

The Co-ordination of Growth in the Tracheal System of Insects

By M. LOCKE

(From the Department of Zoology, Downing Street, Cambridge, and the Department of Zoology, University College of the West Indies, Jamaica)

With one plate (fig. 8)

SUMMARY

The tracheae in *Rhodnius* and many other insects are parallel-sided tubes which branch in such a way that the cross-section of the diffusion path remains constant. Growth in length results mainly from stretching the trachea in the preceding instar. Growth in diameter of the tracheae varies with feeding and the organ supplied. The proportions of the tracheal system are maintained by co-ordination of the growth of the main tracheae and new terminal branches. This is brought about by the property of tracheae which enables them to react to stimuli for growth only upon the spiracle side of the point of application. The stimuli for growth probably originate from the tissues and from the nodes. They determine first the diameter of the tracheae farthest away; that is, the tracheae begin to form at the spiracles. The nodes controlling the diameter of the lateral tracheae are influenced by the blood.

Growth in the tracheal system of *Tenebrio* larvae varies inversely with the oxygen tension both above and below atmospheric.

INTRODUCTION AND PRELIMINARY OBSERVATIONS

THE tracheal system in an insect consists of extensible cuticular tubes, the tracheae, arising from segmentally arranged spiracles. The tracheae branch repeatedly with reduction in diameter and end in the tracheoles, fine intracellular tubes penetrating the tissues of the body. The segmental tracheae may be united by lateral tracheae running in the length of the animal. The pattern of branching in the abdominal tergites of *Rhodnius* is shown in fig. 1. In large insects, particularly those with respiratory movements, the tracheae may be spindle-shaped sacs, oval in cross-section, but in small insects and in terminal ramifications the tracheae are parallel-sided tubes, circular in cross-section. This enabled Krogh (1920) to calculate the cross-sectional area from measurements of the diameter of tracheae at successive levels of branching from the lateral tracheae to the tissues in a *Tenebrio* larva. He found that the sum of the areas of the cross-sections of the branches was equal to the cross-sectional area of the main trachea. Thorpe and Crisp (1947) found the same relationship for three tracheae and their branches in adult *Aphelocheirus*, and assumed it to be true for their calculations on the respiratory efficiency of the rest of the animal. It is remarkable that this simple description of branching in what is otherwise a most complex system should have received so little attention.

The diameters of the tributary tracheae and the main branch were measured

in a number of forks in whole mounts of several insects. The cross-sectional area of a trachea was found to equal that of its branches in all the tubes of circular cross-section examined. Table 1 gives some of these results.

There are exceptions to this simple tree-like branching pattern as in the four-way junctions between the lateral and segmental tracheae, or the plexuses

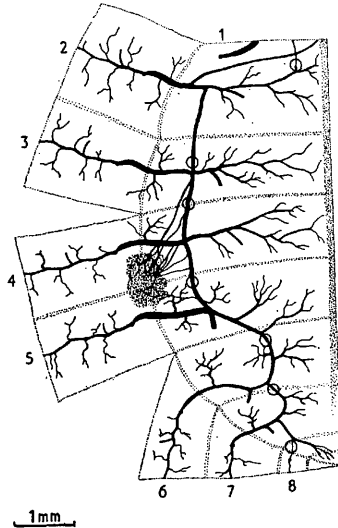


FIG. 1. Diagram of the tracheal system of one side of the abdomen in a 5th instar *Rhodnius* larva, as it usually appears in the preparations described in the text. The larva has been dissected from the ventral surface and the tracheae to the viscera have been cut short. The stippled mass indicates the position of a testis. The tracheae to the tergites are separated on the two sides by the heart but the ventral tracheae unite in nodes. The usual position of the nodes on the lateral tracheae are indicated by small circles. The numbers refer to the appropriate abdominal spiracle.

of tracheae occurring in some insects (e.g. *Dixippus*), but it seems probable that the relation between the branches described above is a general one. Teleologically this is not unexpected if it is assumed that the distance from tissue to spiracle is more or less constant along any one branching system. The rate of diffusion along a tube is proportional to its cross-section. If the total cross-section of the branches grew greater as they divided the cross-section of the main trunk would still remain limiting. If the converse were true the terminal branches would limit diffusion. The latter arrangement might be expected if much oxygen had been lost through the main trunk walls before reaching the tissues.

Tracheal branching in insects may be compared with that in a chilopod, *Lithobius*, in which the cross-section diminishes significantly as branching proceeds (the sum of the cross-sectional areas of the branches divided by the cross-sectional area of the main trunk = 0.85, S.D. 0.13). This branching pattern may be correlated with a respiratory function for the blood. The main tracheae seem more permeable to the red compound of cobalt with di-nitroso-resorcinol than in insects when similar tracheal injection preparations are made of both types of arthropod.

TABLE I

Material	Number of forks measured	Sum of the cross-sectional areas of the branches divided by the cross-sectional area of the main trachea	S.D.
Tergal tracheae of 3rd instar <i>Rhodnius</i>	25	1.00	0.16
" " 4th " "	25	1.00	0.17
" " 5th " "	24	0.97	0.15
Abdominal tracheae of <i>Ephestia</i> larvae	31	1.02	0.18
Tracheae from last instar <i>Calliphora</i> larvae	31	1.00	0.13
Tracheae from the nymphal wing pads of <i>Periplaneta</i>	28	0.99	0.10

The structure and formation of the tracheal cuticle in *Rhodnius* have been described by Locke (1957, 1958). Growth and moulting of the tracheal system in *Rhodnius* have been studied by Wigglesworth (1954). He found that new tracheae and tracheoles were added terminally to the tergal tracheae at each moult. If the increase in cross-section of the main trachea were equal to the total cross-section of the new terminal tracheae, a system branching in the way described above would result. Wigglesworth also described the great plasticity of the tracheal system, which responded by increased growth at moulting to the stimuli of low oxygen tension or the implantation of organs with a high oxygen consumption. Thus it is unlikely that the dimensions and pattern of branching are predetermined at an early stage in development. Now it is not known if this artificially-stimulated terminal growth results in a system branching normally as described above, or whether the effect is a local one only. If the branching pattern after artificially-stimulated growth is a normal one, then terminal growth must be co-ordinated with that of the main trachea in any one branching system.

The problem, then, is to account for the maintenance of the branching pattern in the system as a whole when terminal growth may vary under different conditions, and to relate this to the stimulus to growth given by low oxygen tensions. Experiments of two kinds have yielded information: (1) interference with normal growth by surgical operations upon tracheae, and (2) interference with normal growth by alterations in the external oxygen tension.

MATERIAL AND METHODS

At 25° C 4th instar larvae of *R. prolixus* Stål moult 14–15 days after feeding. Operations were performed upon the tracheae of newly-fed 4th instar larvae and the effect observed upon the 5th instar tracheae. The distended integument is thin and transparent after feeding, with few muscles to hide the stretched tracheae. A fine glass hook was inserted through the integument with a quick flick to cut the tracheae, leaving a barely visible wound in the integument. The tracheae are under tension and the cut ends spring away from the wound of entry. Preparations made 14 days after feeding show two sets of tracheae, the old 4th instar tracheae being still in place within the new system formed around them (see fig. 8, A, opposite p. 383) (Locke, 1958b). The usual procedure was to dissect under Ringer and to fix and stain before removing the insect from the dissecting dish for the preparation of a whole mount. Aqueous Bouin gave satisfactory fