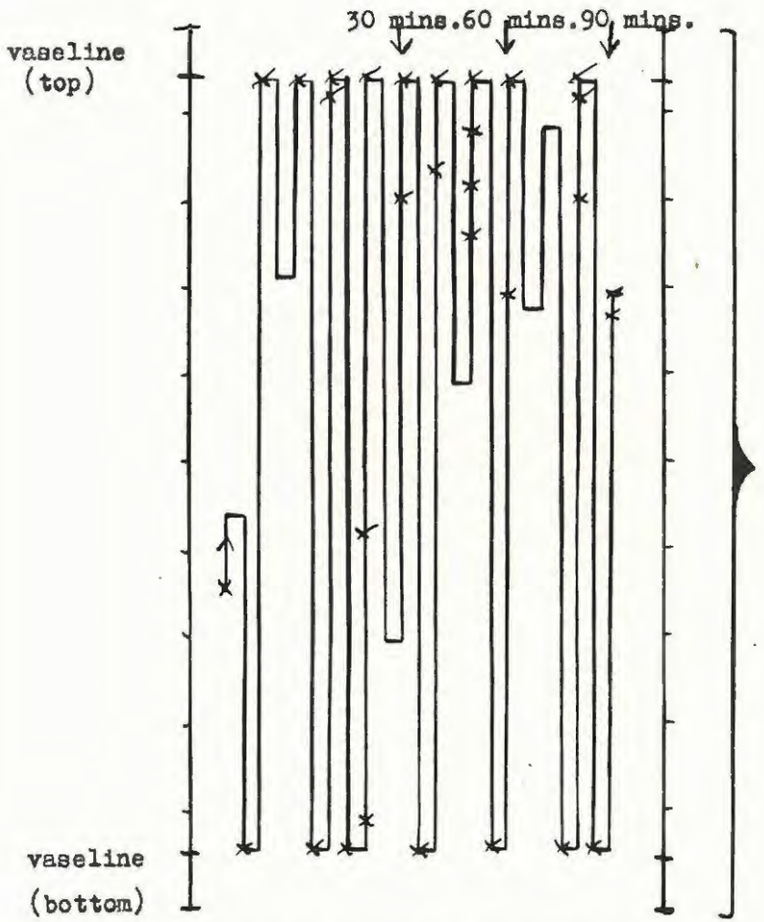
As can be seen from the histograms, the tip appears to override the gravity response but, with the tip eliminated (using vaseline) and the apparatus vertical, most of the larvae still sit at the top (although this response is less marked than with the tip and the geotactic response in conjunction).



TRACK 7. Track made by larva on a vertical rod - tip down.



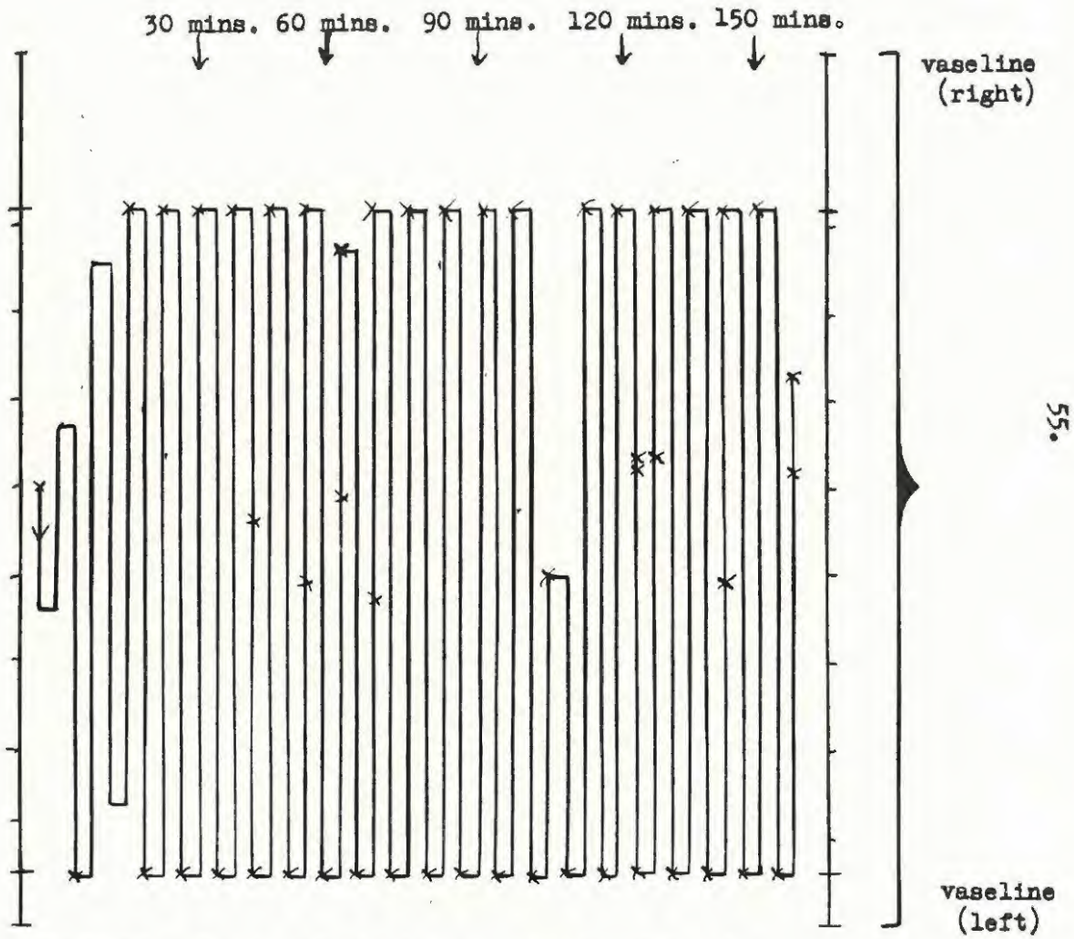
TRACK 8. Track of larva on horizontal rod - tip right.



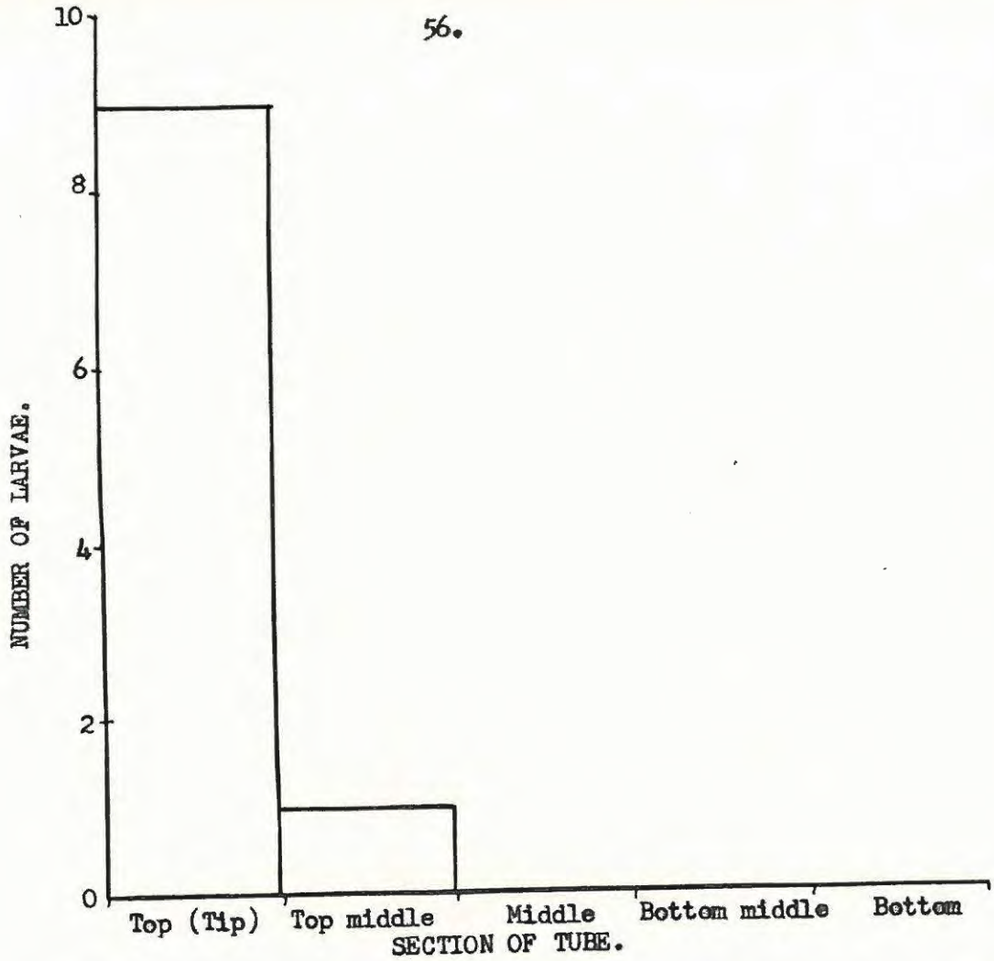
SECTION OF ROD.

1 cm

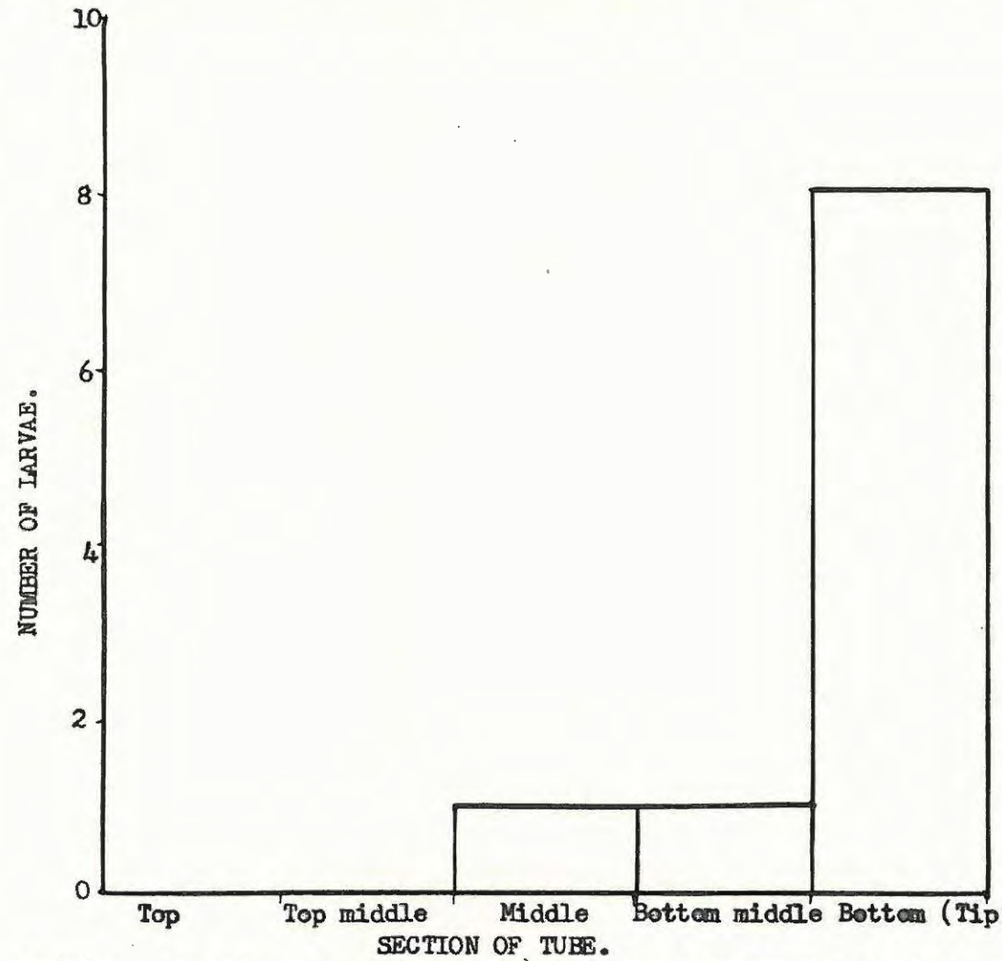
TRACK 9. Track made by larva on vertical rod - vaseline at both ends.



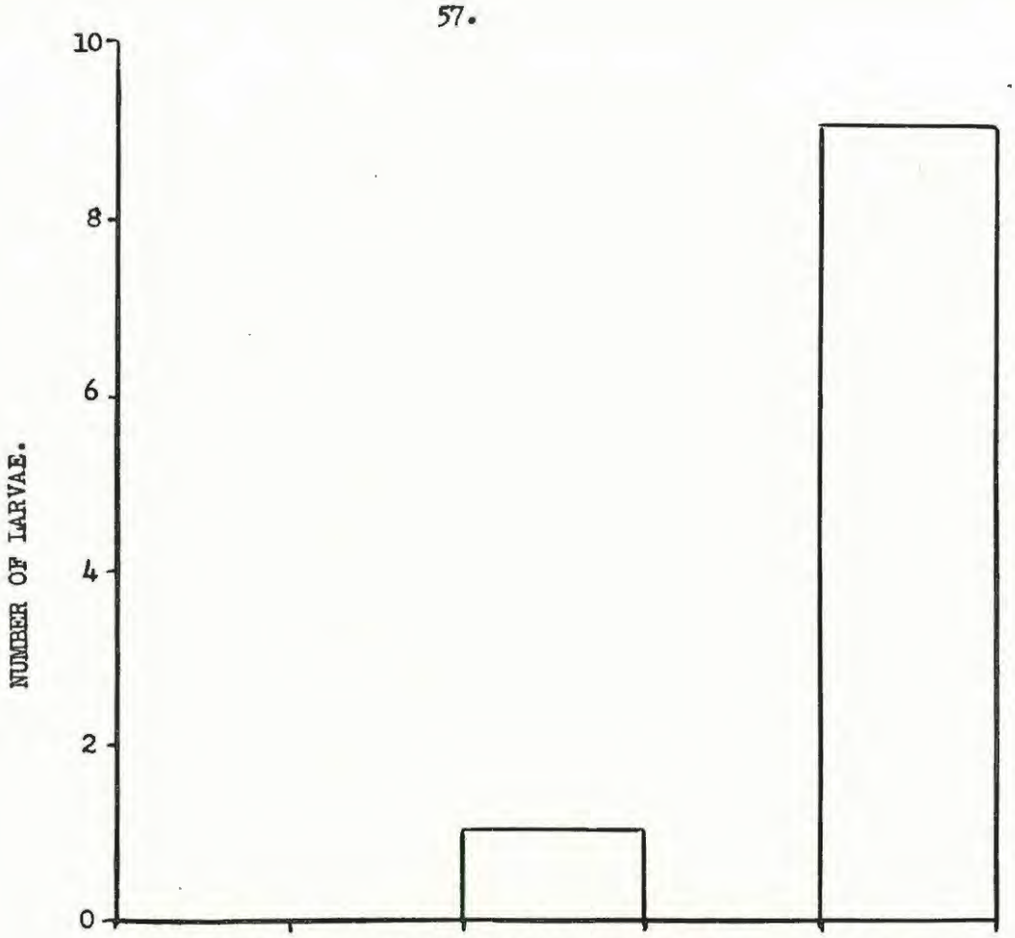
TRACK 10. Track made by larva on horizontal rod - vaseline at both ends.



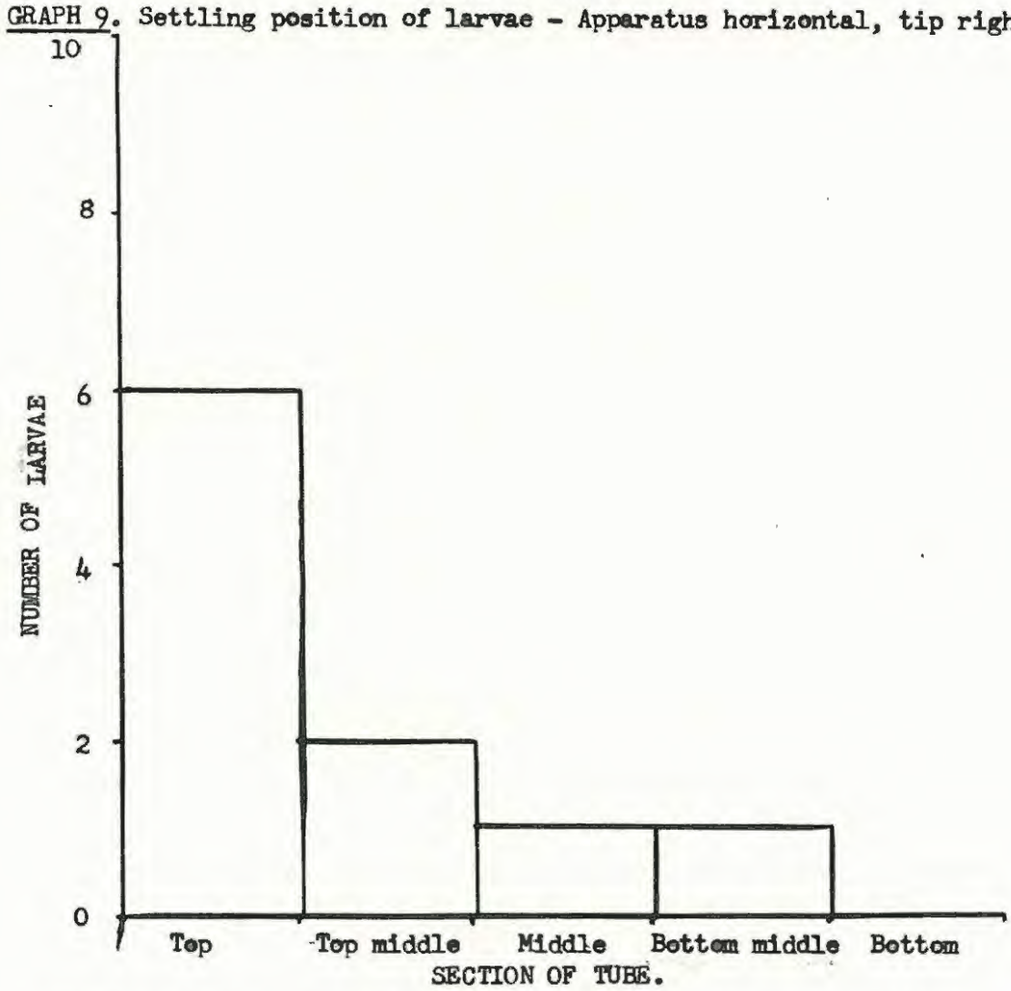
GRAPH 7. Settling positions of larvae - Apparatus vertical, tip up.



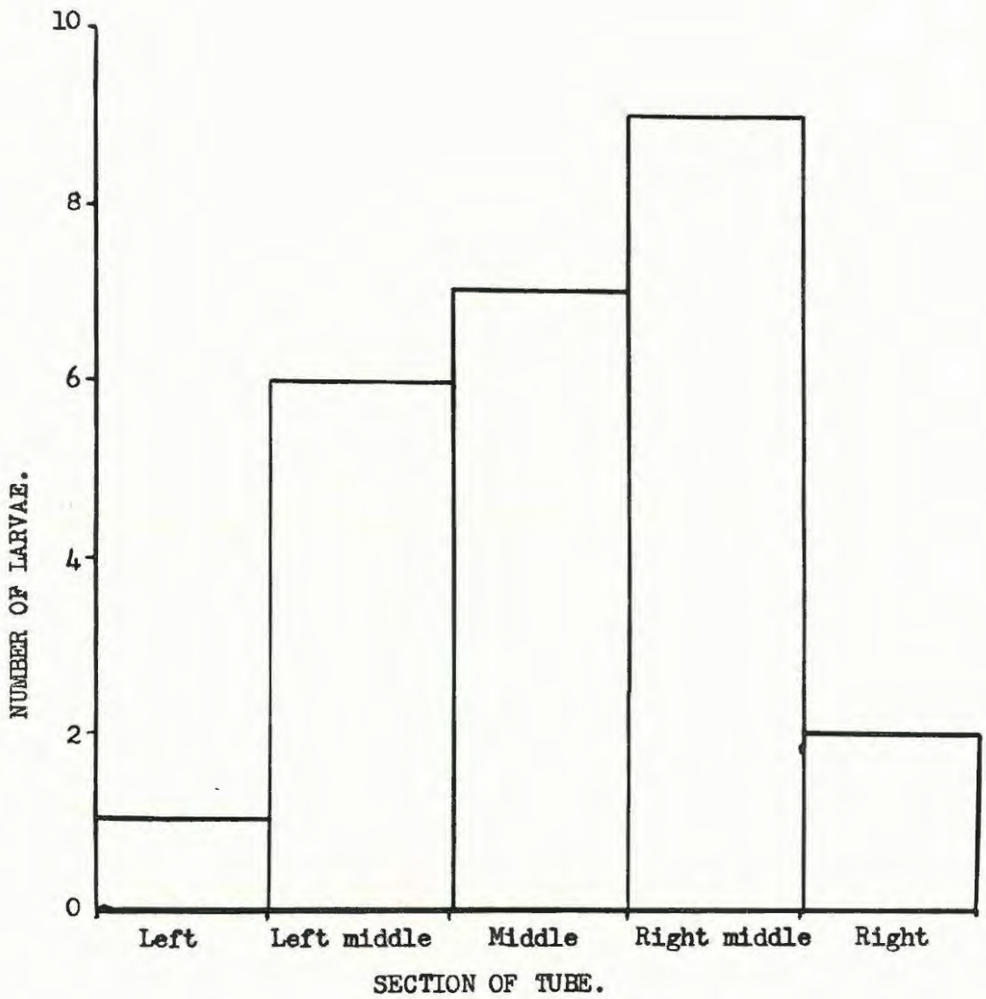
GRAPH 8. Settling positions of larvae - Apparatus vertical, tip down.



GRAPH 9. Settling position of larvae - Apparatus horizontal, tip right.



GRAPH 10. Settling positions of larvae - Apparatus vertical, vaseline at both ends.



GRAPH 11. Settling positions of larvae - Apparatus horizontal, vaseline at both ends.

In the control (horizontal with vaseline at both ends) the ticks settled more or less evenly along the rod. These experiments therefore seem to show that although the larvae demonstrate a type of negative geotaxis, the tip reaction can override the gravity reaction.

The interraction between tip and gravity was further tested with curved rods as used by Lees (1948 a). In this apparatus, the tip was not the highest point and the larvae were so placed that they had to pass over the highest point in order to reach the tip (See Fig. 6). As usual, the experiments were carried out in a constant temperature room under red light. As in the earlier experiments, the rods were kept in outer glass tubes on which was marked a scale to facilitate tracking.

In these experiments, the first point noted was where the larvae finally settled and for this purpose a number of experiments were carried out in which only the final settling position of the ticks was noted. The results were plotted in the form of a histogram (see table in Appendix VIII and also Graph 12).

The results indicate that the larvae are geonegative but, in contrast to the earlier experiments, the negative geotaxis overrides the tip reaction. The question which naturally arises is why?

To attempt to answer this question and to study the nature of the reaction, the curved rod experiments were repeated only this time the ticks were tracked as in the earlier experiments. A typical track from this series of experiments is illustrated in Track 11. In all these tracking experiments the larvae spent most of their time in the top half and in

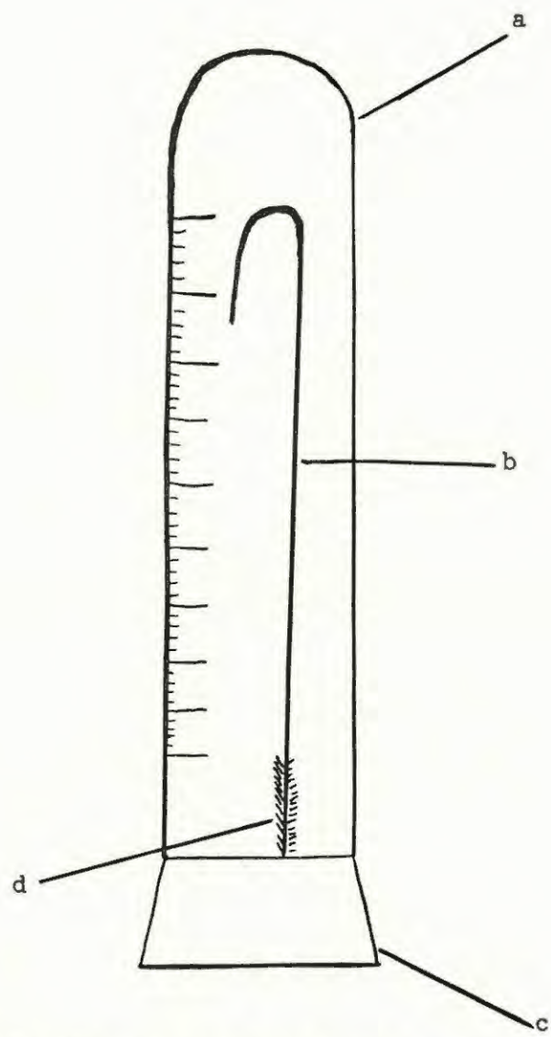
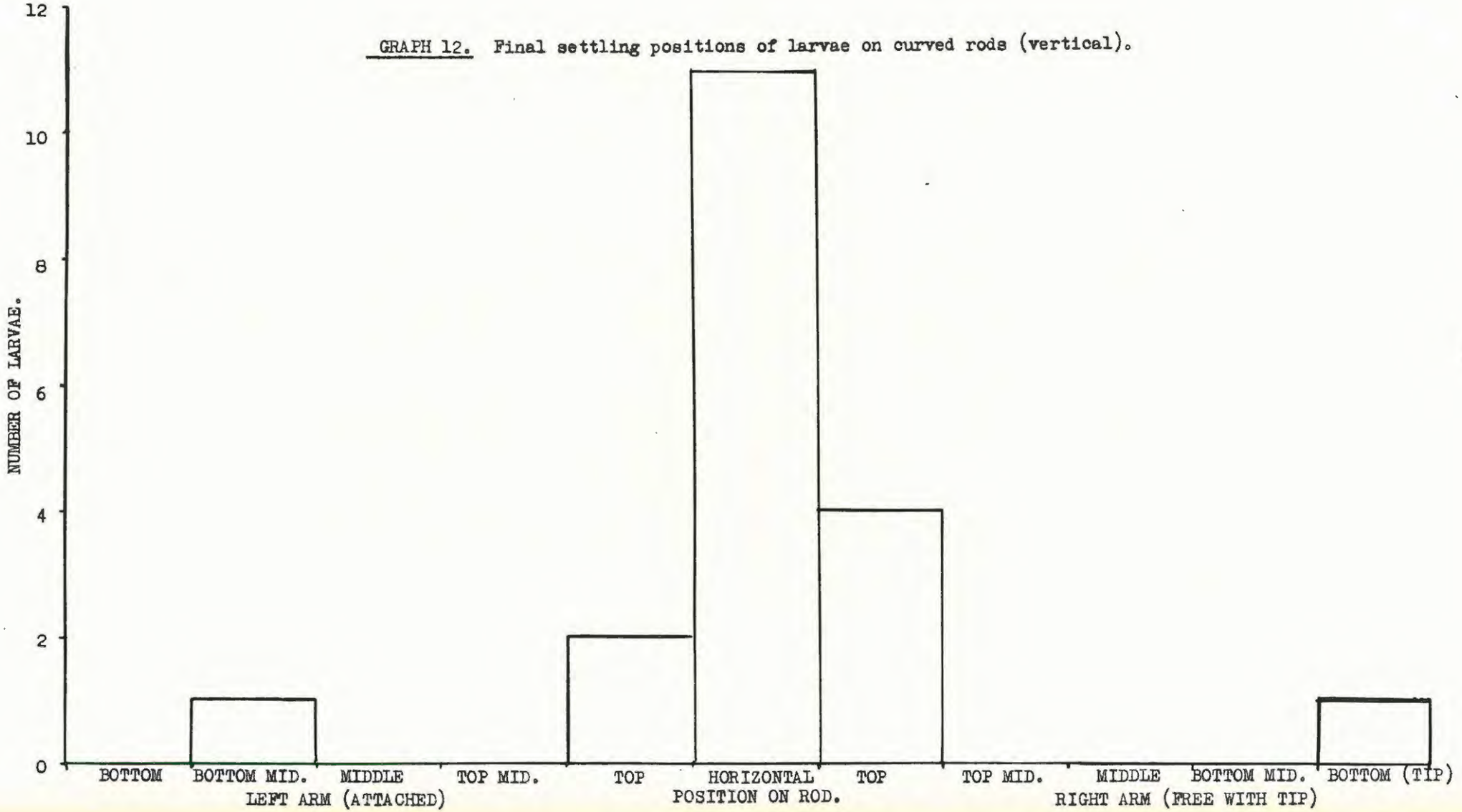
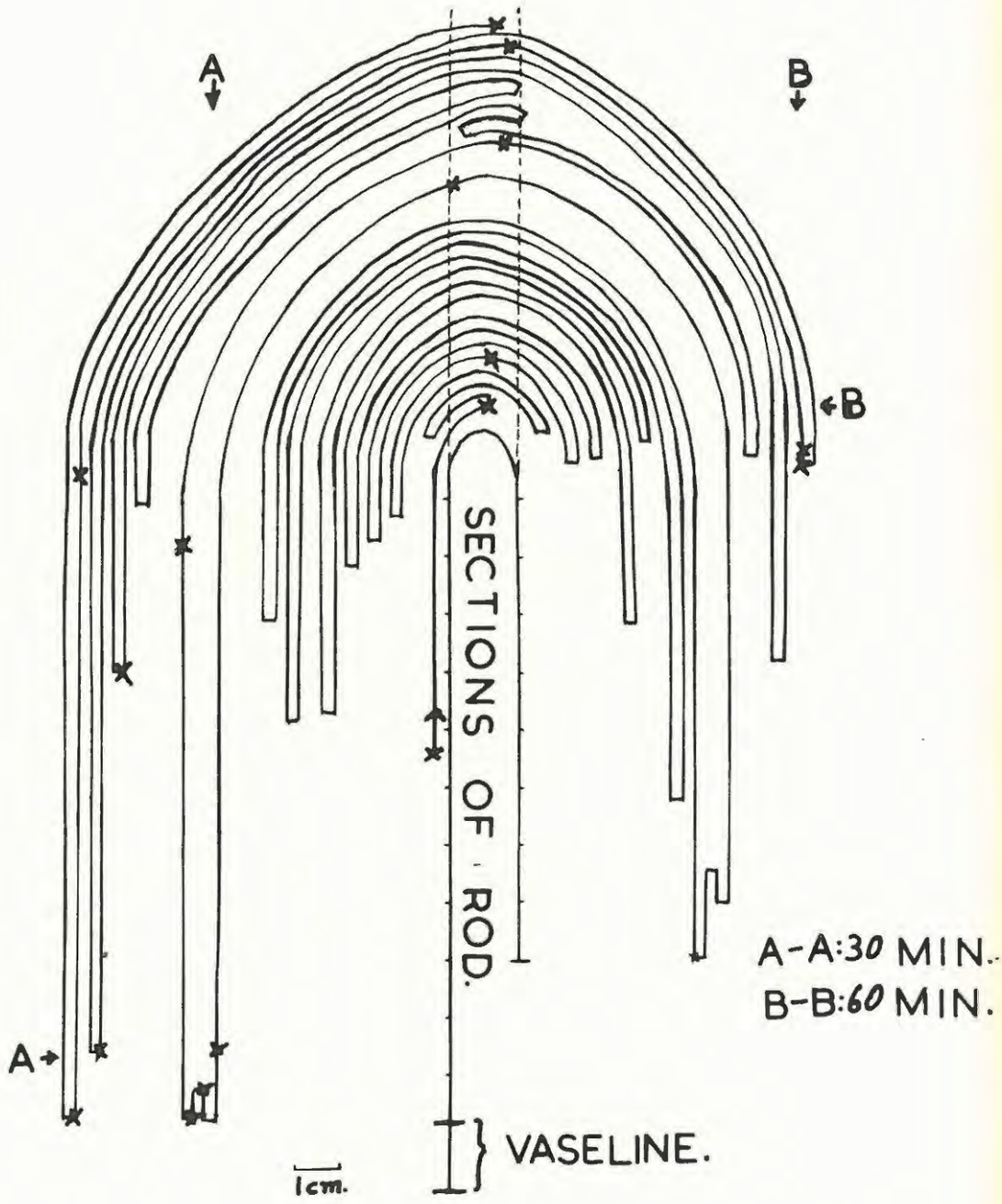


FIGURE 6. Gravity tracking apparatus - curved rod.

a, outer glass tube; b, glass rod;
c, cork; d, vaseline.

GRAPH 12. Final settling positions of larvae on curved rods (vertical).

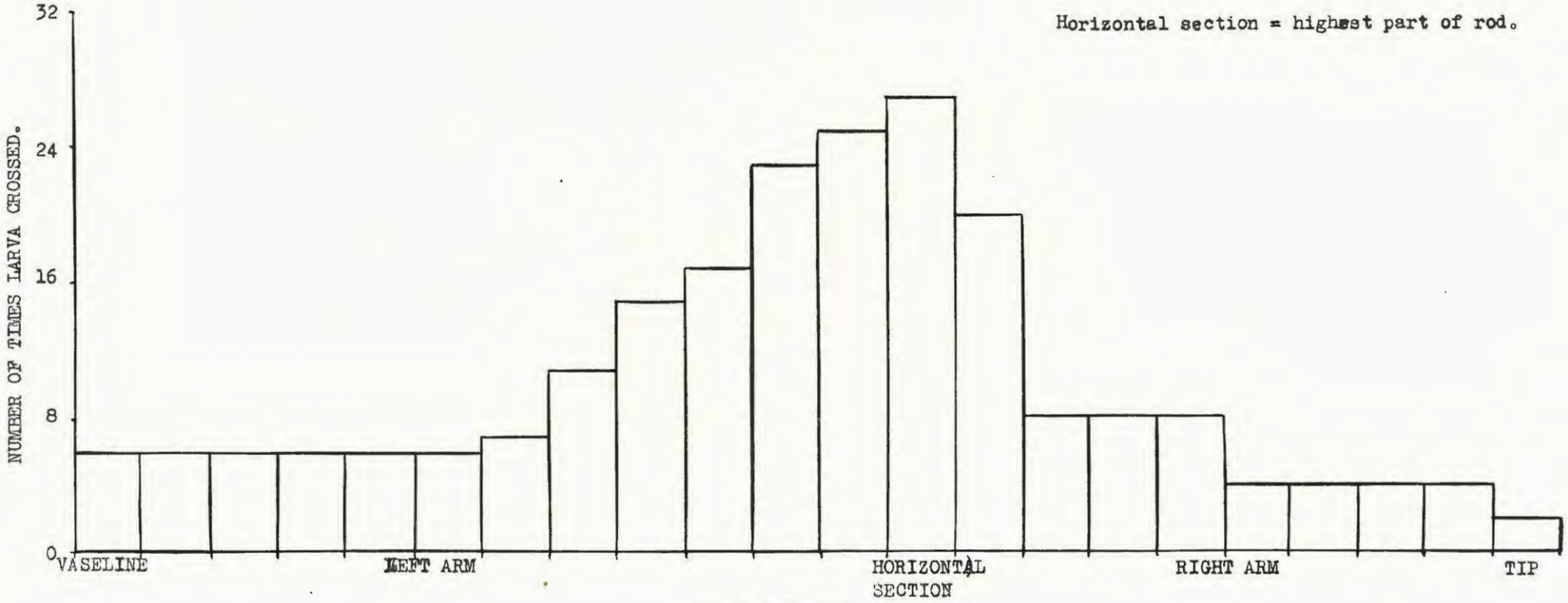




TRACK II. TRACK MADE BY LARVA ON CURVED ROD.

all cases where the final settling position was noted the ticks were found to settle at the top. (See Graph 13 of Track 11). As can be seen from Track 11 the ticks spent most of their time at the top due to a repeated turning upwards. This statement is based on the fact that the larvae did not go from end to end of the apparatus in which case the repeated upward turning could be said to be due to lack of any other direction in which to move. In all the trackings carried out, a tip reaction to the tip was noted but it was also observed that they turned upwards before reaching the tip in some cases. The reaction to the highest point in these experiments was observed to be rather like the normal tip reaction, i.e. a constant turning back to it. From the results obtained, therefore, it appears that movement to the top of a glass rod is due to a geonegative reaction and, once at the top, the larvae remain there due to a combination of this gravity reaction and a tip reaction to the highest point. A possible explanation of this behaviour is discussed later.

The next question to arise was whether the very young larvae were geopositive or geonegative. This was tested as part of the work carried out to find out why the young larvae in the field sit at the base of the vegetation until about a week old. As has been seen in the earlier light experiments, during this first week after hatching the larvae are negatively phototactic but, when about seven days old, they become photopositive. Is it this light reaction alone which causes them to remain at the base of the vegetation (possibly combined with a strong clumping response) or is it a combination of a photonegative



GRAPH 13. Number of times larva crossed different sections of a curved rod.

and a geopositive reaction? The problem was studied using the usual gravity apparatus of a glass tube marked into sections. All experiments were carried out as before, in constant conditions of temperature and humidity and in red light. The results of these experiments are shown in the table in Appendix IX, and the results are plotted in Graph 14. This graph is plotted as was the earlier one using the excess percent system.

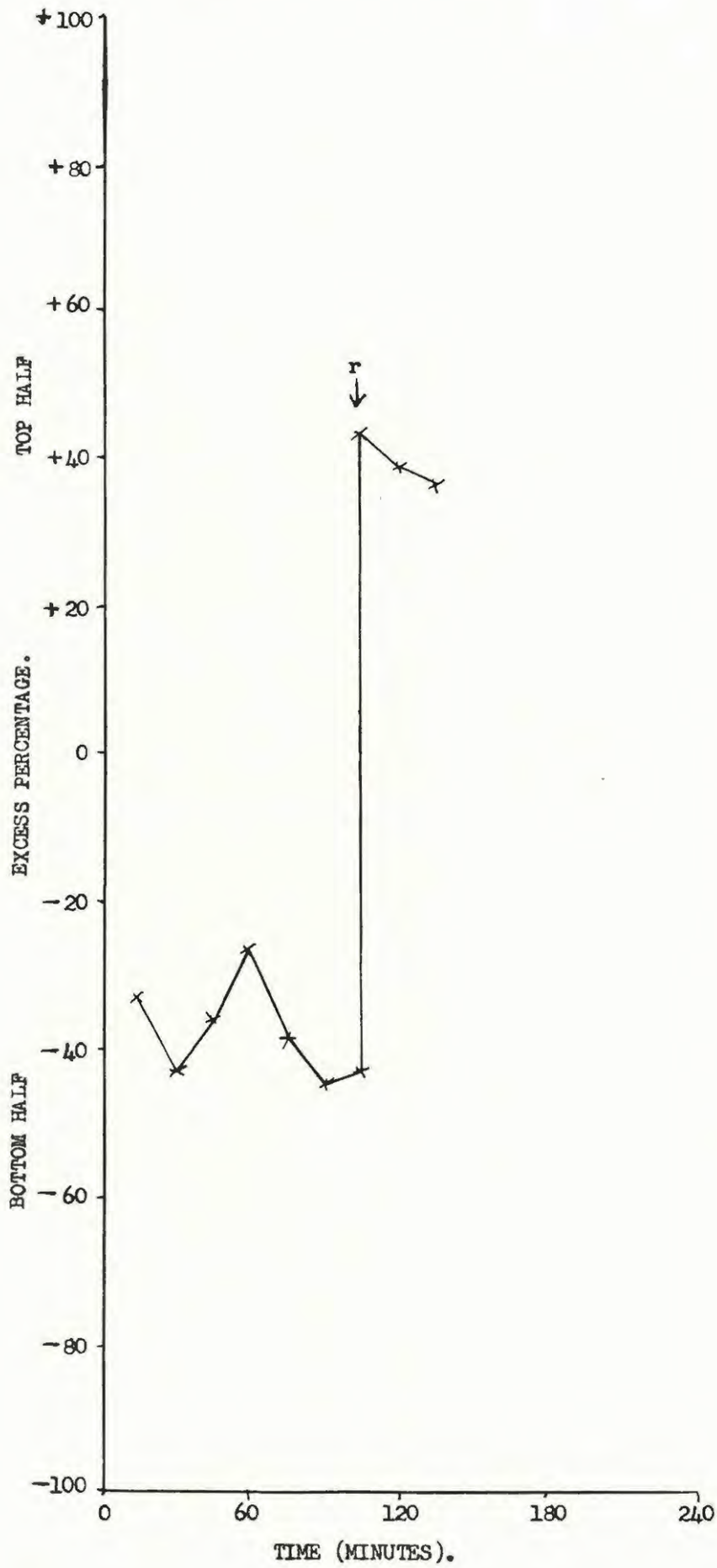
Two criticisms arose from the above experiments: Firstly, the initial readings were taken at 15 minutes and not at the beginning of the experiment, and secondly the larvae were distributed evenly over the apparatus when the experiments were started. However, what does emerge from the results is that the larvae appear to remain more or less as they were placed at the beginning of the experiment.

The question which arose from this observation was: Do the larvae merely tend to settle at the end nearest to which they are placed (or the first end at which they arrive) irrespective of whether the end concerned is at the top or at the bottom - i.e. there is a lack of a gravity reaction in the very young larvae. To attempt to find the answer to this, the following experiments were carried out:

(i) Apparatus vertical with all the larvae placed at the bottom.

(ii) Apparatus vertical with all the larvae placed at the top.

(iii) Apparatus vertical with the larvae distributed evenly over the whole tube.



GRAPH 14. Gravity reactions of 1 - 3 day old larvae.
r, point at which the apparatus was reversed

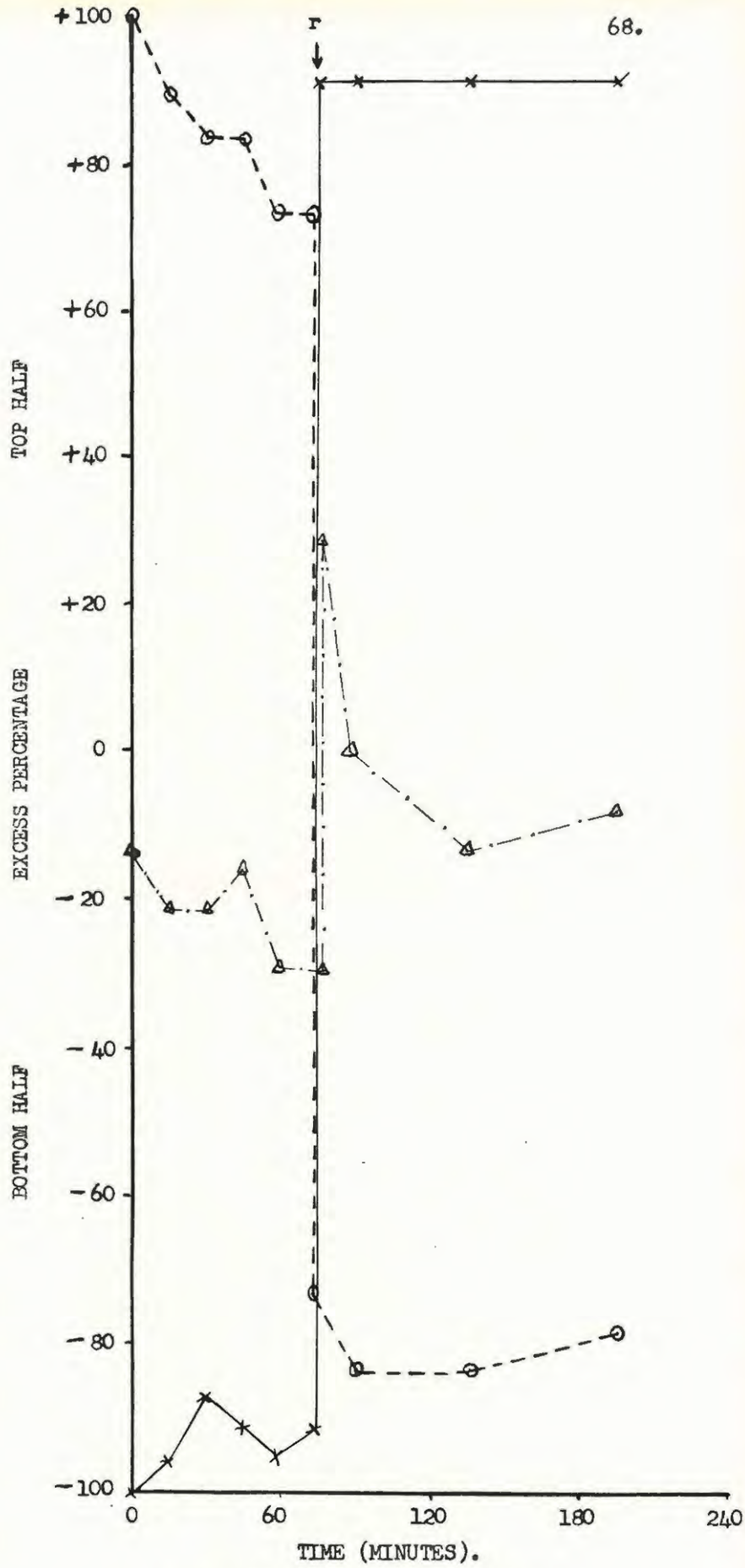
The experiments were again carried out using 1 - 3 day old larvae in glass tubes kept under constant conditions as before. The results can be seen in Graph 15 and the results are tabulated in Appendix X A-C. The larvae thus seem to be unreactive to gravity and appear to settle more or less where they are placed.

It was then decided to test the gravity behaviour of 4 - 6 day old larvae. In the breeding tubes larvae at this age were still at the bottom. The method was unchanged except that here all the larvae were placed at the bottom of the tube as in the experiments with the older larvae.

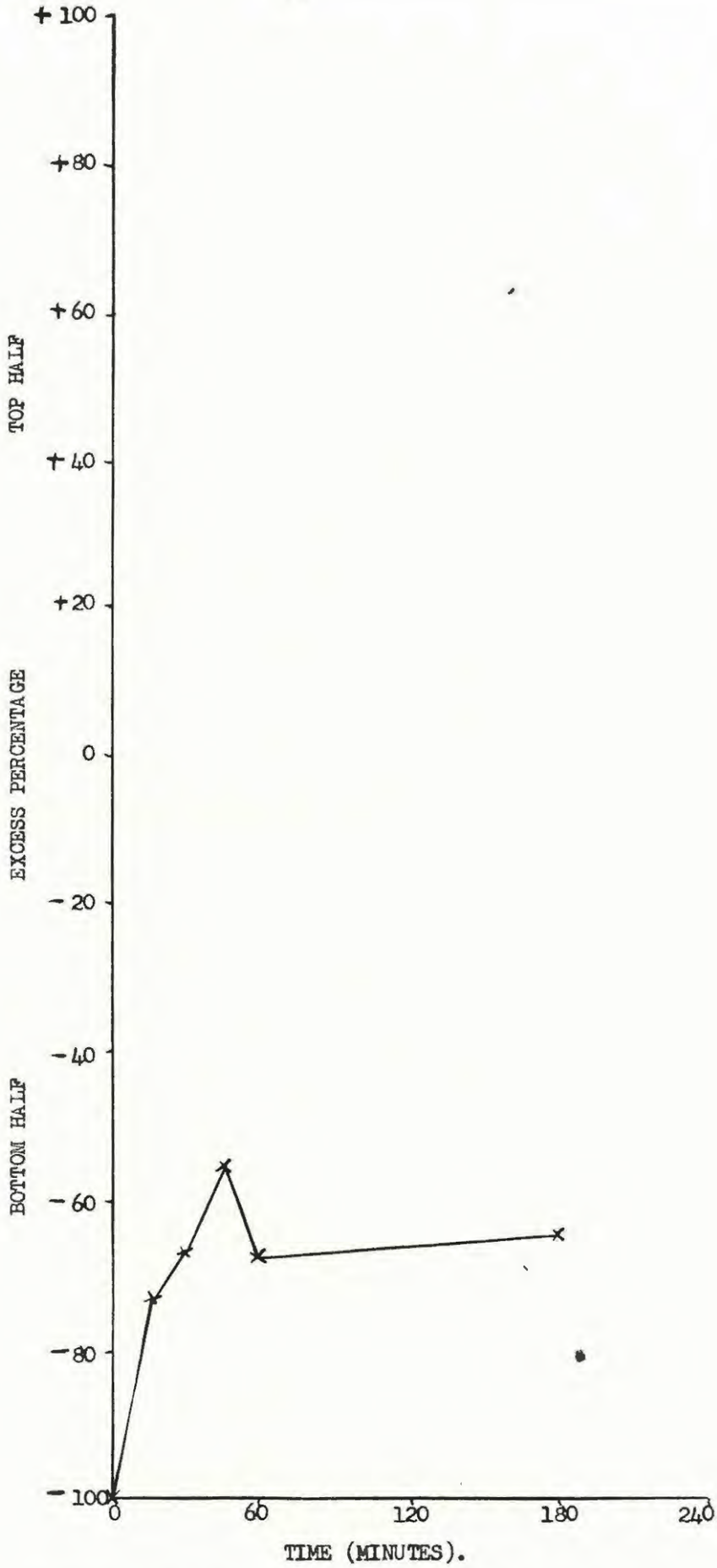
The results were once more presented graphically (See Graph 16 and the table in Appendix XI) The results are rather confused but the 4 - 6 day old larvae appear to show slight but definite negative geotaxis while the 1-3 day old larvae tended merely to move to the nearest end.

In all these experiments, it is necessary to bear in mind the possible effects of clumping on behaviour. This clumping response appears to be particularly strong in the very young larvae and it could therefore be that they move to a forming clump instead of reacting to gravity - it will be shown later that a clump seems to exert some attraction. (See clumping section later).

An attempt was made to understand the gravity behaviour of the larvae under a week old more clearly by means of tracking experiments as this type of experiment in which only one tick at a time was used ruled out the influence of a clump. The method was identical to that used previously on older larvae,



GRAPH 15. Gravity reactions of 1 - 3 day old larvae placed at different positions along apparatus
r, point at which the apparatus was reversed.



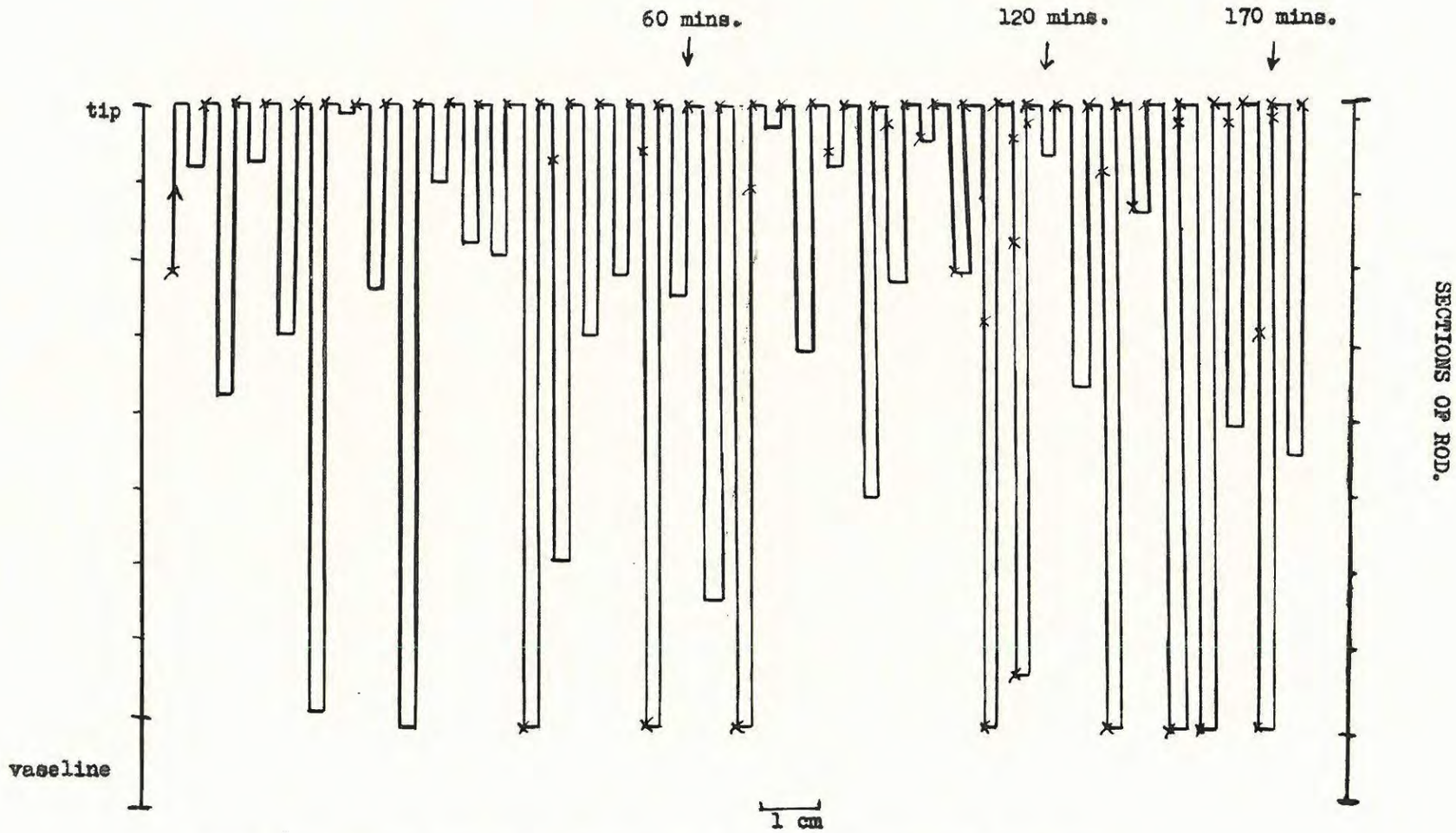
GRAPH 16. Gravity reactions of 4 - 6 day old larvae.

straight rods being used. The experiments were carried out with the tip of the rods up, down, with the rods horizontal and with vertical rods with vaseline at both ends. Typical tracks of the lst 3 types are illustrated (see Tracks 12-14). In the experiments with the tip up the larvae were found to prefer the top (tip) end and, having once experienced the tip, they mostly went right up to the tip each time. In most cases they settled at or near the tip. (For an example of this type of track see Track 12). In the tip down experiments they reacted to the tip and not to gravity (See Track 13 for example of the type), and they also reacted to the tip in the horizontal rod experiments. (see Track 14). From the experiments so far it appears that the very young larvae orientate to a free end rather than to gravity. It was then decided to see how they behaved on a vertical rod with no free end. In these latter experiments only the final settling positions of the larvae were noted and these were then tabulated. (See Table 14 below:

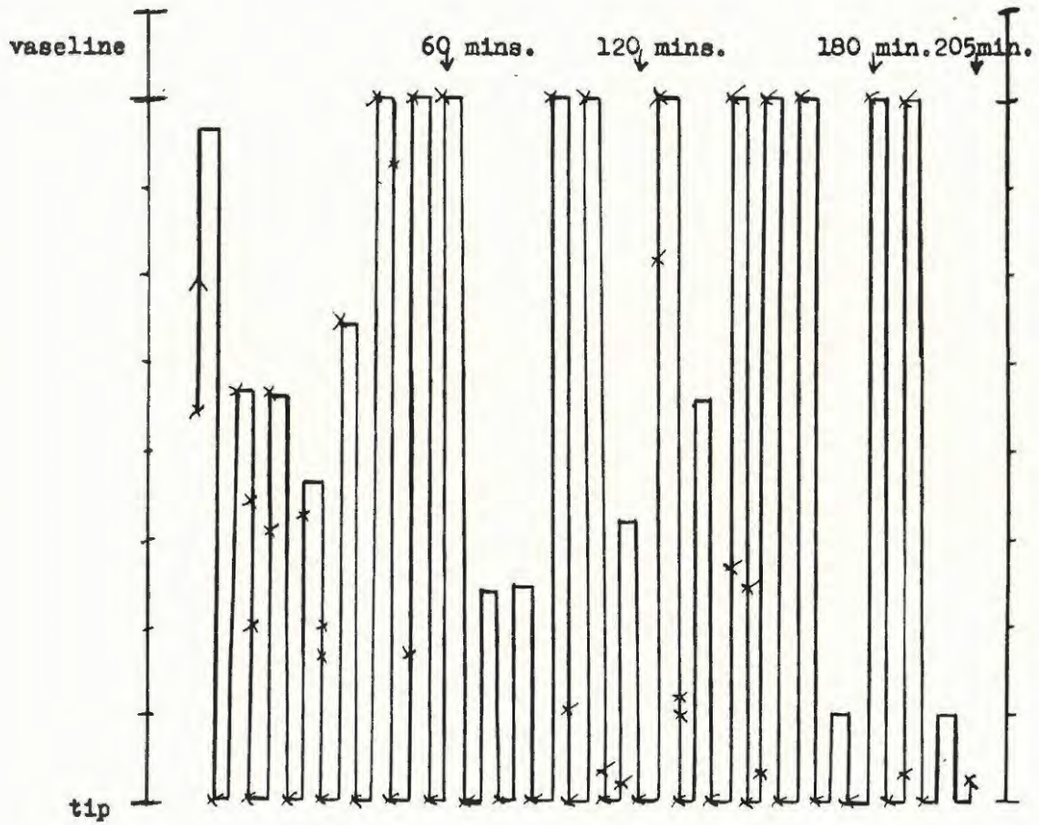
TABLE 14. FINAL SETTLING POSITIONS OF YOUNG (LESS THAN ONE WEEK OLD) LARVAE ON STRAIGHT GLASS RODS.

SETTLING POSITION / POSITION OF ROD	TOP (OR LEFT) FIFTH	TOP (OR LEFT) MIDDLE FIFTH	MIDDLE FIFTH	BOT.OR RIGHT) MIDDLE FIFTH	BOT.(OR RIGHT FIFTH
TIP UP	3	0	1	0	0
TIP DOWN	0	0	0	0	0
TIP LEFT (app.horiz.)	2	0	0	0	0
NO TIP (rod vert.)	0	2	2	4	0

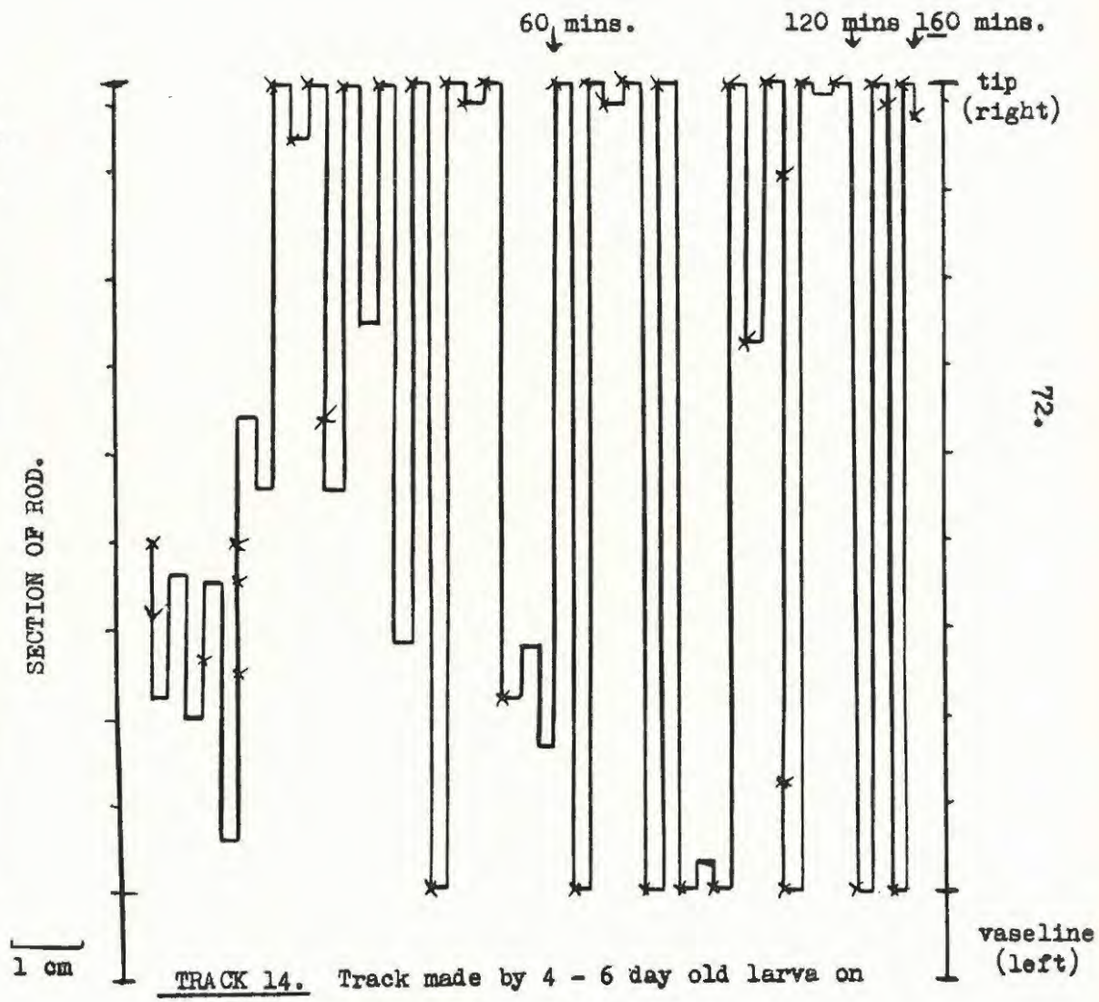
These latter results showed that the very young larvae (a) react to the tip and (b) do not seem



TRACK 12. Track made by 4 - 5 day old larva on vertical rod - tip up.



TRACK 13. Tracks made by 4 - 6 day old larvae on vertical rod - tip down.



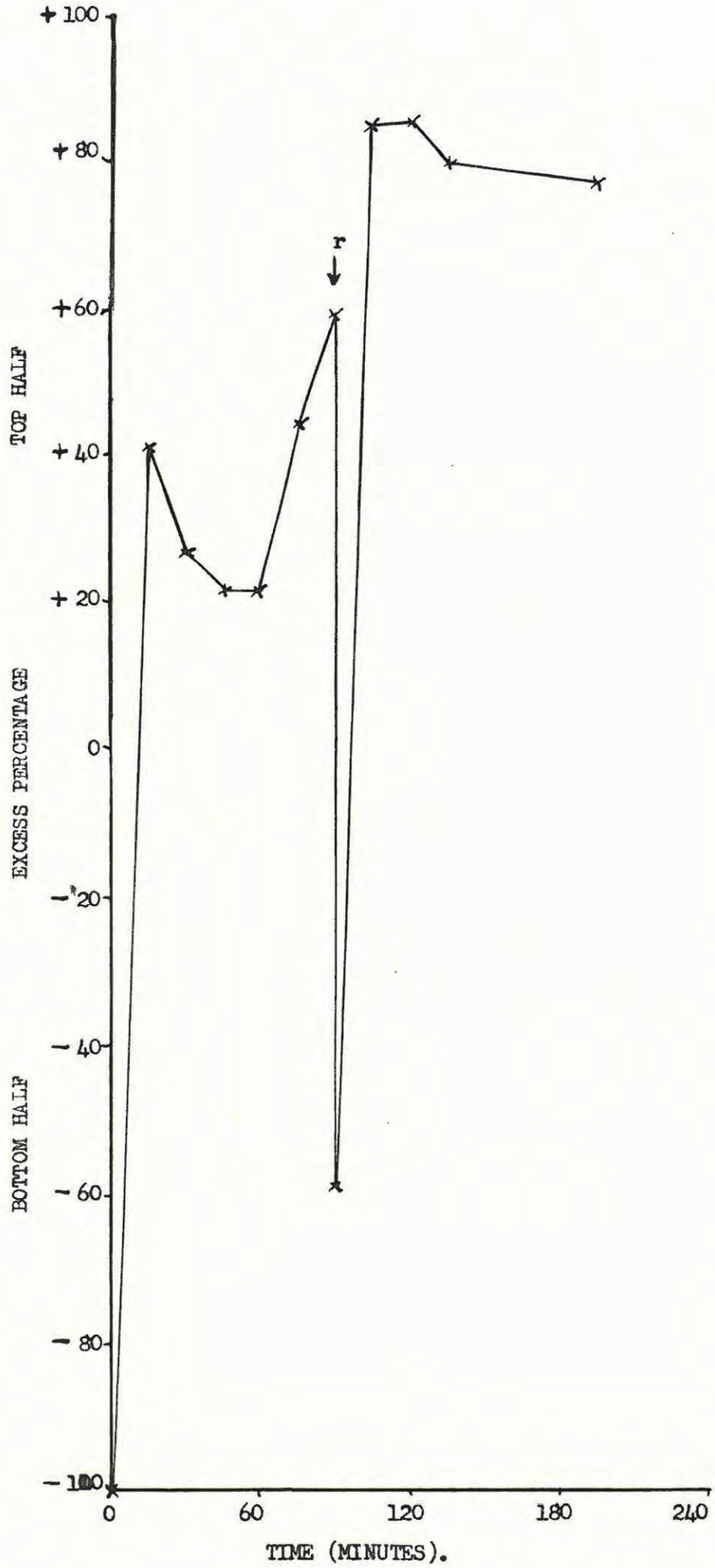
TRACK 14. Track made by 4 - 6 day old larva on horizontal rod - tip right.

to react to gravity.

Finally, when the ticks were just over one week old (about nine days old) the gravity experiments in the tubes were repeated under the usual conditions. This age was chosen as it was the age at which the larvae in the breeding tubes were observed to start to move upwards. The results are shown in Graph 17 and are tabulated in Appendix XII.

It was observed that clumping only commenced after about two hours. By the time that the larvae are about nine days, therefore, they seem to be clearly negatively geotactic. (Tested using Standard Error of Proportion, the result is significant at .001 level).

Lastly the gravity and light interactions of older larvae were investigated and for this purpose the usual glass rod gravity apparatus was used but here the outer glass tube was covered with black paper or black paper with "windows" cut in various positions (e.g. top, middle or bottom). As the outer glass tube was thus covered, the numbers of animals at the various positions could not be counted and therefore another identical glass tube was used which was graduated into quarters. This "counting tube" was kept on hand and, when the numbers of larvae in the various sections were to be counted, the glass rod in its cork was quickly transferred to the counting tube where the counts were made. With practice this method proved to be very successful and accurate counts could be made. The centre rod itself was not marked in case the markings interfered with the movements of the larvae. The light in these experiments was, however, rather weak,



GRAPH 17. Gravity reactions of 9 day old larvae.

r, point at which the apparatus was reversed.

being supplied by a light bulb about one metre away. The apparatus was left for three hours after which the counts were made. The results may be seen in Appendix XIII and in Graphs 18-26).

From these experiments, it appears that:

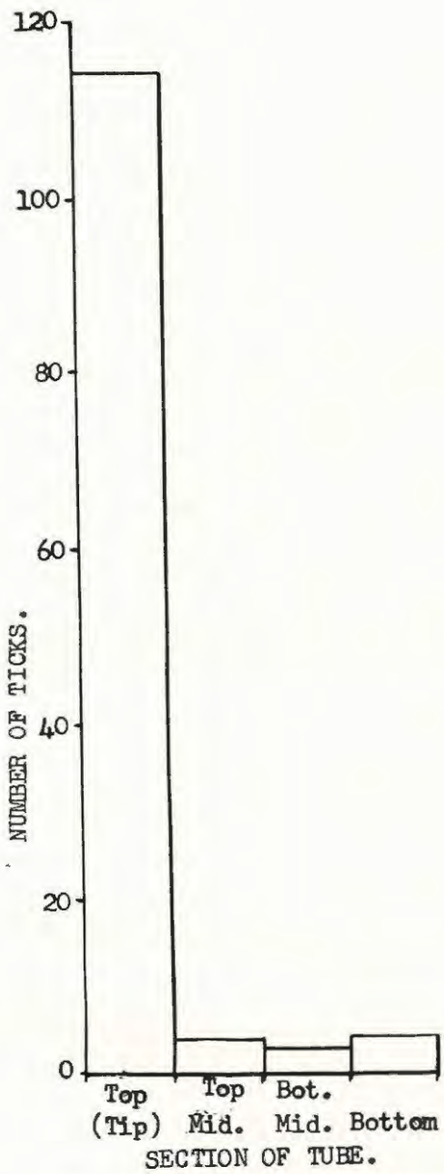
(a) The larvae react to the tip in preference to light or gravity.

(b) With no tip and no light they are negatively geotactic.

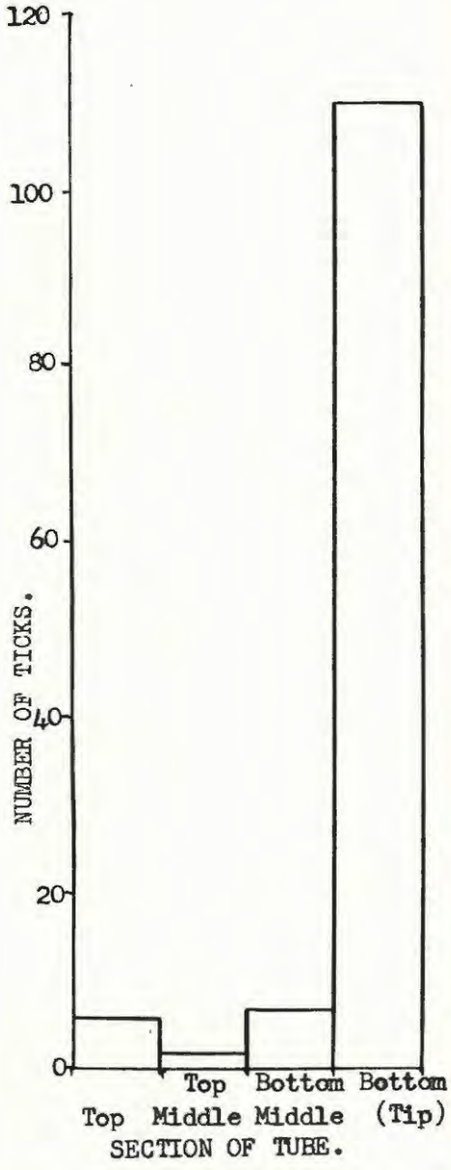
(c) The gravity response overrides the light response.

Conclusions: To summarise the work carried out on the gravity behaviour of B. decoloratus it appears that the larvae less than one week old are unreactive and tend to settle more or less where they were placed. It is suspected, however, that the strong clumping response might have something to do with this especially when they were placed at one or other end of the apparatus. This clumping behaviour is considered later. At about nine days of age, the larvae become strongly geonegative and move upwards. Larvae at about six weeks of age are also negatively geotactic. In some cases it was noticed that the number of larvae at the top of the support fell off after a while.

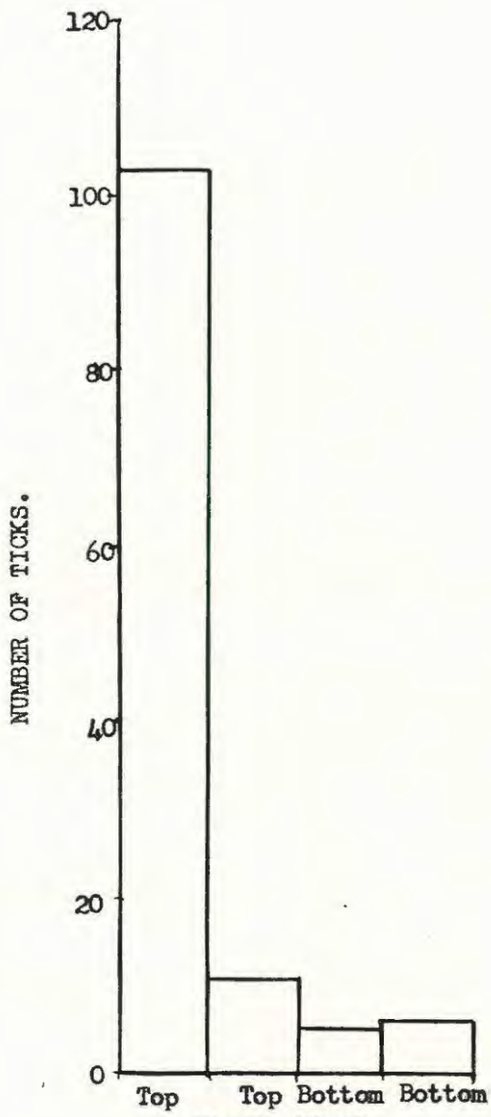
In one series of experiments the larvae showed an apparent gravity rhythm possibly connected to the light/dark cycle, and in a later series, while no such rhythm was obtained a rhythm of activity was observed in which the number of larvae active increased in the late afternoon. The two apparently contradictory results are not, in fact, incompatible, as it was possible for the larvae to become active without moving out of their section in the tube.



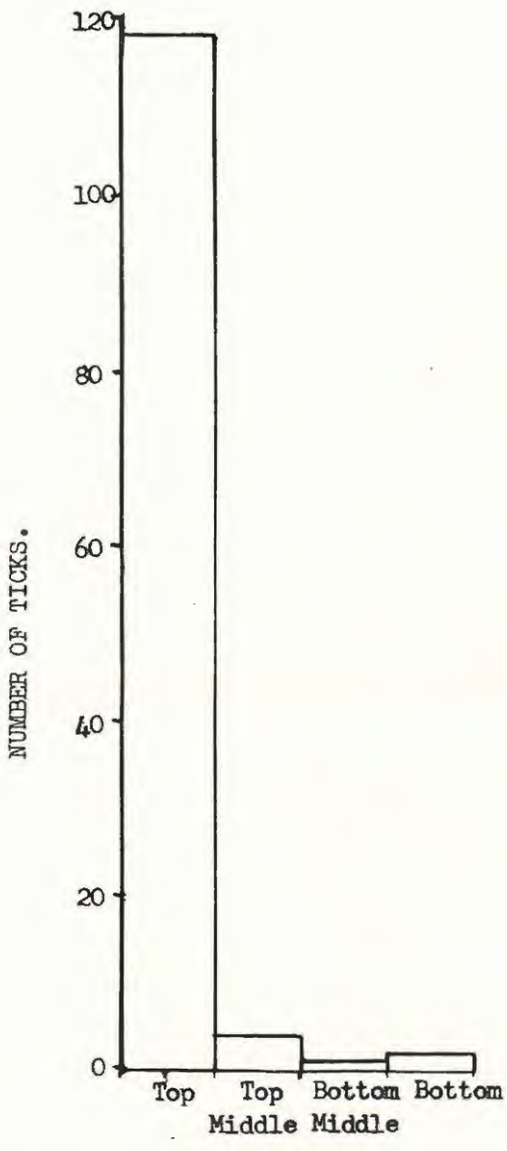
GRAPH 18. Settling positions of larvae - Apparatus vertical, window and tip at the top.



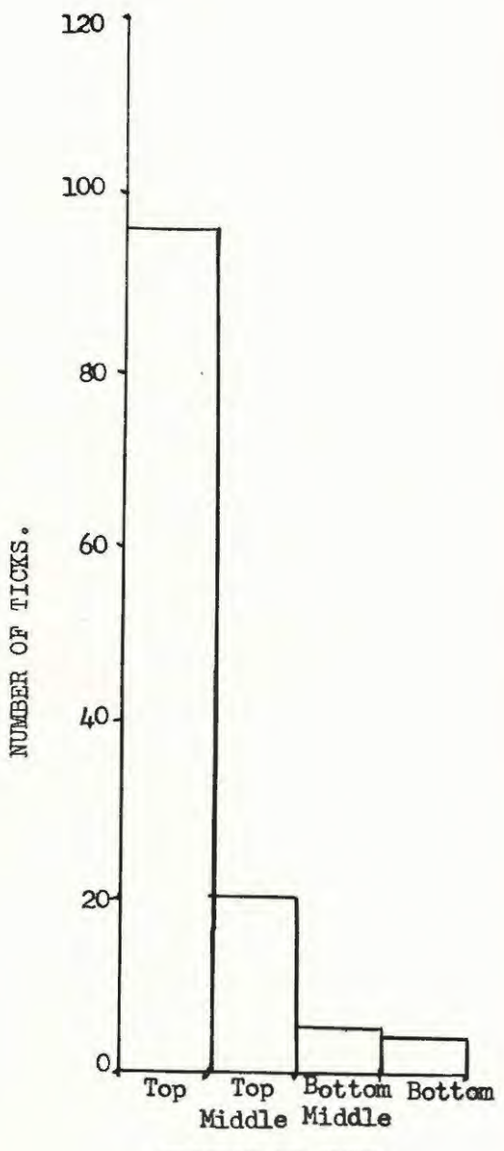
GRAPH 19. Settling positions of larvae - Apparatus vertical, window and tip at the bottom.



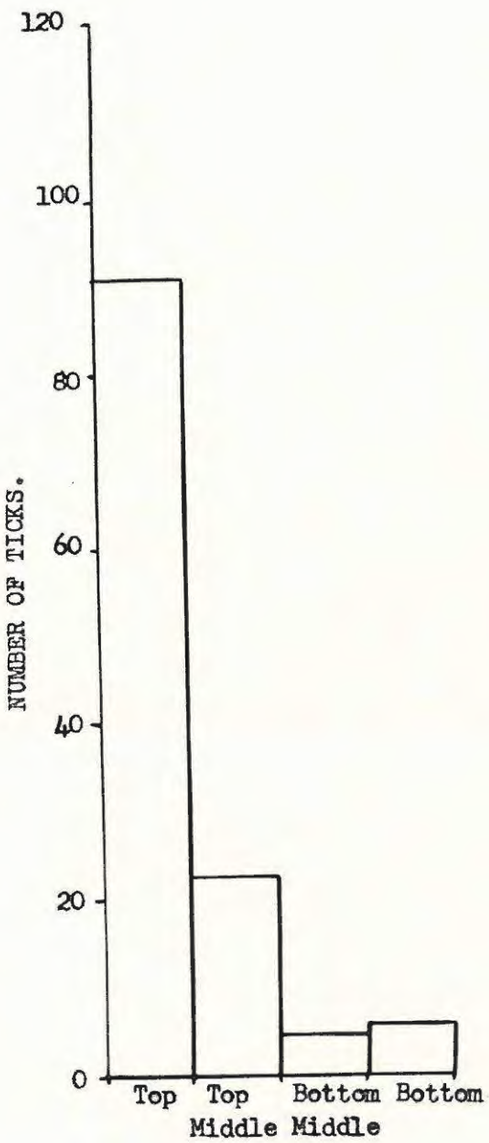
GRAPH 20. Settling positions of larvae - Apparatus vertical, window in middle and tip at top.



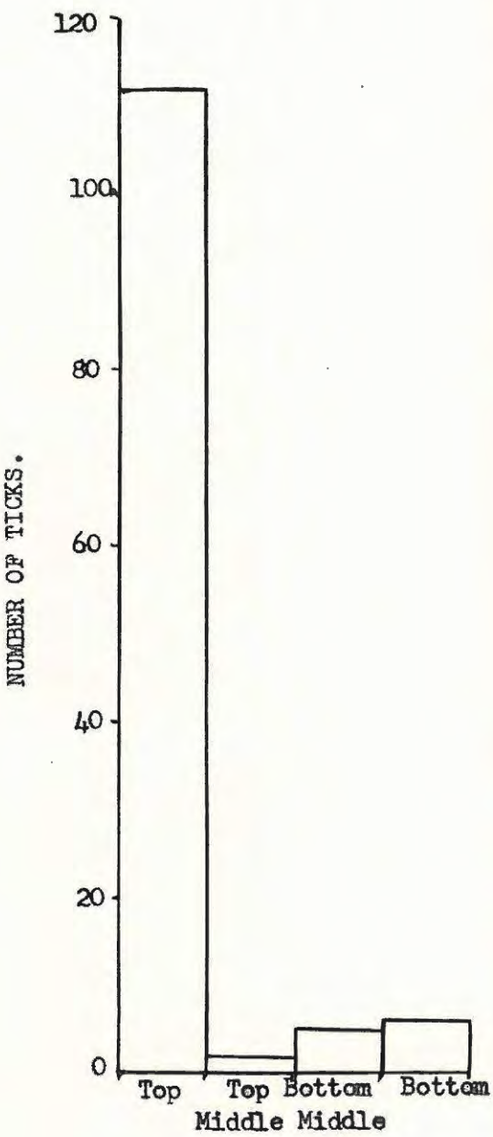
GRAPH 21. Settling positions of larvae - Apparatus vertical, window at top and vaseline at both ends.



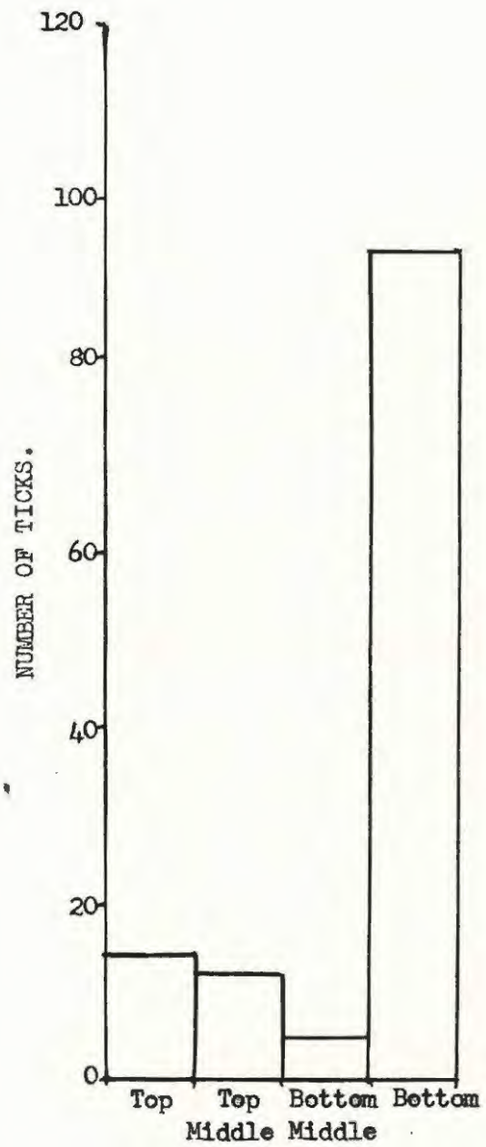
GRAPH 22. Settling positions of larvae - Apparatus vertical, window at bottom and vaseline at both ends.



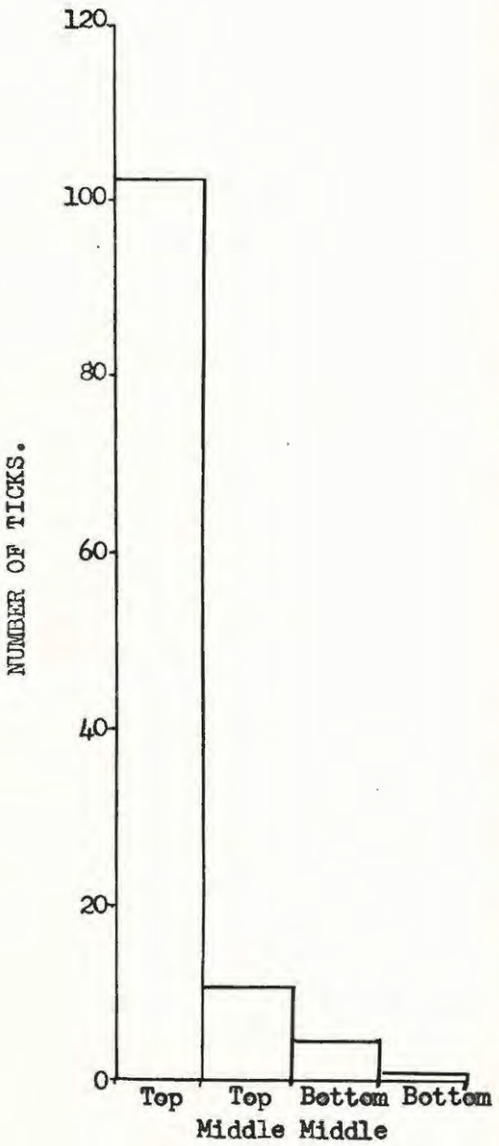
GRAPH 23. Settling positions of larvae - Apparatus vertical, window in middle and vaseline at both ends.



GRAPH 24. Settling positions of larvae - Apparatus vertical, total dark and tip up.



GRAPH 25. Settling positions of larvae - Apparatus vertical, total dark and tip down.



GRAPH 26. Settling positions of larvae - Apparatus vertical, total dark and vaseline at both ends.

Further studies on the gravity behaviour of the larvae were carried out by observing their reactions on glass rods. The results of these experiments agreed with the earlier ones, i.e. the young larvae do not react to gravity while the older ones were negatively geotactic. All larvae, irrespective of age, however, were found to react to the tip. In the older larvae, it was found that the tip reaction overrode the gravity response when straight rods were used but, when corved rods were used, the larvae settled at the highest point. A possible explanation for this is that a larva, being negatively geotactic, moves upwards and settles in a position in which it can sit with its forelegs free (See explanatory diagrams Fig.7). This condition is satisfied by a tip and with a straight rod can only be satisfied by a tip. (See Fig. 7 a and b below). With a curved rod, however, this condition can occur at the highest point as well as at the tip. (see Fig.7c below), and so being geonegative the larvae will go for the highest point rather than the tip on a curved rod.

The tracking experiments showed that the response on rods was due to a constant turning back to the tip (the highest point on a curved rod).

When the interactions between gravity, light and the tip were investigated, it appeared that the tip reaction overrode the other two responses and that the gravity reaction overrode the light response. It is worth noting once more, however, that the light used was not a very strong one.

Another point of interest which emerged was

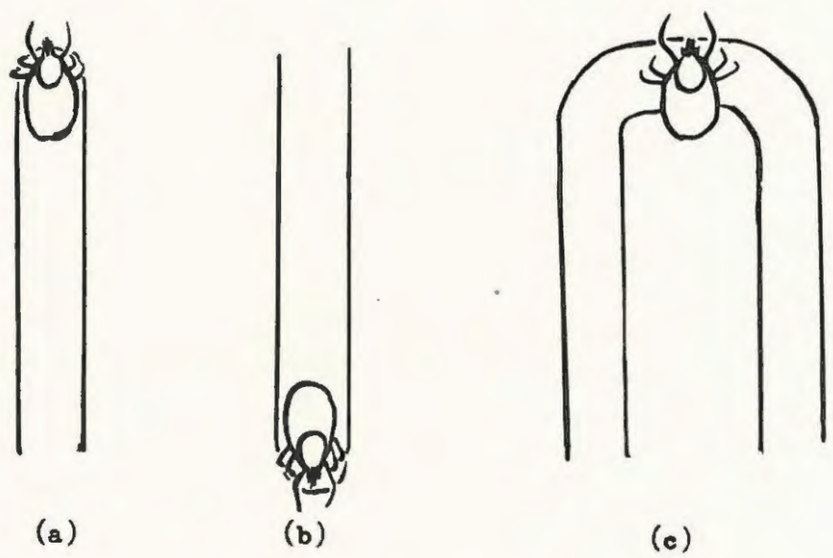


FIGURE 7. Diagrammatic drawings of larvae on straight and curved rods.

that larvae moving downwards would change direction and move upwards with a fall in light intensity (i.e. substitution of light with red light). This ties up with later work carried out on the questing behaviour of the larvae.

APPENDIX IVa

GRAVITY REACTIONS OF 6-8 WEEK OLD LARVAE - LONG TERM EXPERIMENTS

		TIME				NO. OF LARVAE					
READ	CLOCK TIME	Dys.	Hrs.	Mins	LIGHT	TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
1	3.15 p.m.	0	00	00	on	0(0%)	0(0%)	0(0%)	68(100%)	- 100%	68
2	3.30 p.m.	0	00	15	on	47(69%)	5(7.4%)	11(16.2%)	5(7.4%)	53.0%	
3	4.15 p.m.	0	1	00	on	45(66.2%)	9(13.2%)	11(16.2%)	3(4.4%)	58.8%	
4	4.45 p.m.	0	1	30	on	50(73.5%)	6(8.8%)	4(5.9%)	8(11.8%)	64.8%	
5	5.15 p.m.	0	2	00	on	50(73.5%)	5(7.4%)	5(7.4%)	8(11.8%)	61.8%	
6	5.45. p.m.	0	2	30	on	42(61.9%)	10(14.7%)	7(10.2%)	9(13.2%)	53.0%	
7	7.30 p.m.	0	4	15	on	43(63.3%)	10(14.7%)	9(13.2%)	6(8.8%)	55.8%	
8	8.30 p.m.	0	5	15	on	50(73.5%)	3(4.4%)	8(11.8%)	7(.0.2%)	55.8%	
9	9.45 p.m.	0	6	30	off	59(86.7%)	3(4.4%)	1(1.5%)	5(7.4%)	82.4%	
10	6.15 a.m.	0	15	00	off	65(95.5%)	1(1.5%)	0(0%)	2(3%)	94.8%	
11	7.45 a.m.	0	16	30	on	65(95.5%)	0(0%)	0(0%)	3(4.4%)	91.2%	
12	10.30 a.m.	0	19	15	on	64(94.1%)	0(0%)	1(1.5%)	3(4.4%)	88.2%	

(continued)

APPENDIX IVa (continued)

READ	CLOCK TIME	TIME Dys.	Hrs.	Min.	LIGHT	NO. OF LARVAE TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
13	12.30 p.m.	0	21	15	on	65(95.5%)	0(0%)	0(0%)	3(4.4%)	91.2%	68
14	2.30 pm.	0	23	15	on	57(83.8%)	4(5.9%)	3(4.4%)	4(5.9%)	79.4%	
15	4.45 p.m.	1	1	30	on	49(72.0%)	8(11.8%)	8(11.8%)	3(4.4%)	67.6%	
16	6.45 p.m.	1	3	30	on	46(67.7%)	5(7.4%)	7(10.2%)	10(14.7%)	50%	
17	9.45 p.m.	1	6	30	on	57(83.3%)	2(3%)	3(4.4%)	6(8.8%)	73.4%	
18	10.30 p.m.	1	7	15	off	66(97%)	0(0%)	1(1.5%)	1(1.5%)	94.8%)	
19	7.15 a.m.	1	16	00	off	68(100%)	0(0%)	0(0%)	0(0%)	100%	
20	8.15 a.m.	1	17	00	on	67(98.5%)	0(0%)	0(0%)	1(1.5%)	97.0%	
21	10.15 a.m.	1	19	00	on	66(97%)	0(0%)	0(0%)	2(3%)	94.8%	
22	1.15 p.m.	1	22	00	on	66(97%)	0(0%)	0(0%)	2(3%)	94.8%	
23	4.30 p.m.	2	1	15	on	54(79.4%)	9(13.2%)	3(4.4%)	2(3%)	85.2%	
24	5.30 p.m.	2	2	15	on	51(75.1%)	6(8.8%)	4(5.9%)	7(10.3%)	67.6%	
25	7.15 p.m.	2	4	00	on	57(83.8%)	2(3%)	4(5.9%)	5(7.4%)	73.4%	
26	9.15	2	6	00	off	68(100%)	0(0%)	0(0%)	0(0%)	100%	(continued)

APPENDIX IVa (continued)

READ	CLOCK TIME	TIME Dys	Hrs	Min	LIGHT	NO. OF LARVAE TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
27	10.15 p.m.	2	7	00	off	68(100%)	0(0%)	0(0%)	0(0%)	100%	68
28	6.30 a.m.	2	15	15	off	68(100%)	0(0%)	0(0%)	0(0%)	100%	
29	7.30 a.m.	2	16	15	on	68(100%)	0(0%)	0(0%)	0(0%)	100%	
30	8.30 a.m.	2	17	15	on	65(95.5%)	0(0%)	0(0%)	3(4.4%)	91.2%	
31	11.00 a.m.	2	19	45	on	66(97%)	0(0%)	0(0%)	2(3%)	94.8%	
32	1.00 p.m.	2	21	45	on	67(98.5%)	0(0%)	0(0%)	1(5%)	97%	
33	2.30 p.m.	2	23	15	on	68(100%)	0(0%)	0(0%)	0(0%)	100%	

APPENDIX IVb

B. GRAVITY REACTIONS OF 6 - 8 WEEK OLD LARVAE - LONG TERM EXPERIMENTS

READ	CLOCK TIME	TIME			LIGHT	NUMBER OF LARVAE				EXCESS % IN TOP HALF	N
		DYS.	HRS.	MIN.		TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM		
1	9.30am	0	00	00	on	0(0%)	2(2.9%) (2 active)	7(10%) (7 active)	61(87.1%)	-94.2%	70
2	9.45am	0	00	15	on	43(61.4%) (39 active)	3(4.3%) (3 active)	8(11.4%) (8 active)	16(22.9%) (15 active)	+31.4%	
3	10.00am	0	00	30	on	38(54.2%) (35 active)	10(14.3%) (10 active)	6(8.6%) (6 active)	16(22.9%) (15 active)	+37.0%	
4	10.15am	0	00	45	on	41(58.5%) (36 active)	9(12.9%) (9active)	4(5.7%) (4 active)	16(22.9%) (16 active)	+42.8%	
5	10.30am	0	1	00	on	41(58.5%) (36 active)	7(10%) (7 active)	2(2.9%) (2 active)	20(28.6%) (20 active)	+37.0%	
6	11.00am	0	1	30	on	40(57.1%) (28 active)	10(14.3%) (10 active)	4(5.7%) (4 active)	16(22.9%) (15 active)	+42.8%	
7	11.30am	0	2	00	on	42(60%) (32 active)	4(5.7%) (4 active)	2(2.9%) (2 active)	22(31.4%) (21 active)	+31.4%	
8	1.15pm	0	3	45	on	39(55.8%) (18 active)	4(5.7%) (4 active)	3(4.3%) (3 active)	24(34.2%) (22 active)	+23.0%	

(Continued)

APPENDIX IVb (Continued)

READ	CLOCK TIME	TIME DYS.	TIME HRS.	TIME MIN.	LIGHT	NUMBER OF LARVAE TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
9	2.15pm	0	4	45	on	41(58.6%) (8 active)	3(4.3%) (1 active)	2(2.9%) (1 active)	24(34.2%) (18 active)	+37.1%	70
10	3.30pm	0	6	00	on	36(51.3%) (4 active)	2(2.9%) (2 active)	2(2.9%) (1 active)	30(42.9%) (27 active)	+9.4%	188
11	4.00pm	0	6	30	on	41(58.6%) (11 active)	3(4.3%) (3 active)	0(0%) (0 active)	26(37.1%) (18 active)	+25.8%	
12	4.45pm	0	7	15	on	44(62.9%) (9 active)	0(0%) (0 active)	2(2.9%) (2 active)	24(34.2%) (17 active)	+25.8%	
13	5.30pm	0	8	00	on	41(58.6%) (14 active)	3(4.3%) (3 active)	1(1.4%) (1 active)	25(35.7%) (16 active)	+25.8%	
14	6.30pm	0	9	00	on	39(55.6%) (8 active)	2(2.9%) (2 active)	2(2.9%) (1 active)	27(38.6%) (3 active)	+17.0%	
15	7.30pm	0	10	00	on	42(60%) (8 active)	2(2.9%) (2 active)	0(0%) (0 active)	26(37.1%) (5 active)	+25.8%	
16	9.00pm	0	11	30	off	46(65.7%) (6 active)	0(0%) (0 active)	3(4.3%) (0 active	21(30%) (8 active)	+31.4%	
17	10.30pm	0	13	00	off	50(71.4%) (6 active)	0(0%)	0(0%)	20(28.6%) (14 active)	+42.8%	

APPENDIX IVb (continued)

READ	CLOCK TIME	TIME DYS.	TIME HRS.	TIME MIN.	LIGHT	NUMBER OF LARVAE TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
18	6.30am	0	21	00	off	45(64.3%) (0 active)	2(2.9%)	1(1.4%)	22(31.4%) (0 active)	+34.4%	70
19	8.00am	0	22	30	on	49(70%) (2 active)	2(2.9%)	0(0%)	19(27.1%) (2 active)	+45.8%	
20	9.30am	1	00	00	on	48(68.6%) (0 active)	0(0%)	0(0%)	22(31.4%) (5 active)	+37.2%	89
21	10.40am	1	1	10	on	47(67.2%) (0 active)	0(0%)	1(1.4%)	22(31.4%) (1 active)	+34.4%	
22	11.15am	1	1	45	on	49(70%) (1 active)	0(0%)	0(0%)	21(30%) (5 active)	+ 40%	
23	1.30pm	1	4	00	on	51(72.9%) (2 active)	0(0%)	0(0%)	19(27.1%) (0 active)	+ 45.8%	
24	3.00pm	1	5	30	on	51(72.9%) (6 active)	0(0%)	0(0%)	19(27.1%) (2 active)	+ 45.8%	
25	3.30pm	1	6	00	on	52(74.2%) (7 active)	2(2.9%) (0 active)	0(0%)	16(22.9%) (0 active)	+ 54.2%	
26	4.00pm	1	6	30	on	50(71.5%) (7 active)	4(5.7%) (3 active)	1(1.4%) (0 active	15(21.4%) (0 active)	+ 54.4%	

(Continued)

APPENDIX IVb (continued)

READ	CLOCK TIME	TIME DYS.	TIME HRS.	TIME MIN.	LIGHT	NUMBER OF LARVAE TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
27	4.30pm	1	7	00	on	54(77.2%) (11 active)	1(1.4%) (1 active	0(0%)	15(21.4%) (3 active)	+ 57.2%	70
28	5.00pm	1	7	30	on	54(77.1%) (10 active)	0(0%)	0(0%)	16(22.9%) (2 active)	+ 54.2%	
29	5.30pm	1	8	00	on	54(77.2%) (1 active	0(0%)	0(0%)	16(23.8%) (2 active)	+ 54.4%	70
30	7.15pm	1	9	45	on	57(81.4%) (0 active)	0(0%)	0(0%)	13(18.6%) (1 active)	+ 62.8%	
31	9.00pm	1	11	30	off	56(80%) (0 active)	0(0%)	0(0%)	14(20%) (6 active)	+ 60%	
32	10.00pm	1	12	30	off	52(74.3%) (1 active)	0(0%)	0(0%)	18(25.7%) (12 active)	+ 48.6%	
33	6.30am	1	21	00	off	36(51.4%) (2 active)	1(1.4%) (0 active)	2(2.9%) (2 active)	31(44.3%) (5 active)	+ 5.6%	
34	7.30am	1	22	00	on	37(52.8%) (1 active)	0(0%)	3(4.3%)	30(42.9%) (12 active)	+ 5.6%	
35	8.30am	1	23	00	on	35(50%) (0 active)	0(0%)	3(4.3%)	32(45.7%) (0 active)	+ 0%	

(Continued.)

APPENDIX IVb (continued)

READ	CLOCK TIME	TIME DYS	TIME HRS.	TIME MIN.	LIGHT	NUMBER OF LARVAE TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
36	9.30am	2	00	00	on	39(55.7%) (0 active)	0(0%)	0(0%)	31(44.3%) (0 active)	+ 11.4%	70
37	10.30am	2	1	00	on	38(54.3%) (0 active)	1(1.4%) (1 active)	2(2.9%) (0 active)	29(41.4%) (4 active)	+11.4%	
38	11.30am	2	2	00	on	34(48.6%) (1 active)	1(1.4%) (1 active)	0(0%)	35(50%) (0 active)	+ 0%	
39	12.30pm	2	3	00	on	38(54.2%) (0 active)	0(0%)	2(2.9%) (0 active)	30(42.9%) (2 active)	+8.4%	
40	1.30pm	2	4	00	on	37(52.9%) (0 active	0(0%)	1(1.4%) (0 active)	32(45.7%) (0 active)	+5.8%	
41	2.30pm	2	5	00	on	37(52.9%) (0 active)	0(0%)	1(1.4%) (0 active)	32(45.7%) (0 active)	+5.8%	
42	3.30pm	2	6	00	on	37(52.9%) (5 active)	1(1.4%) (0 active)	1(1.4%) (0 active)	31(44.3%) (0 active)	+8.6%	
43	4.30pm	2	7	00	on	41(58.6%) (11 active)	1(1.4%) (0 active)	1(1.4%) (0 active)	27(38.6%) (4 active)	+ 20%	
44	5.30pm	2	8	00	on	45(64.3%) (3 active)	2(2.9%) (0 active)	1(1.4%) (0active)	22(31.4%) (2 active)	+34.4%	

(Contd.)

APPENDIX IV b (continued.

		TIME				NUMBER OF LARVAE						
READ	CLOCK TIME	DYS.	HRS.	MIN.	LIGHT	TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N	
45	9.15pm	2	11	45	on	49(70%) (1 active)	1(1.4%) (1 active)	2(2.9%) (2 active)	18(25.7%) (4 active)	+42.8	70	
46	10.15pm	2	12	45	off	49(70%) (2 active)	0(0%)	1(1.4%) (0 active)	20(28.6%) (3 active)	+ 40%		
47	11.15pm	2	13	45	off	47(67.2%) (2 active)	0(0%)	1(1.4%) (0 active)	22(31.4%) (1 active)	+ 34.4%		

APPENDIX V

GRAVITY REACTIONS OF 6 - 8 WEEK OLD LARVAE

READ	TIME		NUMBER OF LARVAE					
	HRS.	MINS.	TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
I	0	00	0(0%)	0(0%)	0(0%)	183(100%)	- 100%	183
2	0	15	120(65.6%)	55(30.1%)	5(2.7%)	3(1.6%)	+ 91.2%	
3	0	30	122(66.6%)	50(27.3%)	4(2.2%)	7(3.9%)	+ 88%	
Apparatus Reversed								
4	0	45	98(53.6%)	54(30.1%)	18(9.6%)	13(6.7%)	+ 66.2%	
5	1	00	101(55.1%)	44(24%)	23(12%)	15(8.9%)	+ 58.6%	

APPENDIX VI

CONTROL FOR GRAVITY EXPERIMENT-APPARATUS HORIZONTAL

	TIME		NUMBER OF LARVAE					
READ	HRS.	MINS.	TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
1	0	00	7(25%)	5(17.9%)	3(10.7%)	13(46.4%)	- 14.2%	28
2	0	30	6(21.4%)	8(28.6%)	6(21.4%)	8(28.6%)	0%	
3	0	45	7(25%)	7(25%)	8(28.6%)	6(21.4%)	0%	
4	1	00	6(21.4%)	6(21.4%)	8(28.6%)	8(28.6%)	- 14.4%	
5	1	15	7(25%)	6(21.4%)	6(21.4%)	9(32.2%)	- 7.2%	
Apparatus Reversed								
6	1	30	9(32.2%)	7(25%)	5(17.8%)	7(25%)	+ 14.4%)	

APPENDIX VII

SETTLING POSITIONS OF TICK LARVAE ON GLASS RODS IN VARIOUS POSITIONS.

POSN. OF TUBE / SECTN. OF TUBE	VERT.TIP UP	VERT.TIP DOWN	HORIZONTAL TIP RIGHT	VERT.VASELINE AT BOTH ENDS	HORIZONTAL VASELINE AT BOTH ENDS
TOP (OR RT.)	9	0	9	6	2
TOP (OR RT.)MIDDLE	1	0	0	2	9
MIDDLE	0	1	1	1	7
BOTTOM (OR LEFT) MIDDLE	0	1	0	1	6
BOTTOM (OR LEFT)	0	8	0	0	1
TOTAL	10	10	10	10	25

APPENDIX VIII

FINAL SETTLING POSITIONS OF LARVAE ON CURVED GLASS RODS

POSN.	E	D	C	B	A	TOP	A^1	B^1	C^1	D^1	E^1	TOTAL
NO.	0	1	0	0	2	11	2	0	0	0	1	17

APPENDIX IX

GRAVITY REACTIONS OF 1 - 3 DAY OLD TICK LARVAE

READ	TIME HRS.	TIME MINS.	NUMBER OF LARVAE (% IN BRACKETS) TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
1	0	15	17(21.8%)	9(11.5%)	8(10.3%)	44(56.4%)	- 33.4%	78
2	0	30	19(24.4%)	3(3.8%)	5(6.4%)	51(65.4%)	- 43.6%	
3	0	45	21(26.9%)	4(5.1%)	4(5.1%)	49(62.9%)	- 36%	
4	1	00	25(32.1%)	3(3.8%)	3(3.8%)	47(60.3%)	- 26.2%	
5	1	15	24(30.7%)	0(0%)	2(2.6%)	52(66.7%)	- 38.6%	
6	1	30	20(26.3%)	1(1.3%)	2(2.6%)	55(69.8%)	- 44.8%	
7	1	45	21(26.9%)	1(1.3%)	3(3.8%)	53(68%)	- 43.6%	
Apparatus reversed.								
8	2	00	51(65.5%)	3(3.8%)	0(0%)	24(30.7%)	+ 38.6%	
9	2	15	51(65.4%)	2(2.6%)	1(1.3%)	24(30.7%)	+ 36%	

APPENDIX X

A. GRAVITY REACTIONS OF 1 - 3 DAY OLD LARVAE - ALL PLACED AT BOTTOM

	TIME		NUMBER OF LARVAE (% IN BRACKETS)					
READ	HRS.	MINS.	TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
1	0	00	0(0%)	0(0%)	0(0%)	45(100%)	- 100%	45
2	0	15	1(2.2%)	0(0%)	13(28.9%)	31(68.9%)	- 95.6%	
3	0	30	0(0%)	3(6.6%)	8(17.8%)	34(75.6%)	- 86.8%	
4	0	45	1(2.2%)	1(2.2%)	4(8.9%)	39(86.7%)	- 91.2%	
5	1	00	1(2.2%)	0(0%)	0(0%)	44(97.8%)	- 95.6%	
6	1	15	1(2.2%)	1(2.2%)	2(4.4%)	41(91.2%)	- 91.2%	
Apparatus reversed								
7	1	30	41(91.2%)	2(4.4%)	1(2.2%)	1(2.2%)	+ 91.2%	
8	2	15	40(89%)	3(6.6%)	1(2.2%)	1(2.2%)	+ 91.2%	
9	3	15	40(89%)	3(6.6%)	1(2.2%)	1(2.2%)	+ 91.2%	

APPENDIX X

B. GRAVITY REACTIONS OF 1 - 3 DAY OLD LARVAE - ALL PLACED AT TOP

	TIME		NUMBER OF LARVAE (% IN BRACKETS)					
READ	HRS.	MINS.	TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
1	0	00	35(94.6%)	2(5.4%)	0(0%)	0(0%)	+ 100%	37
2	0	15	33(89.2%)	2(5.4%)	1(2.7%)	1(2.7%)	+ 89.2%	
3	0	30	32(86.5%)	2(5.4%)	0(0%)	3(8.1%)	+ 83.8%	
4	0	45	33(89.2%)	1(2.7%)	1(2.7%)	2(5.4%)	+ 83.8%	
5	1	00	30(81.1%)	2(5.4%)	1(2.7%)	4(10.8%)	+ 73.0%	
6	1	15	32(86.5%)	0(0%)	1(2.7%)	4(10.8%)	+ 73.0%	
Apparatus reversed.								
7	1	30	3(8.1%)	0(0%)	0(0%)	34(91.9%)	- 83.8%	
8	2	15	3(8.1%)	0(0%)	0(0%)	34(91.9%)	- 83.8%	
9	3	15	4(10.8%)	0(0%)	0(0%)	33(89.2%)	- 78.4%	

APPENDIX X

C. GRAVITY REACTIONS OF 1 - 3 DAY OLD LARVAE - EVENLY DISTRIBUTED OVER APPARATUS

	TIME		NUMBER OF LARVAE (% IN BRACKETS)					
READ	HRS.	MINS.	TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
1	0	00	28(36.8%)	5(6.6%)	16(21.1%)	27(35.5%)	- 13.2%)	76
2	0	15	17(22.4%)	13(17.1%)	14(18.4%)	32(42.1%)	- 21%	
3	0	30	22(28.9%)	8(10.5%)	9(11.8%)	37(48.8%)	- 21.2%	
4	0	45	23(30.3%)	9(11.8%)	4(5.3%)	40(52.6%)	- 15.8%	
5	1	00	26(34.2%)	1(1.3%)	1(1.3%)	48(63.2%)	- 29.0%	
6	1	15	24(31.6%)	3(3.9%)	3(3.9%)	46(60.6%)	- 29.0%	
Apparatus reversed.								
7	1	30	36(42.1%)	2(2.6%)	0(0%)	38(55.3%)	- 0%	
8	2	15	33(43.4%)	0(0%)	0(0%)	43(56.6%)	- 13.2%	
9	3	15	33(43.4%)	2(2.6%)	0(0%)	41(54%)	- 7.8%	

APPENDIX XI

GRAVITY REACTIONS OF 4 - 6 DAY OLD LARVAE

	TIME		NUMBER OF LARVAE (% IN BRACKETS)					
READ	HRS.	MINS.	TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
1	0	00	0(0%)	0(0%)	4(4.4%)	89(95.6%)	- 100%	90
2	0	15	8(8.8%)	4(4.4%)	16(17.8%)	62(69%)	- 73.6%	
3	0	30	12(13.3%)	3(3.3%)	26(28.9%)	49(54.5%)	- 66.8%	
4	0	45	17(18.9%)	3(3.3%)	20(22.2%)	50(55.6%)	- 55.6%	
5	1	00	11(12.2%)	4(4.4%)	2(2.2%)	73(81.2%)	- 66.8%	
6	3	00	12(13.3%)	4(4.4%)	1(1.1%)	73(81.2%)	- 64.6%	

APPENDIX XII

GRAVITY REACTIONS OF NINE DAY OLD TICK LARVAE

READ	TIME		NUMBER OF LARVAE (% IN BRACKETS)					
	HRS.	MINS.	TOP	TOP MIDDLE	BOTTOM MIDDLE	BOTTOM	EXCESS % IN TOP HALF	N
1	0	00	0(0%)	0(0%)	0(0%)	79(100%)	- 100%	79
2	0	15	48(60.8%)	8(10.1%)	3(3.8%)	20(25.3%)	+ 41.8%	
3	0	30	44(55.7%)	6(7.6%)	8(10.1%)	21(26.6%)	+ 26.6%	
4	0	45	41(51.9%)	7(8.9%)	9(11.4%)	22(27.8%)	+ 21.6%	
5	1	00	45(57%)	3(3.8%)	8(10.1%)	23(29.1%)	+ 21.6%	
6	1	15	52(65.8%)	5(6.3%)	8(10.1%)	14(17.9%	+ 44%	
7	1	30	53(67.1%)	10(12.7%)	4(5.1%)	12(15.1%)	+ 59.6%	
Apparatus reversed.								
8	1	45	64(81.0%)	9(11.4%)	3(3.8%)	3(3.8%)	+ 84.8%	
9	2	00	59(74.4%)	14(17.9%)	1(1.1%)	5(6.3%)	+ 85.2%	
10	2	15	63(79.7%)	8(10.1%)	3(3.8%)	5(6.3%)	+ 79.8%	
11	3	15	65(82.3%)	4(5.1%)	5(6.3%)	5(6.3%)	+ 74.8%	

APPENDIX XIII

REACTIONS OF LARVAE OVER 1 WEEK OLD TO LIGHT AND GRAVITY INTERACTION

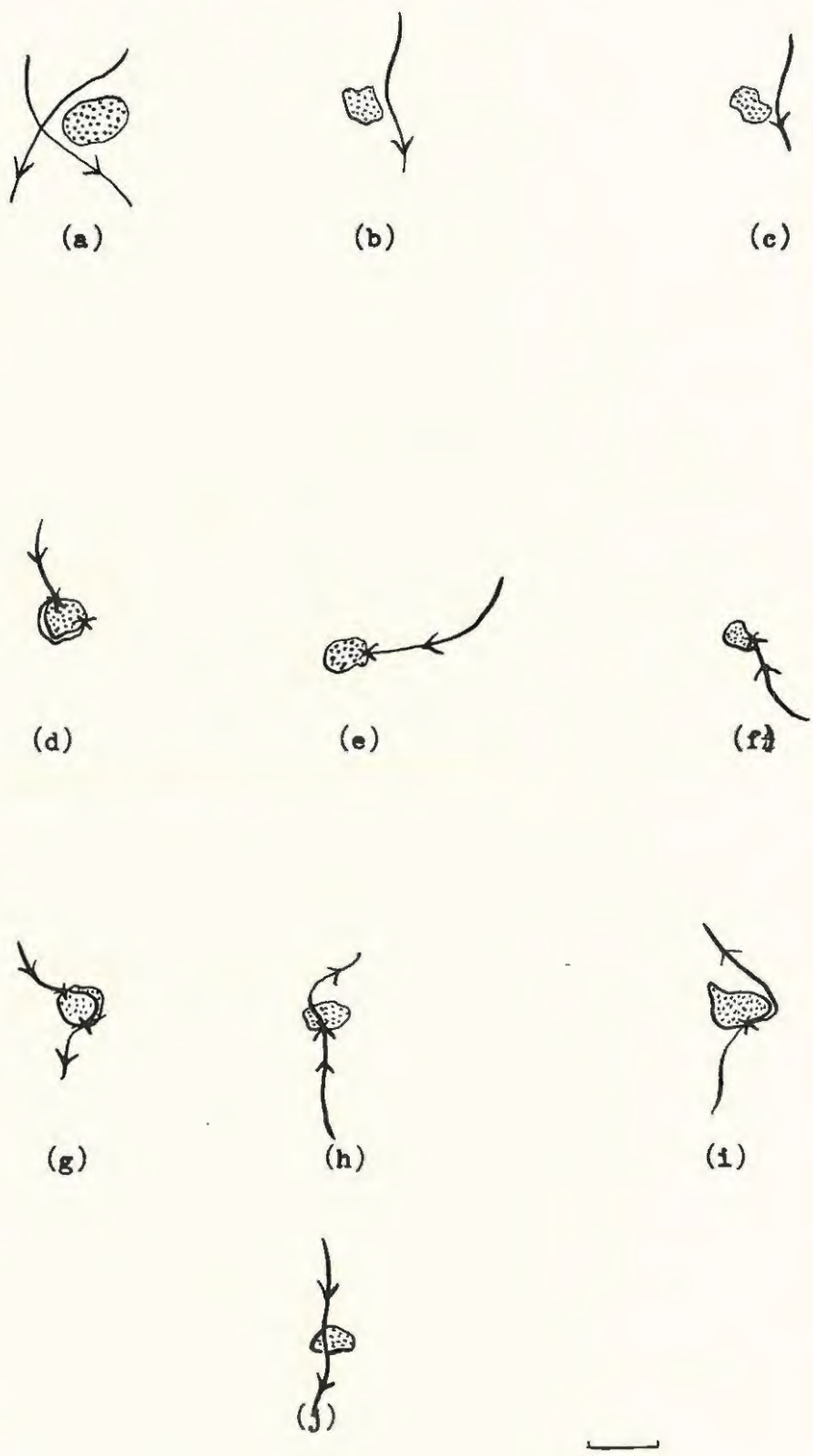
SECTION OF TUBE	TOP ¼	TOP MID. ¼	BOT.MID. ¼	BOT. ¼	N
POSITION OF APPARATUS					
TIP UP; WINDOW AT TOP	114(91.2%)	4(3.2%)	3(2.4%)	4(3.2%)	125
TIP DOWN; WINDOW AT BOTTOM	6(4.8%)	2(1.6%)	7(5.6%)	110(88%)	
TIP UP; WINDOW AT MIDDLE	103(82.4%)	11(8.8%)	5(4%)	6(4.8%)	
VASELINE AT BOTH ENDS; WINDOW AT TOP	118(94.4%)	4(3.2%)	1(0.8%)	2(1.6%)	
VASELINE AT BOTH ENDS; WINDOW AT BOT.	96(76.8%)	20(16.0%)	5(4%)	4(3.2%)	
VASELINE AT BOTH ENDS; WINDOW AT MID.	91(72.8%)	23(18.4%)	5(4%)	6(4.8%)	
VASELINE AT BOTH ENDS; NO WINDOW	103(82.4%)	16(12.8%)	5(4%)	1(0.8%)	
TIP UP; NO WINDOW	112(89.6%)	2(1.6%)	5(4%)	6(4.8%)	
TIP DOWN; NO WINDOW	14(11.2%)	12(9.6%)	5(4%)	94(75.2%)	

V. CLUMPING BEHAVIOUR

The next problem was to find out more about the clumping behaviour of the larvae. The first question which arose was: When clumps form, is it due to the larvae coming into contact with other ticks and sitting or is some chemical factor involved? The apparatus used consisted of petri dishes with plain glass lids sealed on with low melting point wax. Temperature and humidity conditions were constant and observations were made under red light. A group of ticks was allowed to clump and then the reactions to the clump of those larvae which were still active was noted. For this purpose the usual tracking method was employed. Typical results are shown in Tracks 15 A-J.

It was observed that when a tick came into contact with a clump, it nearly always stopped for a while and then either settled there or else moved on (Track 15 J records an exception). It appeared from these observations that the larvae came across the clump purely by chance, as, in a number of cases (Track 15 A-C) a larvae walked very close to a clump without coming into contact with it. In other cases, however, the ticks seemed to deviate from their path and move to the clump (Track 15 E-G). Again, in one experiment the number in a clump was found to increase from three individuals to eleven in five minutes - a much faster rate of increase than one would expect from the population if contact was made by chance. There is therefore some indication of a chemical response.

In an attempt to study this problem further the ticks were allowed to clump and the position of the clump marked with a wax pencil on the outside of

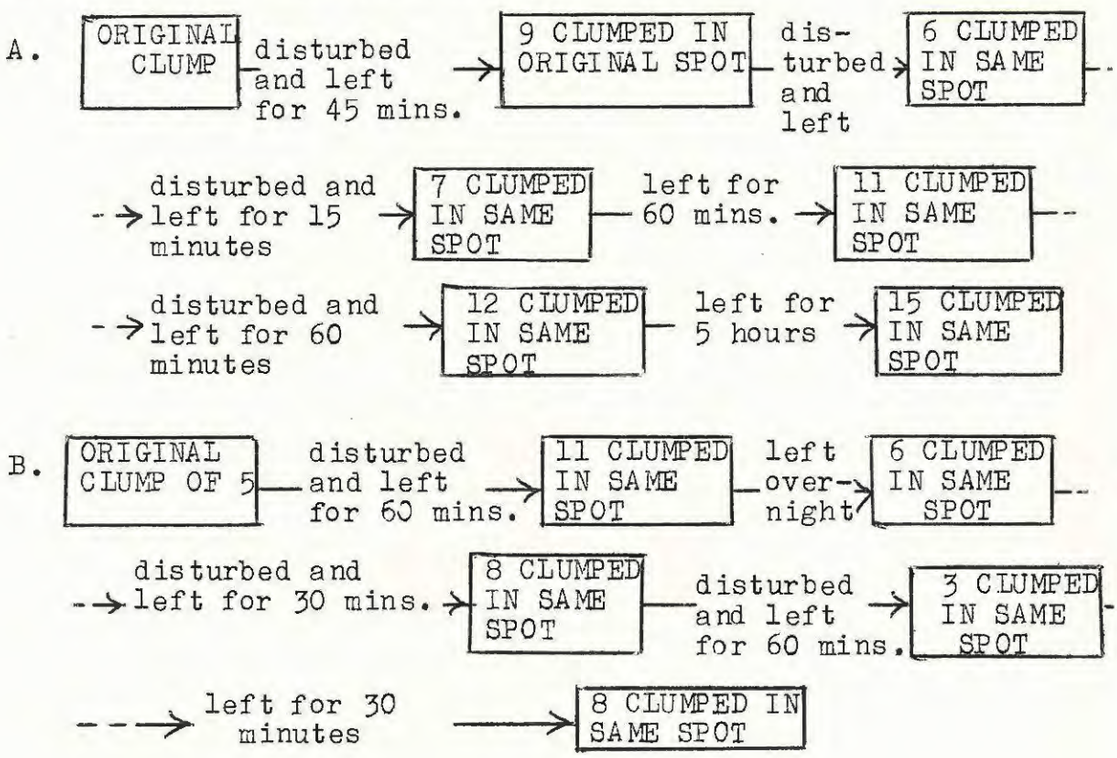


TRACK 15. Reactions of B. decoloratus larvae to clumps of their own species.

Clumps are marked by a shaded area.

Where larvae stop, tracks marked with an "x".

the dish. The clump was then broken up with a soft brush to prevent damaging the larvae. Care was taken to ensure that all the larvae were moved away. The apparatus was then left for a while, after which the position in which the larvae reclumped was noted. The process was then repeated. As a precatuion, the apparatus was frequently reversed after the larvae had been disturbed. This experiment was repeated a number of times and typical examples of the results are shown below:



Although in the majority of cases at least some of the larvae reclumped where the former clump had been, this was not always the case.

It was further thought that it might prove of interest to see whether larvae from another species of tick would clump on the spot where B. decoloratus larvae had clumped. 4½ week old Rhipicephalus evertsi Neu. were used for this purpose. In preliminary experiments, it was established that R. evertsi behaved in the same way as B. decoloratus.

A batch of B. decoloratus larvae was allowed to clump and then the clump was broken up as before. The spot on which they had clumped was marked and the larvae were removed from the apparatus. R. evertsi larvae were then introduced into the apparatus which was then left for a while. It was found that in no case did the R. evertsi larvae settle where the B. decoloratus clump had been. This is not to imply that the R. evertsi avoided the spot, but merely that the spot had no particular attraction for them. In control experiments using two sets of B. decoloratus larvae, it was observed that the second showed a tendency to clump where the first had done so previously. Conclusions: It therefore seems that some "clumping" factor" is produced which marks the spot where ticks have previously clumped and this substance stimulates ticks to settle in this spot again. There are indications that this substance is species specific. There is no evidence that the substance attracts ticks although in some cases larvae were seen to turn towards clumps.

VI. Reactions to Temperature

Experiments to investigate the temperature preferences of B. decoloratus were carried out using a gradient choice chamber as illustrated in Fig. 8. The choice chamber was made of copper and had thermometers placed at intervals along its length. It consisted of a trough fixed onto a thick base and at one end was a water-proof container for ice. For these experiments, the trough was filled with sand and a glass tube 44 cms long and 0.8 cm in diameter was buried in the sand. Ticks were placed in the tube which was then sealed at both ends with corks. The tube was divided into nine equal sections each corresponding to a thermometer set into the copper base of the apparatus. The temperature gradient was created by heating the one end of the apparatus with a bunsen burner and cooling the other end with ice. Ice was also packed onto the sand at the cool end.

A rough idea of the temperature gradient could be obtained from the set of thermometers as they followed a similar but higher gradient to the temperature inside the tube (see Graph 27). An accurate measure of the temperature gradient within the tube was obtained using a copper-constantin thermocouple connected to a Baird and Tatlock suspended coil galvanometer. The thermocouple was graduated by placing the wires first in ice and then in boiling water and then graduating the scale so that the intermediate temperatures could be read off. The thermocouples were not built into the apparatus in case the wires interfered with the counting of the larvae.

Four week old larvae were used and were distributed more or less evenly along the tube at the start of the experiment. The tube was then buried in

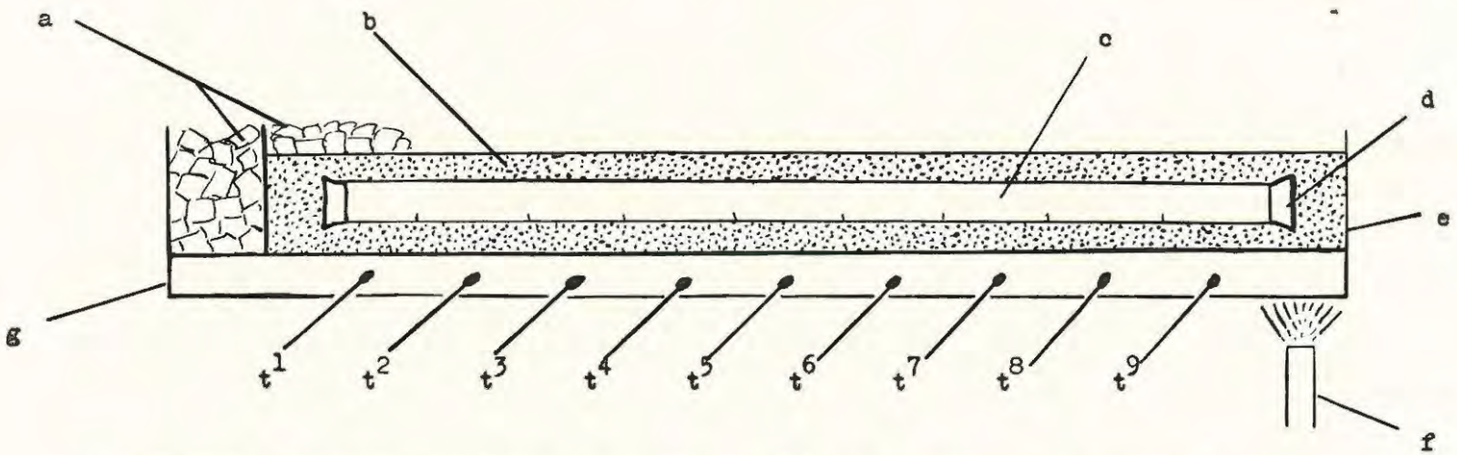
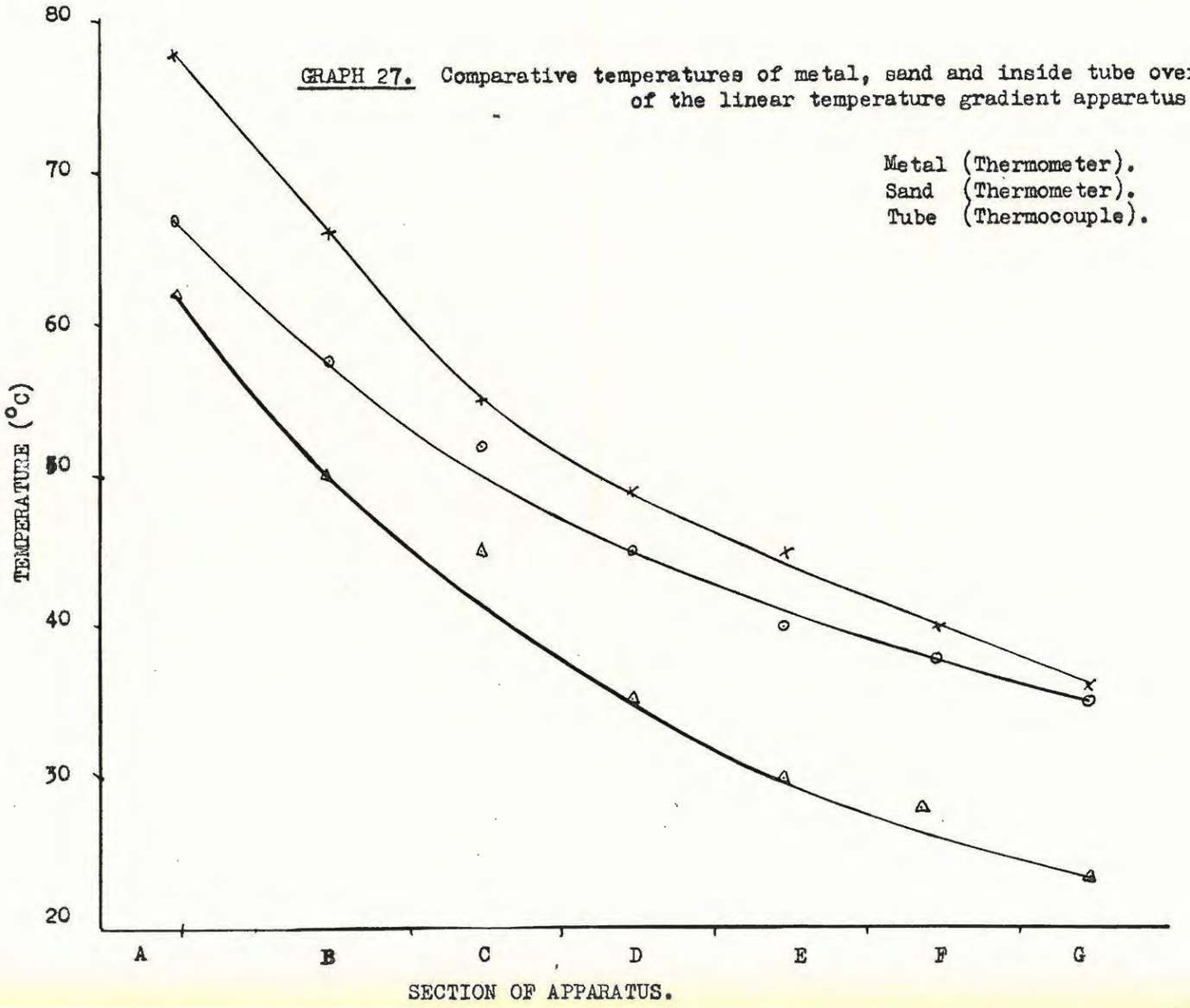


FIGURE 8. Apparatus used to see reactions of larvae in linear temperature gradient. a, ice; b, sand; c, glass tube; d, cork; e, copper trough; f, bunsen burner; g, copper base; t^1 - t^9, thermometers.

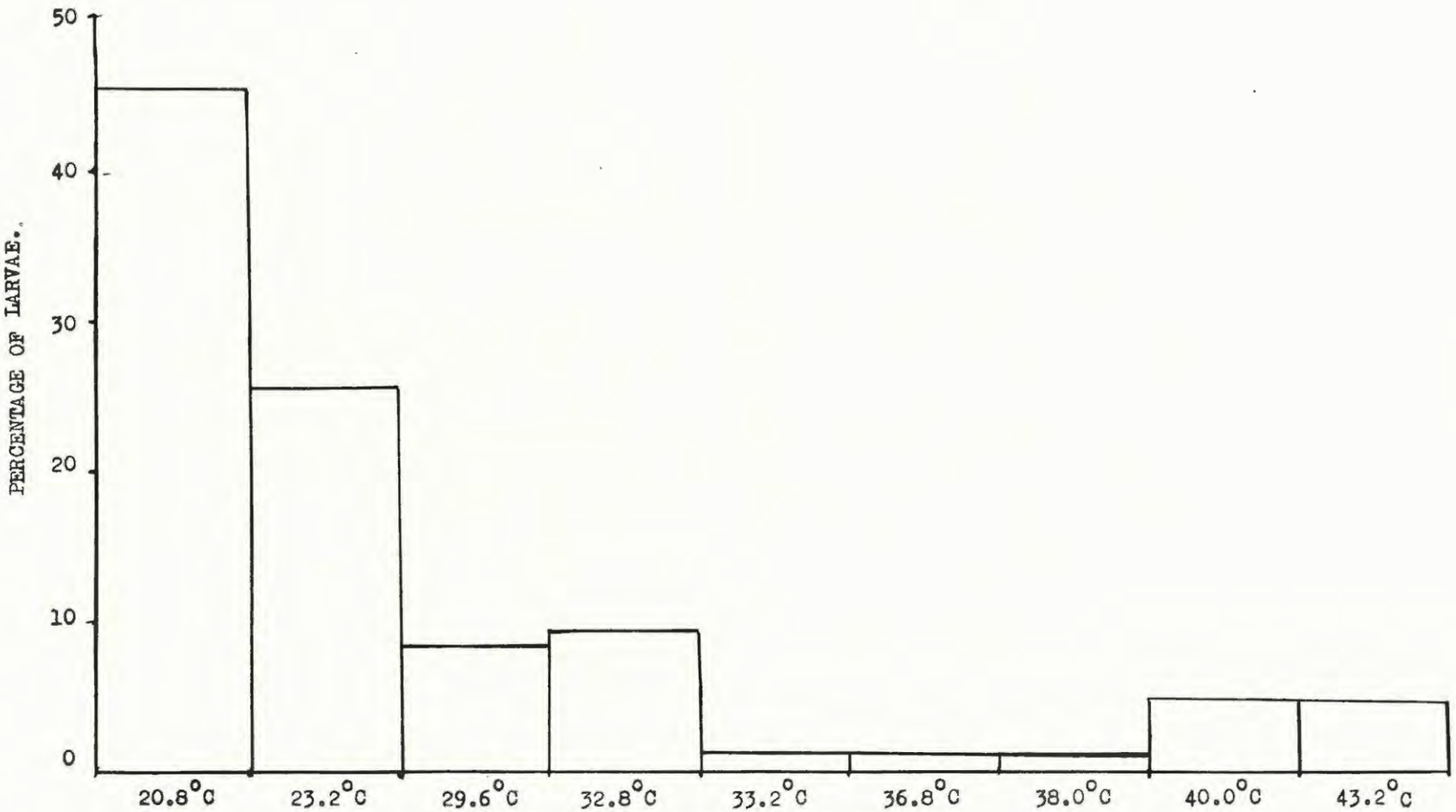


GRAPH 27. Comparative temperatures of metal, sand and inside tube over seven sections of the linear temperature gradient apparatus.

the sand in the trough after a temperature gradient had been set up.. Care was taken to ensure that it was perfectly horizontal. The apparatus was then left for twenty minutes after which the sand was removed from above the tube and the number of ticks in each section counted. Just enough sand was removed to enable counting to be done to keep heat loss at a minimum. On completion of counting, the thermocouple was inserted into the tube and the temperature in each section noted. With practice, counting and temperature reading was accomplished in a very short time.

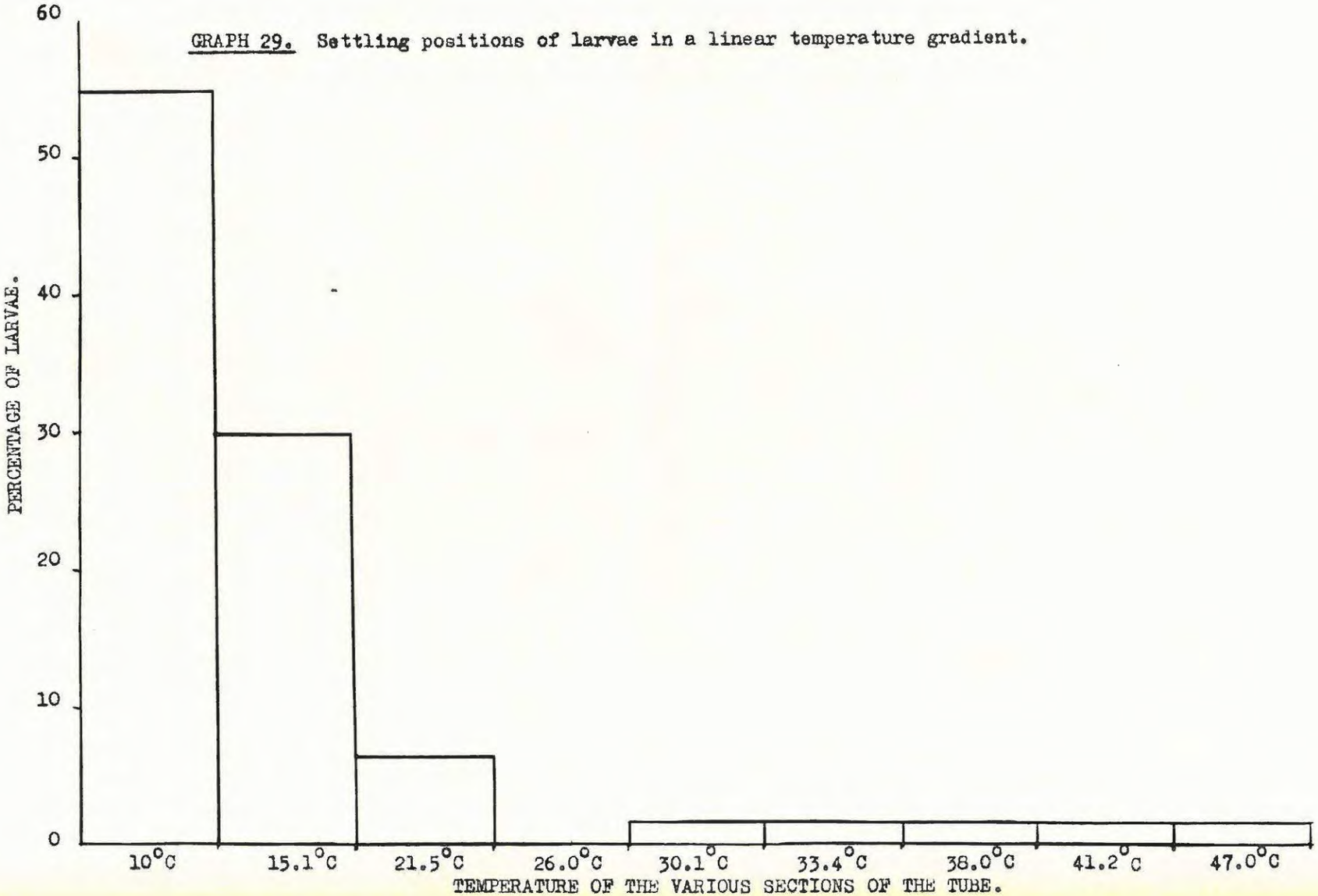
From the results it appeared that the larvae went to the coolest end (see Graphs 28 and 29 compiled from Appendix XIVA and B for illustrations of this behaviour). As the larvae had been distributed over the apparatus at the start it was decided to check that they were in fact reacting to temperature and not merely remaining where they had been placed, by carrying out an experiment in which the number of larvae in the various sections was counted before and after the experiment. This experiment showed that the larvae were moving from their initial positions to the cool end (see Graph 30 and Appendix XV).

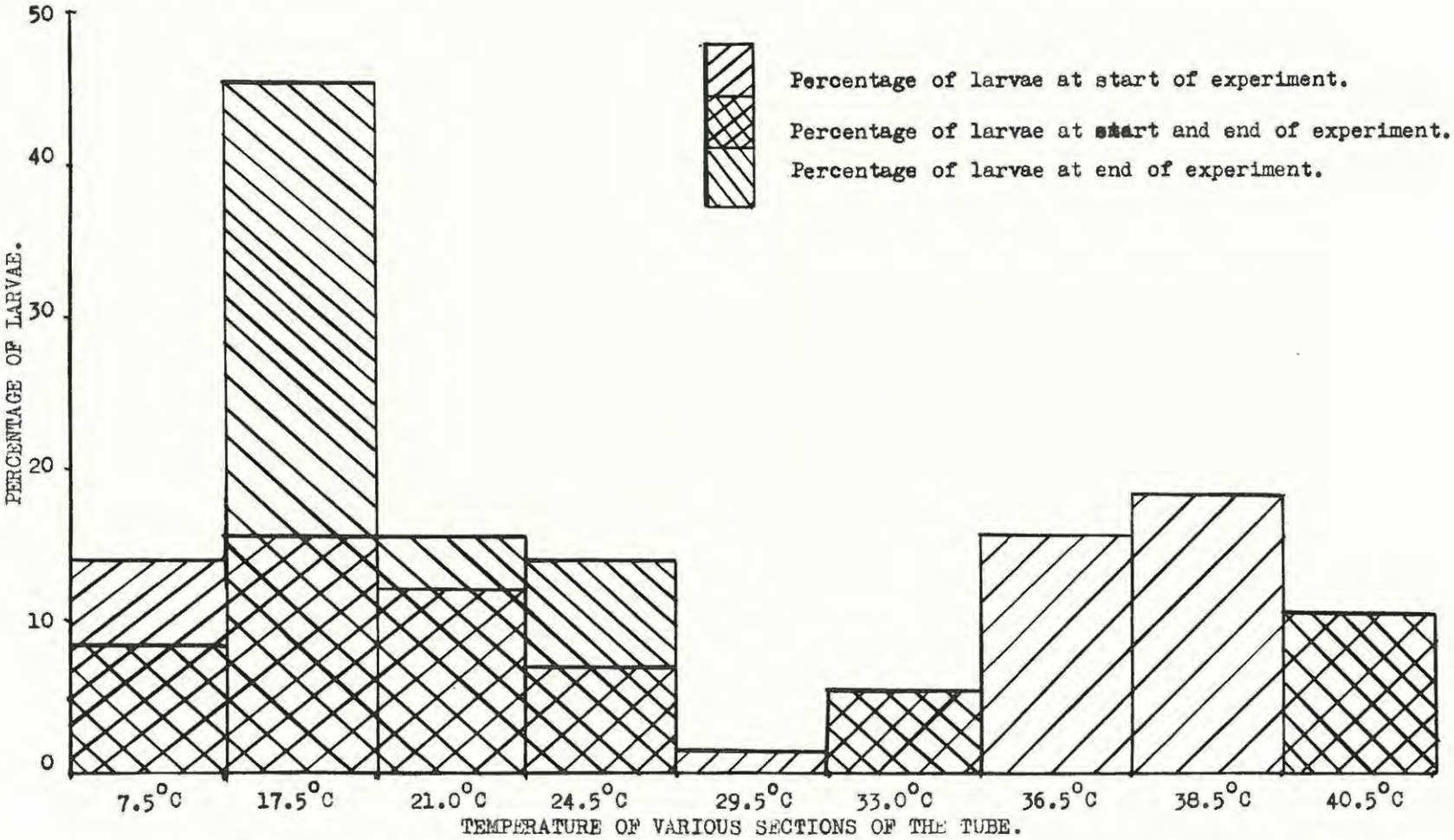
The mechanism of the temperature behaviour was studied by tracking individual ticks in a linear temperature gradient. The apparatus differed from that used previously only in the fact that the glass tube was not covered with sand on top and that it was divided into one tenth of an inch sections to correspond to the graph paper on which the track was plotted. When tracking was completed, the temperature in the various sections was recorded



GRAPH 28. Settling positions of larvae in a linear temperature gradient.

GRAPH 29. Settling positions of larvae in a linear temperature gradient.





GRAPH 30. Starting and final settling positions of larvae in a linear temperature gradient.

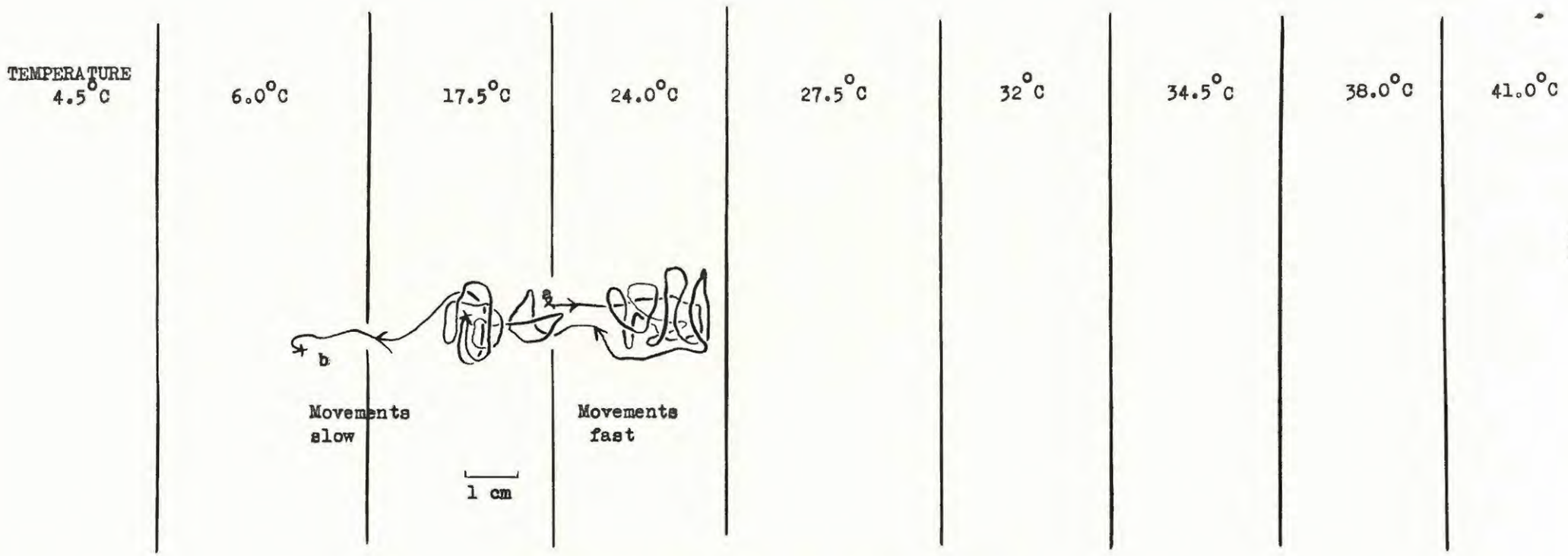
using the thermocouple in the usual way. To prevent loss of heat the uncovered part of the tube was left as small as possible.

In general the picture gained from the tracking experiments was that the larvae, when moving from cool to warm kept turning around and retreating to the cool end. It was often noticed that larvae moving from cool to warm began to weave around as they got into warmer areas (see Tracks 16 and 17 for example.)

When started at the warm end, larvae usually went to the cool sections with less hesitation (see Track 18 for example).

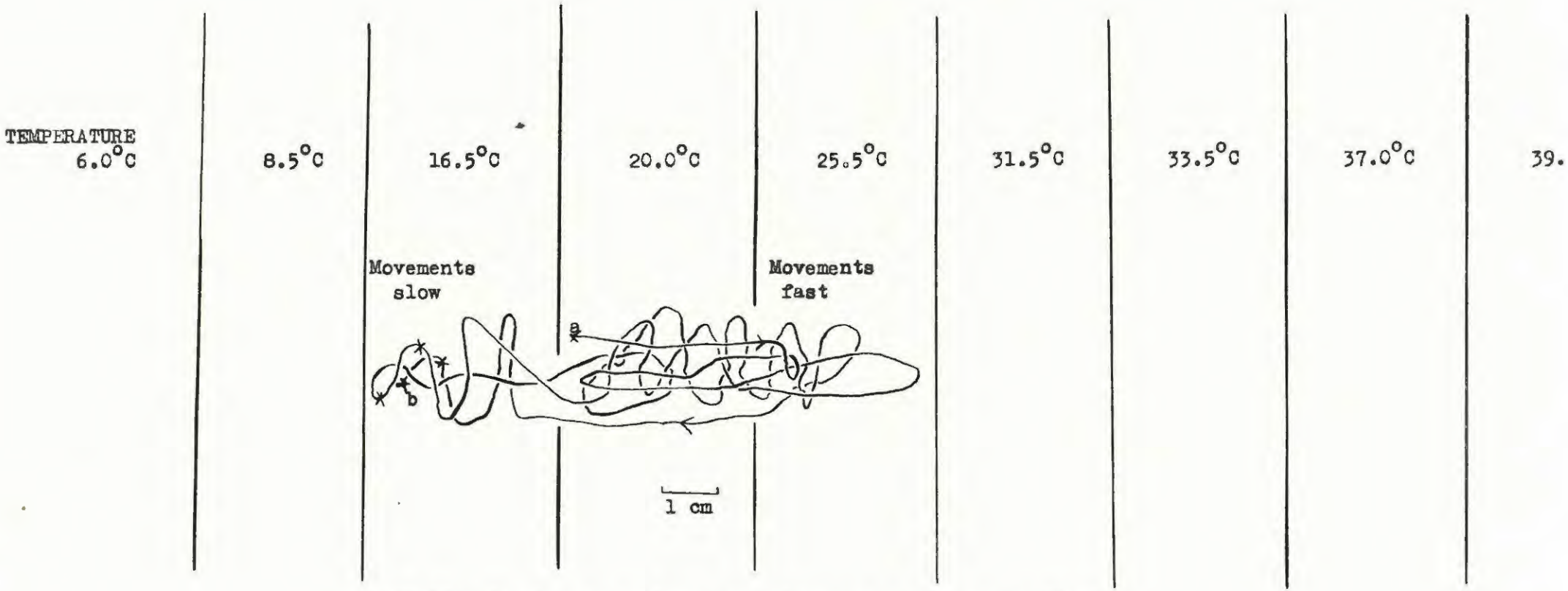
General observations had suggested that larvae moved more quickly in the warm sections than in the cool ones until they finally stopped in the sections with the lower temperatures. The effect of temperature on speed was studied by sealing individual animals into petri-dishes and tracking them in constant temperature rooms at different temperatures under light. Each animal was tracked for ten minutes and then the distance they had covered was measured with an opisometer and the speed calculated.

In each experiment the same tick was tracked at 25°C, 17°C and 9°C then after the track in the lowest temperature had been taken, the tick was again placed in the room at 25°C and tracked as a check that it had not been harmed by the lower temperature. In each case, after being placed in a new temperature, the animal was allowed time to become accustomed to the new temperature. Unfortunately the relative humidity inside the petri dishes could not be controlled.



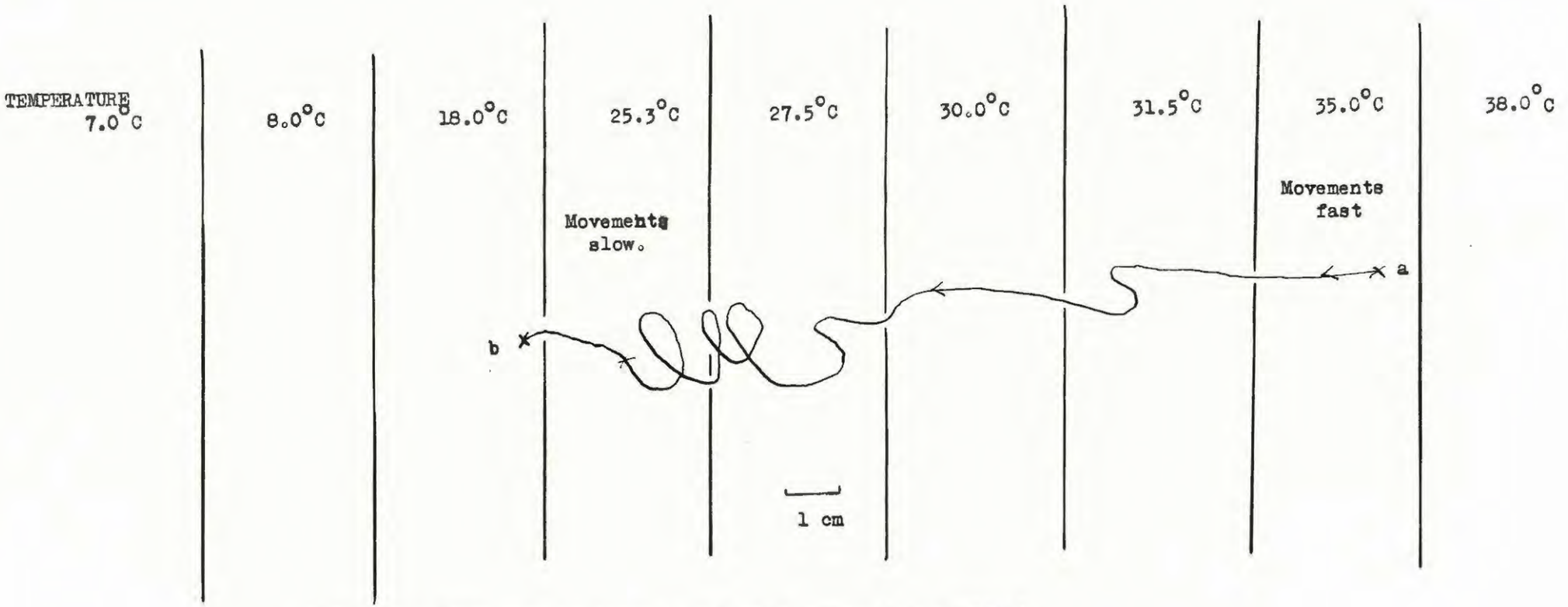
TRACK 16. Track made by larva in a linear temperature gradient.

a, starting point.
b, point at which larva came to rest.



TRACK 17. Track made by larva in a linear temperature gradient.

a, starting point.
b, point at which larva came to rest.



TRACK 18. Track made by larva in a linear temperature gradient.

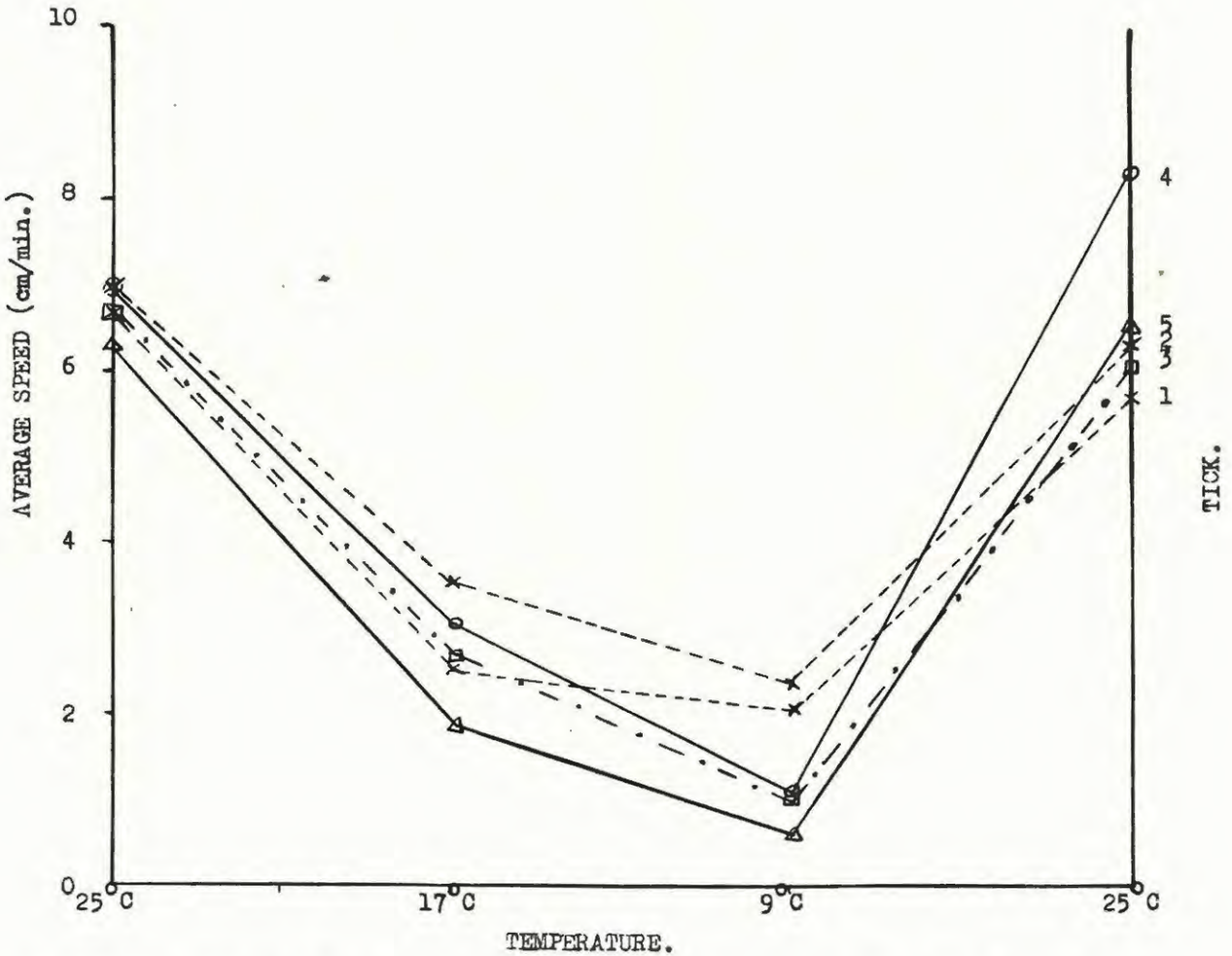
a, starting point.
b, point at which larva came to rest.

The speeds in different temperatures are tabulated in Appendix XVI and a graph of the speeds is given in Graph 31. It can be seen that the larvae move more slowly at lower temperatures.

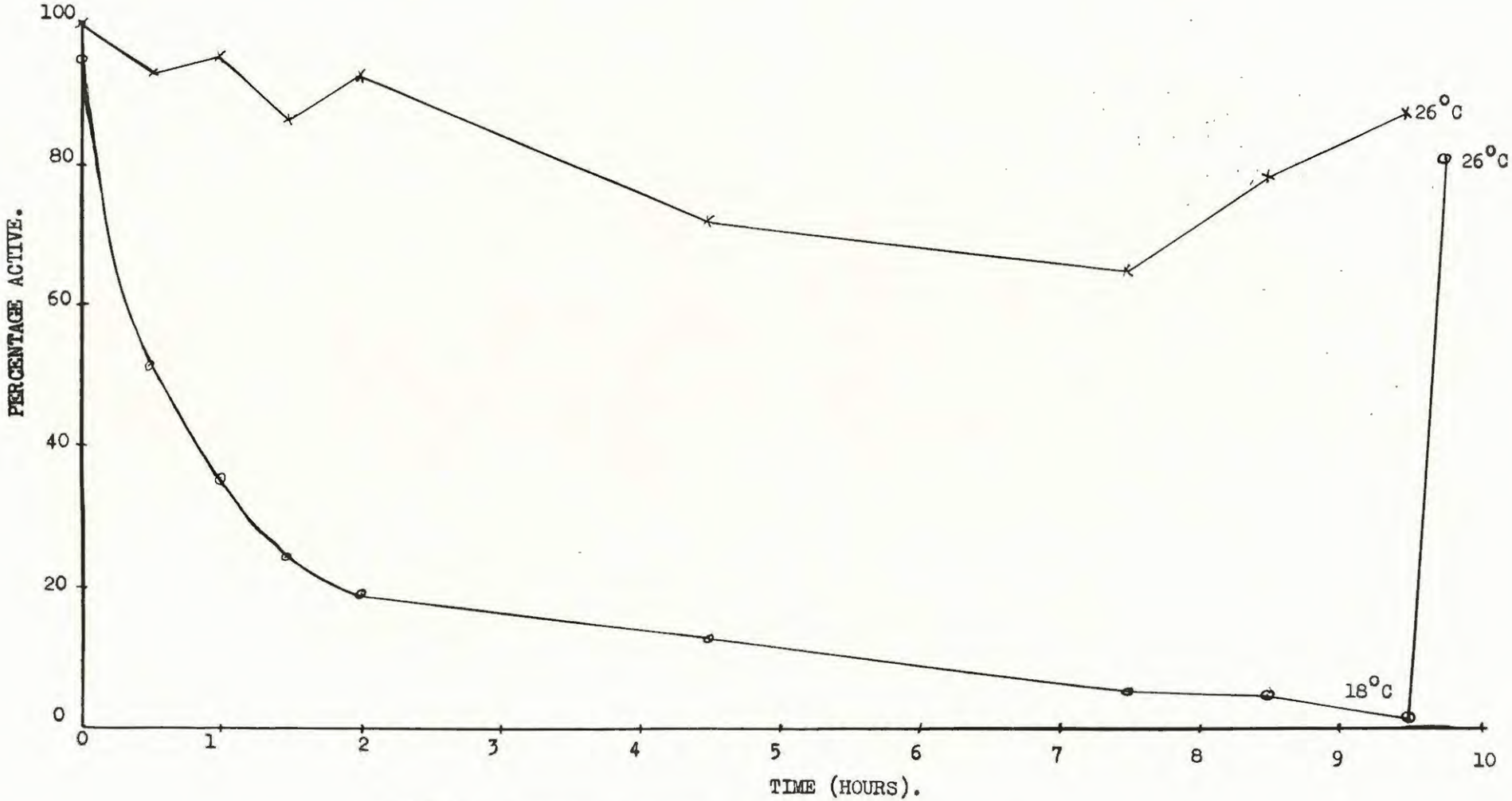
Experiments were also carried out to record the settling times of the larvae at low temperatures. When the settling times at 18°C were compared to that at 26°C it was found that the larvae settled sooner in the lower temperature. (See Appendix XVII and Graph 32). In the experiments at 18°C, the larvae were placed in a room at 26°C to check that they had not been harmed by the low temperature.

Attemps were then made to see whether the larvae exhibited any avoiding reaction to the higher temperatures and for this purpose the temperature gradient apparatus was modified. The trough was divided into halves using an asbestos sheet which had a hole cut through it. The tube in which the animals had been placed passed through this hole. The asbestos created an effective barrier which resulted in a rapid change of temperature inside the tube where it passed through the asbestos. The tube used in these experiments was different from the one used earlier being 2.3 cm in diameter and 6o cm long. It was divided into six sections each section being subdivided into one tenth of an inch sections.

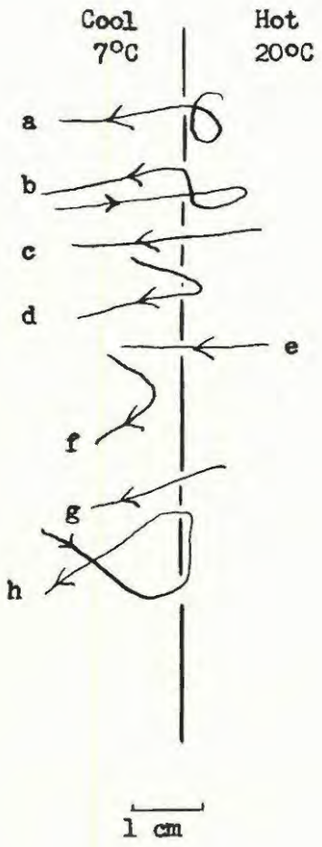
It appears that when moving from cold to warm the larvae show an avoiding reaction at the border or else they turn round and retreat to the cool section very soon after entering the warm side. When moving from warm to cool, however, no avoiding reaction was noticed, although in one case the animal hesitated slightly before entering the cool section. (See Track 19 for illustration).

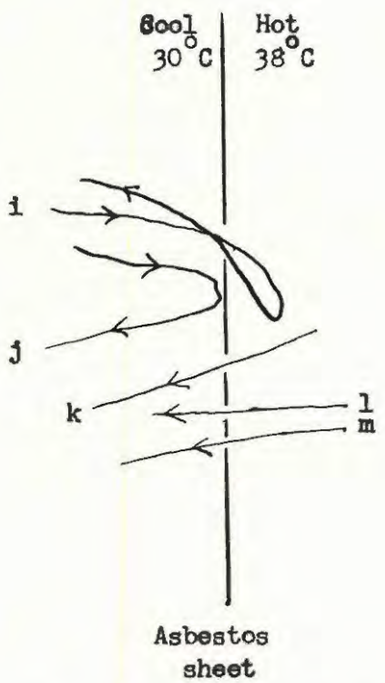


GRAPH 31. The effect of temperature on speed of locomotion of tick larvae.



GRAPH 32. Settling behaviour of larvae at 18o C and 26° C





TRACK 19. Reactions of larvae at border between a high and a low temperature.

Conclusions:

The results of the temperature behaviour experiments indicate that, given a temperature gradient larvae reared at 26 °C tend to settle at the cooler end. This settling is due to an avoidance of higher temperatures combined with the fact that they move more slowly and settle faster in lower temperatures than in higher ones.

APPENDIX XIV

DISTRIBUTION OF LARVAE IN A LINEAR TEMPERATURE GRADIENT

A.

TEMPERATURE (°C)	20.8	23.2	29.6	32.8	33.2	36.8	38	40	43.2	N
NO. OF TICKS	39(45.3%)	21(24.4%)	7(8.1%)	8(9.2%)	1(1.2%)	1(1.2%)	1(1.2%)	4(4.7%)	4(4.7%)	86

B.

TEMPERATURE (°C)	10	15.1	21.5	26	30.1	33.4	38	41.2	47	N
NO. OF TICKS	33(55%)	18(30%)	4(6.5%)	0(0%)	1(1.7%)	1(1.7%)	1(1.7%)	1(1.7%)	1(1.7%)	60

APPENDIX XV

EXPERIMENT TO CHECK THAT LARVAE WERE REACTING TO TEMPERATURE IN LINEAR GRADIENT

SECTION OF TUBE	A	B	C	D	E	F	G	H	I	N
NO. AT START	8(14%)	9(15.9%)	7(12.3%)	4(7%)	1(1.7%)	3(5.3%)	9(15.8%)	10(17.6%)	6(10.5%)	57
NO. AT END	5(8.8%)	26(45.6%)	9(15.8%)	8(14%)	0(0%)	3(5.3%)	0(0%)	0(0%)	6(10.5%)	57
TEMPERATURE (^{o}C)	7.5	17.5	21	24.5	29.5	33	36.5	38.5	40.5	

APPENDIX XVI

AVERAGE SPEEDS OF LARVAE AT DIFFERENT TEMPERATURES

EXPERIMENT TEMPERATURE	1	2	3	4	5
25^{o}C	6.7 cm/min	7.0 cm/min	6.7 cm/min	7.0 cm/min	6.3 cm/min
17^{o}C	2.5 cm/min	3.5 cm/min	2.6 cm/min	3.0 cm/min	1.8 cm/min
9^{o}C	2.0 cm/min	2.3 cm/min	1.0 cm/min	1.0 cm/min	0.6 cm/min
25^{o}C	5.7 cm/min	6.3 cm/min	6.1 cm/min	8.3 cm/min	3.5 cm/min

APPENDIX XVII

SETTLING BEHAVIOUR OF LARVAE AT DIFFERENT TEMPERATURES

A. 26°C

READING	TIME Hrs.	TIME Mins.	NO. ACTIVE	NO. INACTIVE	N
1	0	0	43 (100%)	0 (0%)	43
2	0	30	40 (93%)	3 (7%)	
3	1	00	41 (95.3%)	2 (4.7%)	
4	1	30	37 (86%)	6 (14%)	
5	2	00	40 (93%)	3 (7%)	
6	4	30	31 (72.1%)	12 (27.9%)	
7	7	30	28 (65.1%)	15 (34.9%)	
8	8	30	34 (79.1%) (4 clumped)	9 (20.9%)	
9	9	30	38 (88.4%)	5 (11.6%)	

B.18°C

READING	TIME Hrs.	TIME Mins.	NO. ACTIVE	NO. INACTIVE	N
1	0	00	95 (92.2%)	8 (7.8%)	103
2	0	30	53 (51.5%)	50 (48.5%)	
3	1	00	36 (35%)	67 (65%)	
4	1	30	25 (24.3%)	78 (75.7%)	
5	2	00	20 (19.4%)	83 (80.6%)	
6	4	30	12 (11.7%)	91 (88.3%)	
7	7	30	6 (5.8%)	97 (94.2%) (19 clump)	
8	8	30	5 (5%)	98 (95%) (17 clump)	
9	9	30	2 (1.9%)	101 (98.1%) (18 clump)	
Larvae transferred to 26°C to check that they were unharmed by lower temperature.					
10	9	45	84 (81.6%)	19 (18.4%) (0 clump)	

VII. HOST FINDING BEHAVIOUR

The host finding behaviour of the larvae of B. decoloratus has also been investigated - the aim being to find out which stimuli caused them to "quest" (Lees 1948a). This questing behaviour consists of the larvae becoming very excited, climbing over one another and waving their forelegs about. The questing behaviour of I. ricinus has been studied to some extent by Lees (1948), that of B. annulatus by Krijgsman (1937) while that of B. microplus has been examined briefly by Wilkinson(1953)

In the experiments with B. decoloratus, it was decided to test their reactions to the following stimuli: (a) smell (b) warmth (c) shading (d) moisture (e) vibrations (f) air currents. The apparatus used is illustrated in Figure 9. It consisted of a glass rod surrounded by a perspex screen and covered with polythene sheeting so that the larvae could not detect the observer. For the experiments, numbers of larvae were allowed to ascend the glass rod where they tended to accumulate at the top. Here their reactions to the various stimuli were noted. Observations were made through a stereoscopic microscope. Care had to be taken that the stimulations did not follow each other too rapidly, as the larvae can become adapted to stimuli presented to them at short intervals.

During experiments upon vibrational responses, it was noted that the animals seemed to have two resting states - one which I have termed a "deep resting state" and the other a "light resting state". When in the deep resting state, the larvae would not even respond to heavy "thumps" on the desk unless these were repeated. In this state they were also found to be sluggish in responding to a puff of breath - a stimulus to which they are normally very sensitive.

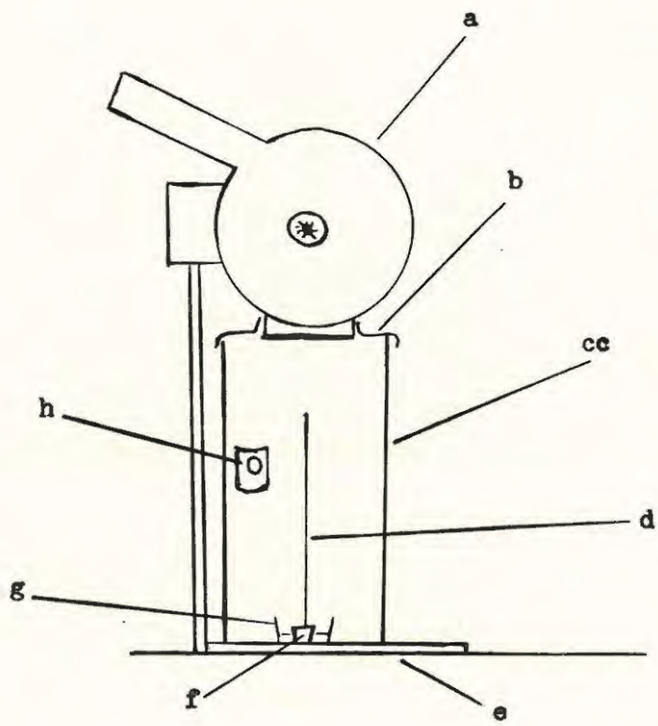


FIGURE 9. Diagram of apparatus used to study host finding behaviour.

a, stereoscopic microscope; b, screen made of polythene sheeting; c, screen; d, glass rod; e, bench; f, cork; g, dish of water; h, "window";

In the light resting state, they react very quickly even to a slight stimulus. The deep state develops when the larvae are left undisturbed for long periods of time - although sometimes they were found in this state after only short periods. It is possible that the light state is a forerunner of the deep resting state.

In all questing experiments, therefore larvae were disturbed just before an experiment and then allowed to settle down for a short time before being used.

(a) Smell. To test the reactions of the ticks to scent, a piece of cotton wool that had been handled was attached to a length of copper wire and pushed carefully through the small "window" cut in the screen. Care was taken to avoid shaking the apparatus and so disturbing the larvae.

TABLE 15. REACTIONS OF THE LARVAE TO SMELL.

NO. REACTING	NO. NOT REACTING	N
199 (94%)	14 (6%)	213

When cotton wool alone (i.e. no smell) was offered on the wire as a control, no reaction was obtained. It thus appeared that the larvae reacted strongly to smells from a potential host.

(b) Warmth. The reactions of the larvae to warmth were observed in the same way as for smell but here the stimulus was provided by a glass tube heated to approximately body temperature. To avoid "smell" on the tube, it was handled with a cloth at the end which was not presented to the animals. Once again care was taken to avoid shaking the apparatus.

TABLE 16. REACTIONS OF THE LARVAE TO WARMTH.

NO. REACTING	NO.NOT REACTING	N
43 (87.7%)	6(12.3%)	49

The larvae were thus seen to react strongly to warmth.

(c) Shading. In order to study the reactions of the larvae to sudden shading, a cardboard sheet was lowered between the apparatus on which the animals were sitting and the light source (a window).

TABLE 17. REACTIONS OF THE LARVAE TO SUDDEN SHADING.

NO. REACTING	NO. NOT REACTING	N
297 (92%)	26 (8%)	323

It may thus be seen that the tick larvae quest strongly when suddenly shaded. In view of this finding, it is interesting to recall that in the earlier gravity tracking experiments it was found that if a tick was moving downwards and the light was switched off and a red one substituted, causing a sudden fall in the light intensity, the tick always changed direction and once more went to the top of the support. However, ticks already moving upwards when the fall in light intensity occurred, continued on their upward path. The possible significance of this is dealt with in the discussion.

(d) Moisture. A negligable reaction was obtained when a moist, unhandled piece of cotton wool was carefully pushed near a clump of ticks. After each of these negative tests, the larvae were tested by blowing on them (which elicited a strong positive reaction) to ensure that they were still reactive.

TABLE 18. REACTIONS OF LARVAE TO MOISTURE.

NO. REACTING	NO. NOT REACTING	N
14 (5.5%)	240 (94.5%)	254

It thus appears that sudden increases in humidity in the immediate vicinity of the larvae do not play any part in stimulating the larvae to quest.

(e) Vibrations. When the bench on which the apparatus stood was rapped sharply, a very strong reaction by the ticks was observed.

TABLE 19. REACTIONS OF THE LARVAE TO VIBRATION.

NO. REACTING	NO. NOT REACTING	N
294 (93.9%)	19 (6.1%)	313

In view of their strong reaction to vibrations, it was decided to study this in more detail. Firstly an investigation was carried out to see whether the larvae on their support would react to airborne vibrations. The apparatus used was identical to that described earlier and vibrations of known frequency were produced using tuning forks. The forks used to test the reactions of the larvae to surface and airborne vibrations were the same distance from the apparatus.

TABLE 20. REACTIONS OF THE LARVAE TO SURFACE AND AIRBORNE VIBRATIONS.

VIBRATION FREQUENCY	% REACTING		N
	SURFACE-BORNE VIBRATIONS	AIRBORNE VIBRATIONS	
256 cps.	91.6%	0.4%	450
512 cps.	93.3%	0.7%	
1000 cps.	88.4%	0.2%	
2048 cps.	0.4%	0.2%	

These results show that the larvae react to surface-borne vibrations (in the lower frequency range) but not to airborne ones. There was an indication too that they were more sensitive to the lower frequencies.

It was thought probable that larvae would show an adaptation to constantly repeated vibrations because, with their limited food reserves, this would be an important factor regarding their survival in the field. The experiments carried out to investigate this were performed as before except that the vibration stimuli were applied and repeated as soon as the larvae had settled down after their last response. The frequency used was 1000 cps. An example of this type of experiment is shown in the table 21 and in Graph 33.

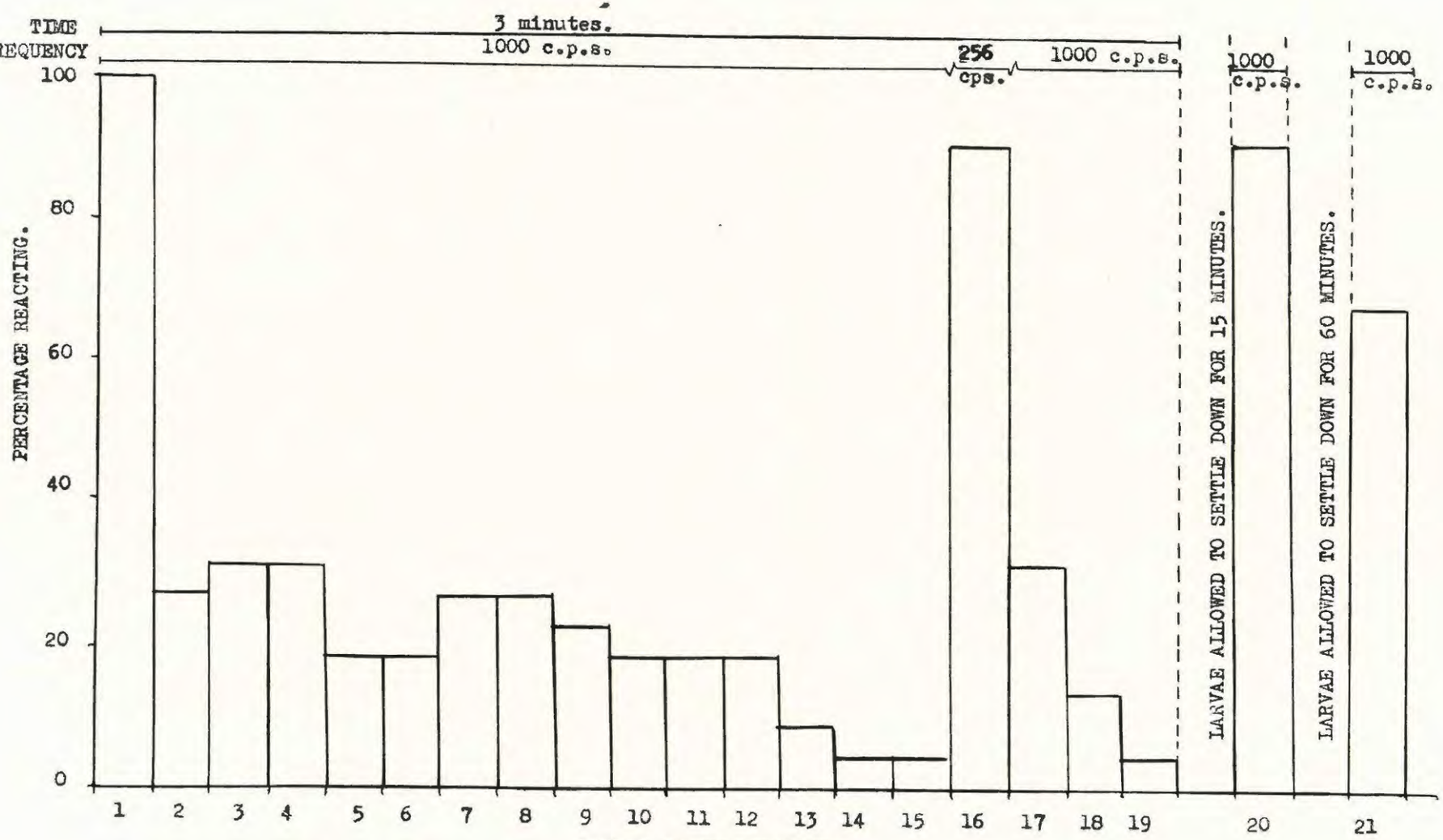
It was found that the larvae became adapted and most did not react to a constantly repeated vibration of a particular frequency. However, when the frequency was changed, the number reacting increased. On reverting once more to the original frequency, the number reacting to the original stimulus rose but then fell more rapidly than at first. Also, if the larvae were allowed to rest for a while, they reacted strongly when stimulated anew.

These experiments suggested that the larvae showed some degree of frequency discrimination and that they became adapted to constantly repeated vibrations of the same frequency. The results could not however be considered critical as, with tuning forks, there was no control of amplitude and there was the possibility that the animals might in fact be unable to detect the higher frequencies due to their lower amplitude.

TABLE 21. EXPERIMENT TO SEE WHETHER THE LARVAE BECOME ADAPTED TO REPEATED VIBRATIONS.

READ.	VIBRATION FREQ.	NO.REACTING	NO. NOT REACTING	N	COMMENTS
1	1000 cps.	22(100%)	0(0%)	22	Reaction strong.
2	"	6(27.3%)	16(72.7%)		"
3	"	7(31.8%)	15(68.2%)		"
4	"	7(31.8%)	15(68.2%)		"
5	"	4(18.2%)	18(81.8%)		"
6	"	4(18.2%)	18(81.8%)		Reaction fairly strong.
7	"	6(27.3%)	16(72.7%)		"
8	"	6(27.3%)	16(72.7%)		"
9	"	5(22.7%)	17(77.3%)		"
10	"	4(18.2%)	18(81.8%)		"
11	"	4(18.2%)	18(81.8%)		Reaction weak
12	"	4(18.2%)	18(81.8%)		"
13	"	2(9.1%)	20(90.9%)		"
14	"	1(4.5%)	21(95.5%)		"
15	"	1(4.5%)	21(95.5%)		"
16	256 cps.	20(90.9%)	2(9.1%)		Reaction strong
17	1000 cps.	7(31.8%)	15(68.2%)		Reaction fairly strong.
18	"	3(13.6%)	19(86.4%)		Reaction weak
19	"	1(4.5%)	21(95.5%)		"
LARVAE UNDISTURBED FOR 15 MINUTES					
20	1000 cps.	20(90.9%)	2(9.1%)		Reaction strong.
LARVAE UNDISTURBED FOR 60 MINUTES.					
21	1000 cps.	15(68.2%)	7(31.8%)		Reaction strong.

All done within a period of three minutes.



GRAPH33 . Reactions of larvae to repeated vibrations .

To try to cut down the number of variables in these experiments, a Heathkit audiogenerator was used. It had a frequency range of from 10 cps. to 100 Kcps. and an accuracy of about 5% The audiogenerator was connected to an amplifier and loudspeaker to produce vibrations of known frequency and amplitude. The loudspeaker was placed face downwards on the bench in order to transmit the vibrations to the ticks on their support via the substrate. This apparatus, while a little better than the tuning forks, obviously was still crude and, for really critical experiments, a much more elaborate arrangement would be necessary.

The method used was, at a fixed frequency, to increase the amplitude until one animal responded and thereafter to increase the amplitude to the maximum which could be produced on the apparatus and to count the number of animals responding. Amplitude readings were from an arbitrary scale on the audiogenerator but this was satisfactory for the comparative method used here. Preliminary experiments were carried out with the apparatus to see whether the larvae reacted to the sound produced by the loudspeaker as well as to the vibrations. In these experiments, the loudspeaker was placed at the same distance from the larvae but on a desk not connected to the one on which the larvae stood. After each reading, the animals were allowed to settle down and readings were so regulated that no adaptation occurred. These preliminary experiments showed that the larvae were much more sensitive to surface borne than to airborne vibrations and, even at the maximum amplitude which could be produced, only a few animals reacted to the airborne stimuli. It was also noted that the larvae responded to a much more limited range of airborne than of surface borne frequencies.

An example of this type of experiment is tabulated below:

TABLE 22. REACTIONS OF LARVAE TO SURFACE-BORNE AND AIR-BORNE VIBRATIONS.

READ	FREQUENCY	AMPLITUDE AT WHICH FIRST TICK RESPONDS		NO. RESPONDING TO MAX. AMPLITUDE OBTAINABLE		N
		SURFACE	AIR	SURFACE	AIR	
1	10 cps.	30(max.)	No response	3	0	20
2	50 cps.	13	No response	4	0	
3	100 cps.	6.75	24.5	4	2	
4	250 cps.	4.75	6	3	3	
5	500 cps.	0*	3.5	9	3	
6	1000 cps.	0	30(max.)	9	2	
7	2000 cps.	0	No response	5	0	
8	3000 cps.	2	-	4	-	
9	4000 cps.	7	-	1	-	
10	5000 cps.	No response	-	0	-	

*Minimal setting of audiogenerator.

The reactions of the larvae to surface borne vibrations were then studied in more detail. These experiments showed that, as the frequency increased to 500 cps. the number of ticks reacting to the maximum amplitude of stimulation increased and then, as the frequency was increased still further, the number reacting dropped. It was also found that as the frequency increased to 500 cps. so the amplitude threshold needed to evoke a response decreased. The threshold then increased at frequencies above 1000 cps. This is illustrated in the table overleaf.

TABLE 23. REACTIONS OF LARVAE TO SURFACE-BORNE VIBRATIONS.

READ.	FREQUENCY	AMPLITUDE AT WHICH FIRST TICK RESPONDS	NO. OF TICKS RESPONDING AT MAXIM AMPLITUDE OBTAINABLE	N
1	10 cps.	No response	0	20
2	50 cps.	6.25	2	
3	100 cps.	2.0	5	
4	250 cps.	0.75	5	
5	500 cps.	0.1	7	
6	1000 cps.	0.75	3	
7	2000 cps.	2.0	3	
8	3000 cps.	6.0	2	
9	4000 cps.	No response	0	
10	5000 cps.	No response	0	
THEN TO SEE THAT THE LARVAE WERE STILL REACTIVE:				
11	500 cps.	0.2	6	

During these experiments, it was noted that some ticks appeared to be more sensitive to vibrations than others, for it was often found that the same tick reacted first to vibrations of different frequencies. When using groups of animals as in these experiments, it must be kept in mind that by their own activity reacting ticks could cause others to quest.

As has been described earlier larvae may exhibit two states of rest. Responses to vibrations in groups of larvae in the light resting state and the deep resting state were examined. One difficulty in this series of experiments was that, within each clump the numbers of individuals in the two states varied, i.e. clumps tended to have members in both light and deep states. This difficulty was overcome by leaving the clump overnight as then most, if not all, of the animals were in a state of deep rest.

In this investigation, each batch of larvae was tested twice - once when in deep resting state (having been left undisturbed overnight) and once when in the light resting state (having been excited with a puff of breath and then allowed to settle down again). Results of one of these experiments is given overleaf.

TABLE 24. REACTIONS OF LARVAE IN LIGHT AND DEEP RESTING STATES TO VIBRATIONS OF DIFFERENT FREQUENCIES.

READ	FREQUENCY	AMPLITUDE AT WHICH 1st TICK RESPONDS		NO. OF TICKS RESPONDING AT MAX.AMPLITUDE OBTAINABLE		N
		LIGHT REST	DEEP REST	LIGHT REST	DEEP REST	
1	10cps.	No response	No res.	0	0	20
2	50cps.	30 (max.)	"	1	0	
3	100cps.	8.75	No res.	2	0	
4	250cps.	3.0	9.5	2	1	
5	500cps.	0.9	5.75	4	1	
6	1000cps.	1.15	No res.	2	0	
7	2000 cps.	1.08	"	2	0	
8	3000cps.	No response	"	0	0	
9	4000cps.	"	"	0	0	
10	5000cps.	"	"	0	0	

The results showed that larvae in the deep rest state were unreactive but, having been "activated" and allowed to settle into the light resting state, they became more reactive.

(f) Air currents: The reactions of the larvae to air currents were studied with the same apparatus as in the other questing experiments except that a tube was fastened to the "window" in the screen so that blasts of air could be trained on the tick clump at the tip of the support(See Fig.10). The air currents were from a compressed air supply. Using this method, with a manometer to measure the strength of the blasts, air currents of known strength and duration could be applied. The rod on which the larvae were sitting was strong enough to prevent it from vibrating in the air current.

The first experiments were carried out using a short blast of air to see whether it would cause the larvae to quest.

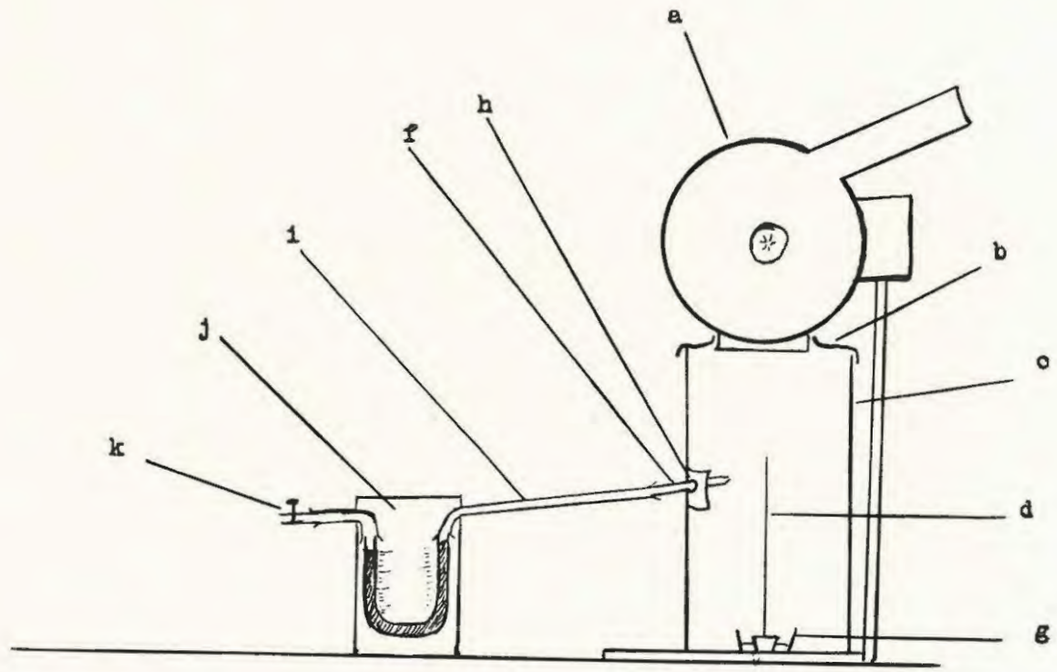


FIGURE 10. Apparatus used to study the reactions of tick larvae to air currents.

a, stereoscopic microscope; b, polythene sheeting screen; c, screen; d, glass rod; e, bench; f, glass tube with narrow mouth to direct the currents of air; g, dish of water; h, "window"; i, rubber tubing; j, manometer and scale; k, tap of compressed air supply.

TABLE 25. REACTION OF THE LARVAE TO AN AIR CURRENT OF SHORT DURATION.

NO.REACTING	NO. NOT REACTING	N
168 (91.8%)	15 (8.2%)	183

It can thus be seen that the larvae began questing in response to sudden air currents.

Further experiments were undertaken to study the behaviour of the larvae in steady air currents, and their responses to changes in the velocity of such currents. Observations were made on a clump at five minute intervals. In general it was found that, with an increase in air current velocity, the number of ticks active and/or questing increased. However, if the air current remained constant, the larvae became adapted to it, ceased questing and usually became inactive as well.

Before these experiments were undertaken, it had been expected that, with increasing velocity of steady air currents, the number of ticks at the top of the rod would decrease. Although this was found to be true in some cases, an increase in the number at the top of the support often occurred irrespective of whether the air current velocity was increased or decreased. Thus there is no direct correlation between air current velocity and numbers of larvae at the top of the rod. This is illustrated in the table overleaf.

TABLE 26. REACTIONS OF LARVAE TO A CONTINUOUS AIR CURRENT OF VARYING VELOCITY.

READ.	TIME Mins)	AIR PRESSURE	NO.OF TICKS AT TOP OF SUPPORT	COMMENTS
1	0	0	16	Evenly distributed
2	5	0	21	"
3	10	2	12	all active; 11 questing
4	15	2	11	0 active; 0 questing
5	20	2	8	1 active; 0 questing
6	25	2	8	1 active; 0 questing
7	30	4	9	all active; all questing
8	34	4	9	0 active; 0 questing
9	35	6	16	3 active; 3 questing
10	39	6	15	3 active; 3 questing
11	40	8	11	All active all questing
12	44	8	11	4 active; 4 questing
13	45	30	18	14 active; 14 questing
14	49	30	18	3 active 3 questing

† All on protected side of support

A number of points arise from the type of experiment illustrated in the table above: (i) Ticks which did not respond when the velocity of the air current was increased might have been protected from the blast and (ii) many animals tended to move up and down the rod throughout the experiment and therefore it was possible for ticks to come into the experimental zone shortly before a reading was taken. This may explain why a small percentage of animals was often found to be active and/or questing after the air current had been constant for quite a while.

To try further to understand this behaviour tracking experiments were carried out similar to those used in the gravity experiments, and the reactions of the larvae to a "narrow" air current were noted (see Fig.11 and Track17). From these tracking experiments and from general observations, it appeared that the larvae on the vertical supports moved upwards until they arrived at the border of the air current. They then stopped and either descended, stayed at the border or moved to the protected side of the support and carried on upwards. When there were large numbers of ticks on the rod, they were found to congregate at the border and then, when the air current was switched off, they would carry on upwards (or downwards if they were above the exposed area.)

It was also often found that larvae which were evenly distributed at the top would, when the air current was switched on, move to the protected side and remain there until the current was switched off. When the air current ceased, they would either descend or would once more become evenly distributed around the rod.

Conclusions: These investigations on the questing behaviour of B. decoloratus larvae show that they will quest to smell, heat, shading, vibrations and air currents but not to moisture. Work on the reactions of the larvae to vibrations indicates that they react more strongly to surface borne than to airborne vibrations and that they react most to vibrations in the 500-1000 cps. ranges. From observations on groups of ticks, it seems that individual larvae differ in their sensitivity

DRAWN FROM PHOTOGRAPHS.

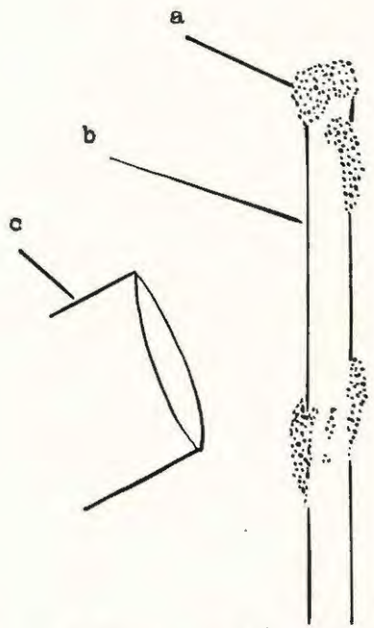


FIGURE 11 A. Air current on. Clumps of ticks can be seen collecting at the border of the air current and sheltering on the protected side of the rod. Note - no ticks on exposed side in experimental zone.

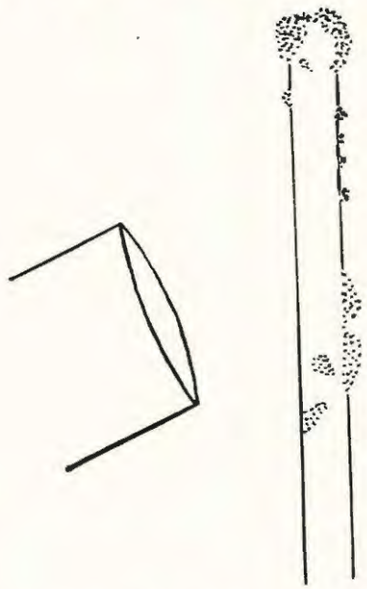


FIGURE 11 B. As for A but, in addition note small groups of larvae moving up and down on the protected side of the rod.

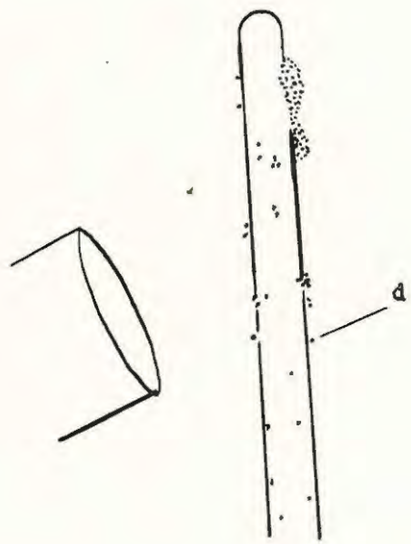
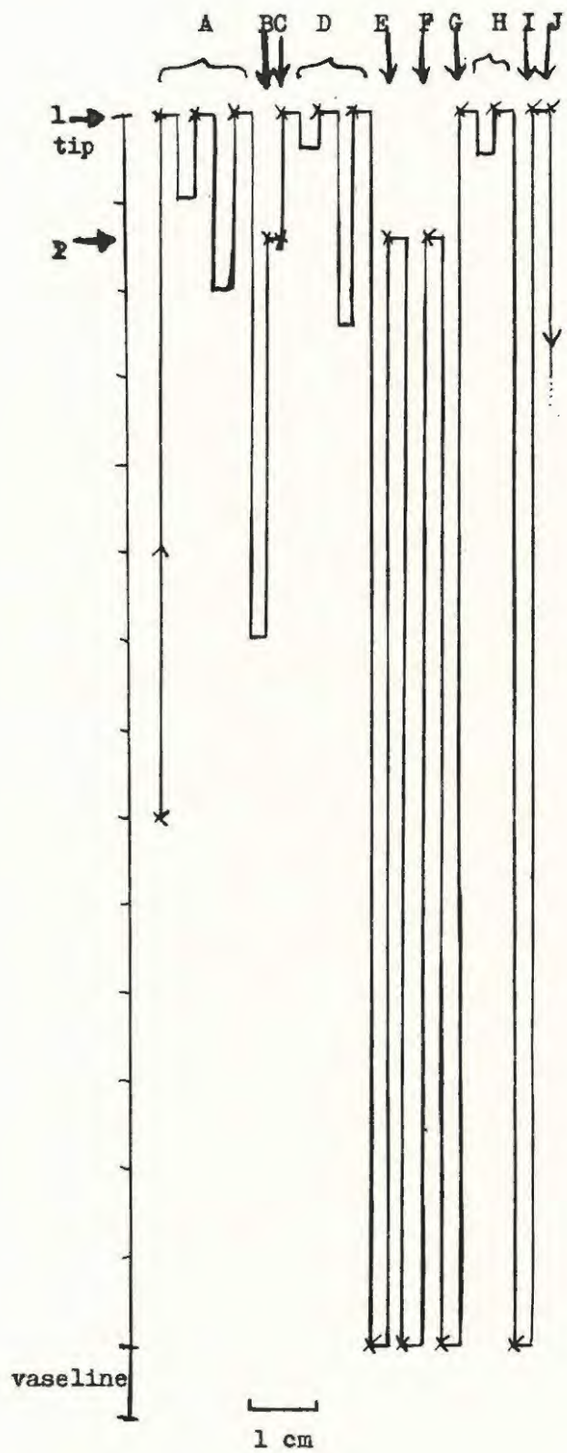


FIGURE 11 C. Air current switched off. Clumps at the former borders (see A and B) break up and larvae move up and down over all sides of the rod.

a, clump of larvae; b, glass rod; c, glass tube directing air current; d, tick larva.



A^1 D^1 H^1 Normal tip reaction with no air current (p=0)

B^2 Air current velocity p=1. At B^2 larva stops and seeks at lower border of the air current zone.

C^2 Air current velocity p=0. When air current was switched (at C^2) off, larva continued upwards.

E^2 Air current velocity p=1 At E^2 tick stops, quests then retreats downwards.

F^2 Air current velocity still p=1. At F^2 larva again stops, quests but then it moves to the protected side of the rod and goes down again.

G^2 Air current off (ie. p=0). Tick continues up to top.

I^1 Air current velocity p=1. Larva quests when current first switched on, then moves to the protected side where it settled.

J^1 Air current switched off (ie. p=0). When the current was switched off, the tick became active moved to the previously exposed side and went down.

TRACK 17. Track showing reactions of a tick larva on a vertical rod (tip up) to air currents.

to vibrations - as one would expect. It also appears that larvae have two resting states - one in which they react quickly to stimuli and one (after a long undisturbed period) in which they are not so reactive at first. It should be noted, however, that the apparatus used in the vibration experiments was too crude to give anything more than an indication of their behaviour.

Detailed studies on the reactions of the larvae to air currents revealed that they would shelter from these currents on the protected side of their support or in some other protected spot (e.g. beneath the "windy" area).

VIII. ACTIVITY RHYTHM

The previous gravity experiments had given indications of some kind of rhythm in the late afternoon and, while this rhythm could be observed in one series of gravity experiments as an increase or decrease in the number of larvae at the top of the apparatus, it was not obvious in the second series. However, even in this second series, when the number active was plotted, a rise was seen in the late afternoon. In the gravity experiments, as explained in Chapter III, due to the method used, it was possible for the total number of individuals active to increase while the number of animals in each half remained constant e.g. ticks within a section could become active and move around within that section - an action which would not be accounted for by the system used, as it was only when they moved out of one half and into the other that the change would be recorded. The rhythm appeared to consist of many of the inactive larvae becoming active - a process which would result in the previously observed partial or complete break-up of clumps, and sometimes in the movement of the larvae to a different section of the apparatus.

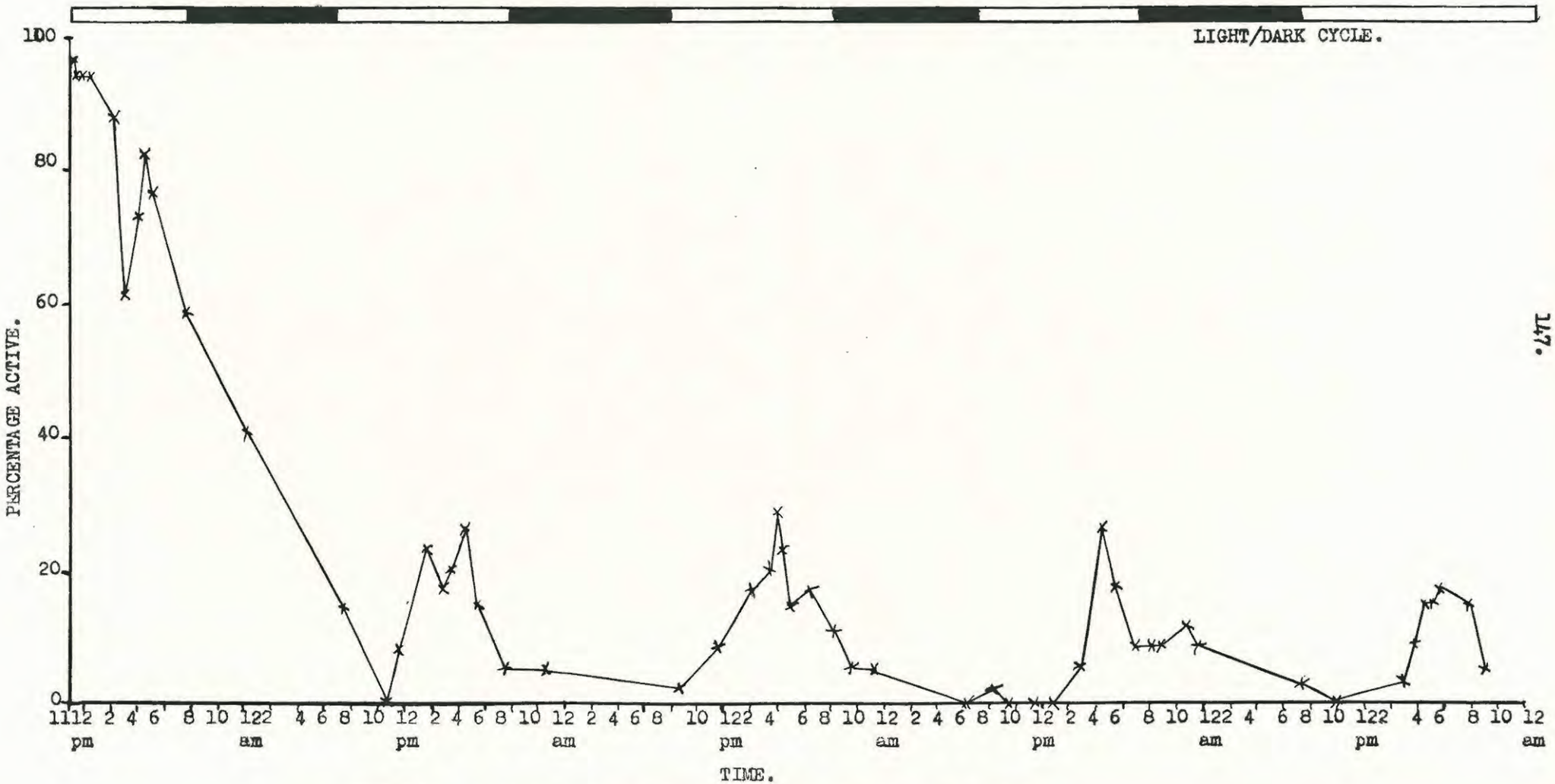
It was decided to investigate this suggested activity rhythm in more detail. The method used was to keep a batch of larvae in a petri dish which had a plain glass lid sealed on with low melting point wax. The experiments were, as usual, carried out under constant conditions of humidity and temperature. To see the effect of light and dark on the suspected rhythm one series of experiments was carried out with the light/dark cycle more or less sunchronised with day and night while the other series was carried out under constant light.

The results are tabulated in Appendix XVIII, XIX and XX and summarised in Graphs 34, 35 and 36.

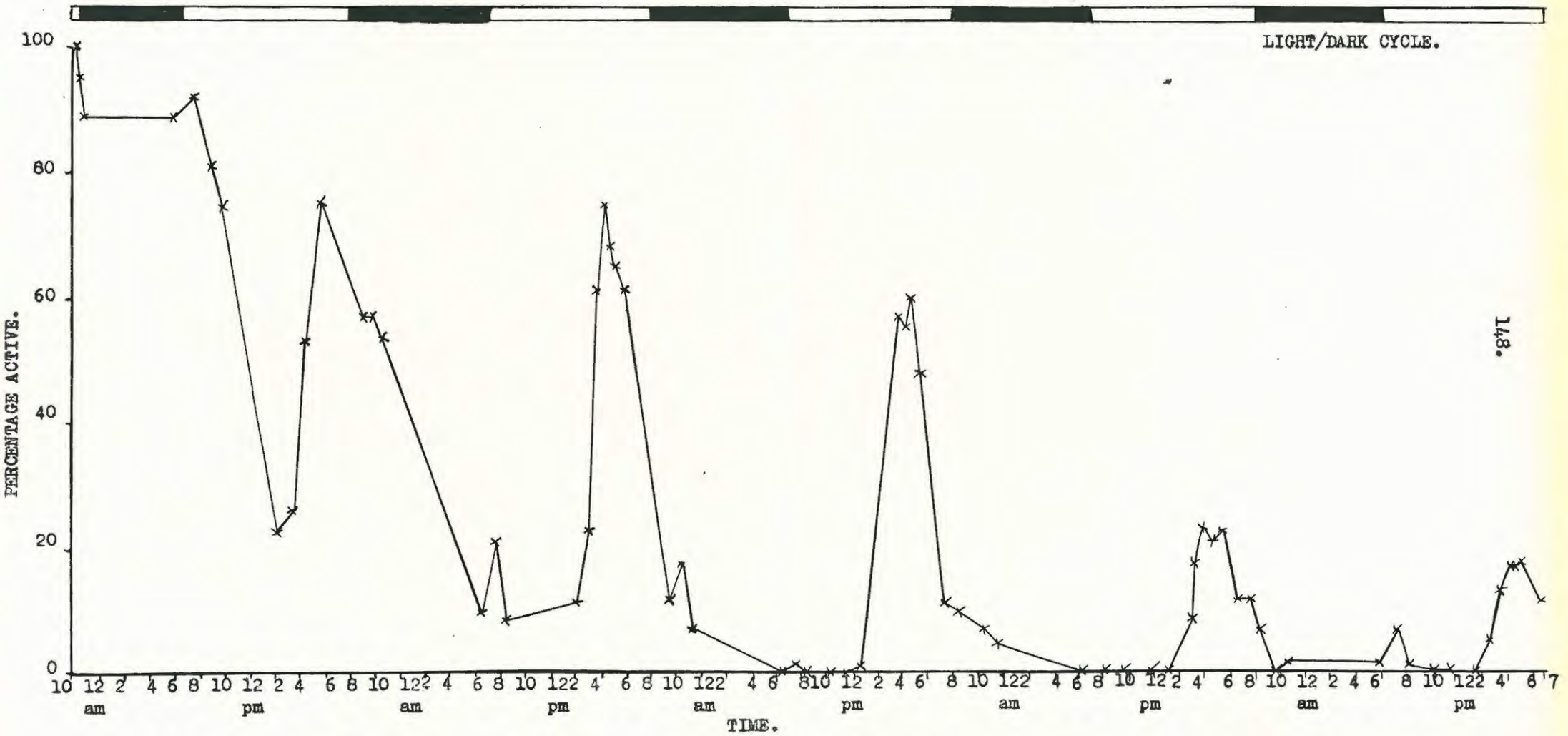
In the experiments with alternating light and dark, a definite increase in activity can be seen at about 4 - 6 pm. In Graph 35, the effect is much more noticable than it is in Graph 34 and in the former it can be seen that the effect becomes less pronounced as the days pass. The effect can still be seen in the experiments carried out under constant light (see Graph 36) but tend to be more drawn out.

No further analysis was undertaken of this effect. Of the environmental variables, the only one which might possibly have fluctuated was that of light intensity with varying mains voltage. No direct observations were made upon this point, but the magnitude of any such effect is likely to have been so small that the present observations almost certainly reflect an inherent activity rhythm of the larvae.

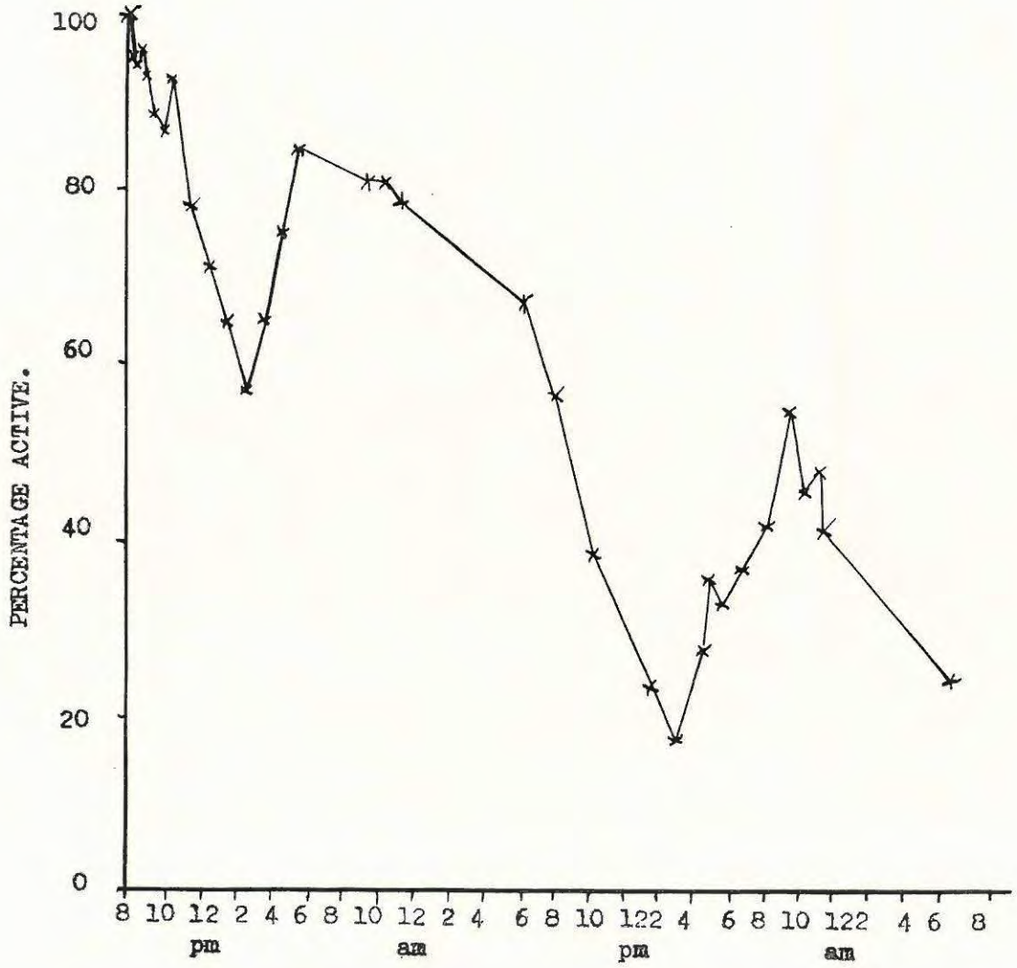
Conclusions: It appears that the larvae show a rhythm of activity in the late afternoon which, while to some extent connected with the light/dark cycle, seems to be inherent. The nature of the rhythm is considered further in the general discussion.



GRAPH 34. Activity of tick larvae through the 24 hour period.



GRAPH 35. Activity of tick larvae through the 24 hour period.



GRAPH 36. Activity of tick larvae through the 24 hour period in constant light.

APPENDIX XVIII

ACTIVITY OF LARVAE THROUGH THE 24 HOUR PERIODS IN ALTERNATING LIGHT AND DARK.

READ	CLOCK TIME	TIME DYS.	HRS.	MINS.	LIGHT	NO.ACTIVE	NO.INACTIVE	N
1	11.15 a.m.	0	00	00	on	33(97.1%)	1(2.9%)	34
2	11.30 a.m.	0	00	15	on	32(94.1%)	2(5.9%)	
3	11.45 a.m.	0	00	30	on	32(94.1%)	2(5.9%)	
4	12.45 p.m.	0	1	30	on	32(94.1%)	2(5.9%)	
5	2.15 p.m.	0	3	00	on	30(88.2%)	4(11.8%)	
6	3.15 p.m.	0	4	00	on	21(61.8%)	13(38.2%)	
7	4.15 p.m.	0	5	00	on	25(73.5%)	9(26.5%)	
8	4.45 p.m.	0	5	30	on	28(82.4%)	6(17.6%)	
9	5.15 p.m.	0	6	00	on	26(76.5%)	8(23.5%)	
10	7.45 p.m.	0	8	30	on	20(58.8%)	14(41.2%)	
11	12.15 a.m.	0	13	00	off	14(41.2%)	20(58.8%)	
12	7.30 a.m.	0	20	15	on	5(14.7%)	29(85.3%)	
13	11.00 a.m.	0	23	45	on	0(0%)	34(100%)	
14	11.45 a.m.	1	00	30	on	3(8.8%)	31(91.2%)	
15	1.45 p.m.	1	2	30	on	8(23.5%)	26(76.5%)	
16	3.00 p.m.	1	3	45	on	6(17.6%)	28(82.4%)	
17	3.30 p.m.	1	4	15	on	7(20.6%)	27(79.4%)	
18	4.30 p.m.	1	5	15	on	9(26.5%)	25(73.5%)	
19	5.30 p.m.	1	6	15	on	5(14.7%)	29(85.3%)	
20	7.30 p.m.	1	8	15	on	2(5.9%)	32(94.1%)	
21	10.45 p.m.	1	11	30	off	2(5.9%)	32(94.1%)	
22	8.45 a.m.	1	21	30	on	1(2.9%)	33(97.1%)	
23	11.30 a.m.	2	0	15	on	3(8.8%)	30(91.2%)	
24	2.00 p.m.	2	2	45	on	6(17.6%)	28(82.4%)	
25	3.30 p.m.	2	4	15	on	7(20.6%)	27(79.4%)	
26	4.00 p.m.	2	4	45	on	10(29.5%)	24(70.6%)	

(continued)

APPENDIX XVIII (continued)

READ	CLOCK TIME	Time DYS.	HRS.	MINS.	LIGHT	NO.ACTIVE	NO.INACTIVE	N
27	4.30 p.m.	2	5	15	on	8(23.5%)	26(76.5%)	34
28	5.00 p.m.	2	5	45	on	5(14.7%)	29(85.3%)	
29	5.30 p.m.	2	6	15	on	6(17.6%)	28(82.4%)	
30	8.15 p.m.	2	9	00	on	4(11.8%)	30(88.2%)	
31	9.15 p.m.	2	10	00	off	2(5.9%)	32(94.1%)	
32	11.15 p.m.	2	12	00	off	2(5.9%)	32(94.1%)	
33	6.15 a.m.	2	19	00	off	0(0%)	34(100%)	
34	8.15 a.m.	2	21	00	on	1(2.9%)	33(97.1%)	
35	9.15 a.m.	2	22	00	on	0(0%)	34(100%)	
36	11.00 a.m.	2	23	45	on	0(0%)	34(100%)	
37	12.30 p.m.	3	1	15	on	0(0%)	34(100%)	
38	2.30 p.m.	3	3	15	on	2(5.9%)	32(94.1%)	
39	4.15 p.m.	3	5	00	on	9(26.5%)	25(73.5%)	
40	5.15 p.m.	3	6	00	on	6(17.6%)	28(82.4%)	
41	6.45 p.m.	3	7	30	on	3(8.8%)	31(91.2%)	
42	8.00 p.m.	3	8	45	off	3(8.8%)	31(91.2%)	
43	8.45 p.m.	3	9	30	off	3(8.8%)	31(91.2%)	
44	10.30 p.m.	3	11	15	off	4(11.8%)	30(88.2%)	
45	11.15 p.m.	3	12	00	off	3(8.8%)	31(91.2%)	
46	7.15 a.m.	3	20	00	on	1(2.9%)	33(97.1%)	
47	9.45 a.m.	3	22	30	on	0(0%)	34(100%)	
48	2.30 p.m.	4	4	15	on	1(2.9%)	33(97.1%)	
49	3.15 p.m.	4	5	00	on	3(8.8%)	31(91.2%)	
50	4.15 p.m.	4	6	00	on	5(14.7%)	29(85.3%)	
51	4.45	4	6	30	on	5(14.7%)	29(85.3%)	

APPENDIX XVIII (continued)

READ	CLOCK TIME	TIME DYS.	HRS.	MINS.	LIGHT	NO.ACTIVE	NO.INACTIVE	N
52	5.15 p.m.	4	7	00	on	6(17.6%)	28(82.4%)	34
53	7.45 p.m.	4	9	30	on	5(14.7%)	29(85.3%)	
54	8.45 p.m.	4	10	30	on	2(5.9%)	32(94.1%)	

APPENDIX XIX

ACTIVITY OF LARVAE THROUGH THE 24-HOUR PERIODS IN ALTERNATING LIGHT AND DARK

READ.	CLOCK TIME	TIME DYS.	TIME HRS.	TIME MINS.	LIGHT	NO. ACTIVE	NO. INACTIVE	N
1	10.30 p.m.	0	00	00	on	62(100%)	0(0%)	62
2	10.45 p.m.	0	00	15	off	59(95.2%)	3(4.8%)	
3	11.00 p.m.	0	00	30	off	55(88.7%)	7(11.3%)	
4	6.30 a.m.	0	8	00	off	55(88.7%)	7(11.3%)	
5	7.45 a.m.	0	9	15	on	57(91.9%)	5(8.1%)	
6	9.00 a.m.	0	10	30	on	50(80.6%)	12(19.4%)	
7	10.15 a.m.	0	11	45	on	46(74.2%)	16(25.8%)	
8	2.00 p.m.	0	15	30	on	14(22.6%)	48(77.4%)	
9	3.15 p.m.	0	16	45	on	16(25.8%)	46(74.2%)	
10	4.30 p.m.	0	18	00	on	33(53.2%)	29(46.8%)	
11	5.45 pm.	0	19	15	on	46(74.2%)	16(25.8%)	
12	9.15 p.m.	0	22	45	off	35(56.5%)	27(43.5%)	
13	10.00 p.m.	0	23	30	off	35(56.5%)	27(43.5%)	
14	10.45 p.m.	1	00	15	off	33(53.2%)	29(46.8%)	
15	6.30 a.m.	1	8	00	off	6(9.7%)	56(90.3%)	
16	7.30 a.m.	1	9	00	on	13(21%)	49(79%)	
17	8.15 a.m.	1	9	45	on	5(8.1%)	57(91.9%)	
18	2.00 p.m.	1	15	30	on	7(11.3%)	55(88.7%)	
19	3.00 p.m.	1	16	30	on	14(22.6%)	48(77.4%)	
20	3.45 p.m.	1	17	15	on	38(61.3%)	24(38.7%)	
21	4.15 p.m.	1	17	45	on	46(74.2%)	16(25.8%)	
22	4.45 p.m.	1	18	15	on	42(67.7%)	20(32.3%)	
23	5.15 p.m.	1	18	45	on	40(64.5%)	22(35.5%)	
24	6.00 p.m.	1	19	30	on	38(61.3%)	24(38.7%)	

(continued)

APPENDIX XIX (continued

READ.	CLOCK TIME	TIME DYS.	TIME HRS.	TIME MINS.	LIGHT	NO.ACTIVE	NO.INACTIVE	N
25	9.15 p.m.	1	22	45	off	7(11.3%)	55(88.7%)	62
26	10.15 p.m.	1	23	45	off	10(16.1%)	52(83.9%)	
27	11.15 p.m.	2	00	15	off	4(6.5%)	58(93.5%)	
28	6.30 a.m.	2	7	30	off	0(0%)	62(100%)	
29	7.30 a.m.	2	8	30	on	1(1.6%)	61(98.4%)	
30	8.30 a.m.	2	9	30	on	0(0%)	62(100%)	
31	10.15 a.m.	2	11	15	on	0(0%)	62(100%)	
32	11.30 a.m.	2	13	00	on	0(0%)	62(100%)	
33	12.45 p.m.	2	14	15	on	1(1.6%)	61(98.4%)	
34	4.00 p.m.	2	17	30	on	35(56.5%)	27(43.5%)	
35	4.30 p.m.	2	18	00	on	32(54.8%)	30(45.2%)	
36	5.00 p.m.	2	18	30	on	37(59.7%)	25(40.3%)	
37	5.30 p.m.	2	19	00	on	29(46.8%)	33(53.2%)	
38	7.30 p.m.	2	21	00	on	10(16.1%)	52(83.9%)	
39	8.30 p.m.	2	22	00	off	6(9.7%)	56(90.3%)	
40	10.45 p.m.	3	00	15	off	4(6.5%)	58(93.5%)	
41	11.30 p.m.	3	1	00	off	3(4.8%)	59(95.2%)	
42	6.30 a.m.	3	8	00	off	0(0%)	62(100%)	
43	8.00 a.m.	3	9	30	on	0(0%)	62(100%)	
44	9.15 a.m.	3	10	45	on	0(0%)	62(100%)	
45	11.45 a.m.	3	13	15	on	0(0%)	62(100%)	
46	1.00 p.m.	3	14	30	on	0(0%)	62(100%)	
47	3.00 p.m.	3	16	30	on	5(8.1%)	57(91.9%)	
48	3.30 p.m.	3	17	00	on	11(17.7%)	51(82.3%)	
49	4.00 p.m.	3	17	30	on	14(22.6%)	48(77.4%)	

(Continued)

APPENDIX XIX (continued)

READ.	CLOCK TIME	TIME DYS	TIME HRS	TIME MINS.	LIGHT	NO.ACTIVE	NO.INACTIVE	N
50	4.45 p.m.	3	18	15	on	13(21%)	49(79%)	62
51	5.30 p.m.	3	19	00	on	14(22.6%)	48(77.4%)	
52	6.45 p.m.	3	20	15	on	7(11.3%)	55(88.7%)	
53	7.45 p.m.	3	21	15	on	7(11.3%)	55(88.7%)	
54	8.30 p.m.	3	22	00	off	4(6.5%)	58(93.5%)	
55	9.45 p.m.	3	23	15	off	0(0%)	62(100%)	
56	10.30 p.m.	4	00	00	off	1(1.6%)	61(98.4%)	
57	6.00 a.m.	4	7	30	off	1(1.6%)	61(98.4%)	
58	7.15 a.m.	4	8	45	on	2(3.2%)	60(96.8%)	
59	8.15 a.m.	4	9	45	on	1(1.6%)	61(98.4%)	
60	10.00 a.m.	4	11	30	on	0(0%)	62(100%)	
61	11.30 a.m.	4	12	30	on	0(0%)	62(100%)	
62	1.30 p.m.	4	14	30	on	0(0%)	62(100%)	
63	2.30 p.m.	4	15	30	on	3(4.8%)	59(95.2%)	
64	3.30 p.m.	4	16	30	on	8(12.9%)	54(87.1%)	
65	4.15 p.m.	4	17	15	on	10(16.1%)	52(83.9%)	
66	4.45 p.m.	4	17	45	on	10(16.1%)	52(83.9%)	
67	5.15 p.m.	4	18	15	on	11(17.7%)	51(82.3%)	
68	7.00 p.m.	4	20	00	on	7(11.3%)	55(88.7%)	
69	7.45 p.m.	4	20	45	on	6(9.7%)	56(90.3%)	
70	8.30 p.m.	4	21	30	off	1(1.6%)	61(98.4%)	
71	9.00 p.m.	4	22	30	off	2(3.2%)	60 (96.8%)	
72	10.00 p.m.	4	23	30	off	2(3.2%)	60(96.8%)	

APPENDIX XX

ACTIVITY OF LARVAE THROUGH THE 24-HOUR PERIODS IN CONSTANT LIGHT

READ.	CLOCK TIME	TIME DYS.	TIME HRS.	TIME MINS.	NO.ACTIVE	NO.INACTIVE	N
1	8.00 a.m.	0	00	00	148(100%)	0(0%)	148
2	8.15 a.m.	0	00	15	141 (95.3%)	7(4.7%)	
3	8.30 a.m.	0	00	30	140(94.6%)	8(5.4%)	
4	8.45 a.m.	0	00	45	142(96%)	6(4%)	
5	9.00 a.m.	0	1	00	138(93.2%)	10(6.8%)	
6	9.30 a.m.	0	1	30	131(88.5%)	17(11.5%)	
7	10.00 a.m.	0	2	00	129(87.2%)	19(12.8%)	
8	10.30 a.m.	0	2	30	137(92.6%)	11(7.4%)	
9	11.30 a.m.	0	3	30	115(77.7%)	33(22.3%)	
10	12.30 p.m.	0	4	30	106(71.6%)	42(28.4%)	
11	1.30 p.m.	0	5	30	95(64.2%)	53(35.8%)	
12	2.30 p.m.	0	6	30	84(56.8%)	64(43.2%)	
13	3.30 p.m.	0	7	30	96(64.9%)	52(35.1%)	
14	4.30 p.m.	0	8	30	111(75%)	37(25%)	
15	5.30 p.m.	0	9	30	125(84.5%)	23(15.5%)	
16	9.15 p.m.	0	13	15	119(80.4%)	29(19.6%)	
17	10.15 p.m.	0	14	15	119(80.4%)	29(19.6%)	
18	11.15 p.m.	0	15	15	116(78.4%)	32(21.6%)	
19	6.30 a.m.	0	22	30	98(66.2%)	50(33.8%)	
20	8.15 a.m.	1	00	15	85(57.4%)	63(42.6%)	
21	10.15 a.m.	1	2	15	57(38.5%)	91(61.5%)	
22	1.30 p.m.	1	5	30	34(23%)	114(77%)	
23	3.00 p.m.	1	7	00	26(17.6%)	122(82.4%)	
24	4.15 p.m.	1	8	15	41(27.7%)	107(72.3%)	
25	4.45 p.m.	1	8	45	52(35.1%)	96(64.9%)	

(continued)

APPENDIX XX (continued)

READ.	CLOCK TIME	TIME DYS.	HRS.	MINS.	NO.ACTIVE	NO.INACTIVE	N
26	5.15 p.m.	1	9	15	48(32.4%)	100(67.6%)	148
27	6.30 p.m.	1	10	30	54(36.5%)	94(63.5%)	
28	8.00 p.m.	1	12	00	61(41.2%)	87(58.8%)	
29	9.15 p.m.	1	13	15	80(54.1%)	68(45.9%)	
30	10.15 p.m.	1	14	15	67(45.3%)	81(54.7%)	
31	11.00 p.m.	1	15	00	70(47.3%)	78(52.7%)	
32	11.30 p.m.	1	15	30	61(41.2%)	67(58.8%)	
33	6.30 a.m.	1	22	30	34(23%)	114(77%)	

IX. DISCUSSION

It is intended to deal with the discussion in two ways - firstly to compare the present results with those of other workers and secondly to consider the possible importance of the laboratory findings to the animals in the field.

In the present investigation it was found that B. decoloratus larvae less than one week old were negatively phototactic while those over one week old were positively phototactic. The mechanism by which the young larvae stay in the dark appears to involve an avoiding reaction (taxis)(or phobic reaction, Carthy 1958) to the light, combined with the fact that they settle faster (i.e. an activity orthokinesis) and form clumps in the dark. Newly formed clumps can easily be broken up on exposure to light but, in the "mature" clump, the clumping response apparently overrides the negative phototaxis. This might be due to some extent to the fact that the larvae in a clump shade each other (at least partially). The older larvae, on the other hand, avoid the dark and move towards the light.

These results are in general agrement with those of Krijgsman (1937) who states that B. annulatus larvae are photonegative when less than four days old but then become positively phototactic. He also found that Rhipicephalus sanguineus Latrielle was photonegative until six or seven days old after which it became positively phototactic (to moderate light intensities.) Wilkinson (1953) suspects that Krijgsman was, in fact, dealing with B. microplus and not B. annulatus. Wilkinson himself made some rather superficial observations on B. microplus larvae and his results indicate that this tick too is photo-positive and he also concludes that "the tendency to

remain in a group, once formed, is stronger than the stimulus of the light." Unfortunately he does not state how old the larvae were. Lees (1948a)states that Totze obtained a positive phototaxis for I. ricinus, but that McLeod could not confirm this. Lees himself, working with unfed I. ricinus nymphs, concluded that they were photonegative when newly moulted but that they became indifferent with age. Using I. persulcatus, Minorov is reported by Lees to have obtained a weak positive phototaxis under conditions of low light intensity and a negative phototaxis when the light intensity was higher.

Lees (1948a)found that the humidity behaviour of unfed I. ricinus nymphs depended on the conditions under which they had been reared. When reared under moist conditions, they tended to avoid the damp but when reared under dry conditions, the ticks lost this avoiding reaction and tended to settle in the moist air. Krijgsman (1937) found with B.annulatus larvae that, when reared in low humidities they were positively hygrotactic but that when reared under high humidities they were indifferent to humidity.

In the present series of experiments, no positive answer was obtained as to whether B. decoloratus reacted to humidity or not. It is thus still not clear why they were positively hygrotactic in the first series of experiments but indifferent in the later series, especially since no correlation could be found between their humidity behaviour and their water balance, age or the season during which the female had been collected.

Results of different workers on the reactions of various tick species to gravity also differ greatly. Lees (1948a)and MacLeod (as quoted by Lees) found that

I. ricinus nymphs were negatively geotactic and Minorov (as cited by Lees) found the same result with I. persulcatus. Krijgsman (1937) with B. annulatus and Wilkinson (1953) with B. microplus claim that larvae of these species are indifferent to gravity. It should be pointed out, however, that the results of Lees and Minorov were complicated by the presence of a "tip reaction".

The present work on B. decoloratus points to their being negatively geotactic when over seven days of age but indifferent when less than one week old. They also showed a strong reaction to the tip when on a glass rod. It appears that the tip reaction overrides the gravity reaction when on a straight rod but that, when on a curved rod, the gravity reaction is dominant and it is suggested that this might somehow be connected with the positioning of the forelegs. Whereas Krijgsman and Wilkinson conclude that light overrides gravity, the present work indicates the reverse. It must be noted here, however, that only weak illumination was used in the experiments with B. decoloratus.

In a linear temperature gradient, B. decoloratus larvae were found to behave in the same way as did the nymphs of I. ricinus (Lees 1948a) In both cases, ticks reared at about 25°C were found to move to the cool end of the gradient. This behaviour in B. decoloratus was found to result from an avoidance (taxis) of the hot end, combined with a slowing down and final settling in the cool end (kinesis). Krijgsman (1937), like Lees, concludes that the reactions to temperature depend on the rearing conditions but he concludes that B. annulatus larvae prefer the warm.

The investigation on the gravity behaviour of B. decoloratus suggested the occurrance of an activity rhythm and this is discussed later.

A study made of the clumping behaviour of blue tick larvae indicates that some chemical factor might be important in clump formation and that this substance is probably specific in its action. Wilkinson (1953) commented on the possibility of secretions playing some part in clump formation and suggests that in tick larvae the secretion of the Sensilla Sagittiformia "may permit recognition, leading to the clump-forming response observed in nature."

The suggested activity rhythm obtained in the gravity experiments was studied in more detail to attempt to understand whether it was exogenous or endogenous (Cloudsley-Thompson 1961). It should be mentioned here that despite the paper by Kleitman (1949) and Cloudsley-Thompson (1961) there still seems to be some disagreement about the exact definition of the terms "cycle", "rhythm" and "periodicity". For convenience sake, the term "rhythm" is used here in the broad sense as used by Cloudsley-Thompson.

The results obtained suggested an inherent (or endogenous) activity rhythm resulting in an increase in the number of animals active in the late afternoon. The rhythm appeared to be correlated with the light/dark cycle (which was more or less synchronised with day and night). This, however, is a common feature of such rhythms (Cloudsley-Thompson 1961). While no other known papers mention a rhythm of activity in the laboratory, Wilkinson (1953) mentions that in the field " a conspicuous feature of the observations" (on B. microplus) was the movement

usually at dusk or before dawn of the larval groups into more exposed positions, often terminal, on the grass stalks". He suggests that light intensity governs this movement but the present studies on B. decoloratus indicate that, possibly combined with the effect of light, an inherent activity might also be involved. Dr. K. Jooste (unpublished data) working on ticks associated with domestic cattle in Rhodesia obtained maximal collections of larvae in the field at about 4 - 5 p.m. a fact which tends to support the idea that larvae are most active at this time of day.

The host finding or "questing" behaviour of unfed B. decoloratus larvae was also studied and it was found that they reacted to various stimuli by "questing" with their forelegs. The findings of the various workers on different species of Boophilus are compared with those found in the present investigation in Table 27 below:

TABLE 27. COMPARISON OF THE STIMULI EVOKING "QUESTING" IN BOOPHILUS SPP. AS REPORTED BY VARIOUS WORKERS.

SPECIES STIMULUS	B.ANNULATUS (B.Microplus?) Knjgsman	B. MICROPLUS Wilkinson	B.DECOLORATUS Author
Vibrations	+	+	+
Warmth	+	-	+
Scent	+	+	+
Air currents		-	+
Shadows		+	+
Moisture	+	-	-

The reactions of B. decoloratus to vibrations and air currents were studied in detail and it was found that the larvae became adapted to repeated vibrations of the same frequency and there are indications that they can distinguish between vibrations

of different frequencies. They also reacted much more strongly to substrate-borne vibrations than to air-borne ones. However, owing to the crudity of the apparatus only generalisations can be made. Observations on their reactions to air currents showed that they quested to air currents but, if the current was of constant velocity, they became adapted to it, ceased questing and became inactive. When the air current was strong, they took cover or avoided it.

The point which now arises is: how do these reactions help the animal in the field?

It appears that when the larvae hatch, they remain at the base of the vegetation where it is dark due to a negative phototaxis combined with a strong tendency to clump and an indifference to gravity. After about seven to ten days, the tendency to clump weakens somewhat and this, combined with a strong positive phototaxis and a negative geotaxis, causes the clump to break up and the larvae to move to the tips of the vegetation. The mechanism whereby they react to gravity seems to be a kinesis resulting in their continually turning to the top of their support and a taxis or "tip reaction".

Once at the tip (and/or) top of their support, the larvae are warned of the approach and proximity of potential hosts by vibrations, sudden shading, warmth, scent and air-currents. Connected with the finding that the larvae quest to sudden shading is the finding described in the light section that ticks moving downwards on their support will change direction and move to the top with a fall in light intensity. Obviously, some of these stimuli could be caused by agencies other than a host and thus,

to avoid needless activity and consequent waste of their limited food reserves, the larvae show adaptation to repeated stimuli (e.g. vibrations and air-currents) Wilkinson (1953) doubts that surface-borne vibrations play an important part in forewarning larvae of the approach of a potential host but the strong reactions obtained in the present work suggest that it might quite likely play such a role - as has, in fact, been suggested by Kuijgsman (1937). Carthy (1958) too, mentions the possible importance of surface-borne vibrations to animals.

It was found that, when the velocity of the air currents increased and became too strong, the larvae tended to move to a sheltered spot, e.g. the protected side of the support, an important factor under windy conditions in the field. Some larvae in the laboratory were, in fact, blown from the support - an observation which suggests that this method might play some part in the horizontal distribution of tick larvae by wind. Wind is well known to be an important means of dispersal among Tetranychus spp. - the red spider mite. (Fleschner et al, 1956).

While on their support waiting for a host, the larvae appear to have two resting states - one in which they are reactive and one in which they are not so reactive immediately. It is interesting to speculate whether perhaps this unreactive state helps in the conservation of food reserves and is perhaps tied up with the activity rhythm, ie. two interesting questions arise: (a) Do the larvae show a rhythm of these two resting states tied up with the activity rhythm?

(b) Does this increase in activity in the late afternoon have any importance in host-finding and survival? i.e. does their being in the unreactive state help conserve food reserves by cutting down activity and yet permit their becoming active in the late afternoon (when it is cool and hosts are actively feeding) and thus permit them to be in a state of readiness to get a host. Wilkinson also suggests that the rhythm in B.microplus might help in host-finding and in addition suggests its importance in obtaining water from the atmosphere and from pools of dew.

Admittedly this is mere speculation but when their chances of getting a host are considered and when one considers the short amount of time available to the larvae when a potential host passes, combined with the very real necessity to conserve food reserves, one must realise the value such behaviour would bestow.

On one point the laboratory results differ from the field results on B. microplus by Wilkinson. This is in the fact that Wilkinson obtained two activity peaks whereas only one significant peak was obtained with B. decoloratus.

The attraction of larvae to a clump, perhaps by some chemical substance, is of importance as it would result in clumps composed of the same species of tick forming at the tips of the vegetation. This would mean that there would be a much better chance that males and females of the same species would get onto a particular host. In cases where mating occurs on the host (as it does in the Ixodidae) this is obviously of great importance.

Lees (1948a)states that if I. ricinus nymphs were reared in warm conditions and were then tested, they went to the cool end of the temperature gradient apparatus. He found, however, that if the ticks were reared under normal field conditions (i.e. exposed to lower temperatures) they did not tend to go to the cool end so readily. He suggests that the reason for this is that the "cold-adapted" ticks are not trapped so easily at the cold end by "cold coma". These results can perhaps be reviewed in another way. If ticks were to hatch in summer, when temperatures were high, this type of behaviour would result in their moving to cool areas. However, if they were to hatch in winter, they would to some extent sit in the warmer areas. Both patterns of behaviour would be of value

X SUMMARY

The behaviour of unfed larvae of Boophilus decoloratus (Koch) was studied in the laboratory. Experiments were carried out to discover their reactions to light, humidity, gravity and temperature. In addition, studies were made on their clumping behaviour, their activity through each 24 hour period and on their "questing behaviour".

(a) Light. It appeared that larvae less than one week old were negatively phototactic while those over one week old were positively phototactic. The mechanism keeping the young larvae in the dark was found to comprise an avoidance of light (taxis) combined with an activity orthokinesis leading to settling and clumping in the dark. The older larvae are attracted to the light (a mechanism involving a telotaxis) and avoid the dark but no speed or activity orthokinesis appears to be involved. The larvae did not seem able to distinguish between red light and dark.

(b) Humidity. At first a positive hygrotaxis was obtained but thereafter no significant hygrotaxis was observed. All efforts to find out why such a change in behaviour had occurred, failed.

(c) Gravity. Larvae less than one week old were found to be unreactive to gravity while larvae more than one week old were negatively geotactic. The gravity reaction consists of a kinesis resulting in their repeated turning to the top of the support and a tip reaction to the tip of the support. When in opposition the tip reaction overrides the gravity reaction except on a curved rod when the gravity reaction is dominant. It is thought that this apparent contradiction in results might somehow be connected with the animals' forelegs being free. The gravity reaction was found

to override the light reaction.

(d) Temperature. Larvae reared at 25°C always went to the cool end when placed in a linear temperature gradient. The mechanism involved an avoidance of high temperatures (taxis) combined with a speed and activity orthokinesis, i.e. they move more slowly and settle sooner in the lower temperatures.

(e) Clumping. Larvae, especially those less than one week old, showed a marked tendency to clump. It is suspected that some chemical stimulus is involved and there are indications that this "clumping substance" is specific.

(f) Host finding behaviour. B. decoloratus larvae react strongly to vibrations, scent, warmth, shading and air currents. It was found, however, that they did not react to moisture. They showed a tendency to adapt themselves to repeated stimuli (e.g. vibrations and air-currents). They were found to react much more strongly to substrate-borne vibrations than to air-borne ones. Investigations on the sensitivity of the larvae to vibrations of different frequencies showed that they were more sensitive to vibrations in the 250 - 1000 cps. region. Studies n their reaction to air currents indicated that the larvae would move to a protected area when these currents became too strong.

(g) Activity rhythm. An increase in activity was observed at about 4 - 6 p.m. each day. This activity rhythm was clearly marked in a constant temperature room in which light and dark were more or less synchronised with day and night. This rhythm was also observed under constant light although it tended to be more drawn out. The increase in activity often caused a partial or complete break-up of clumps.

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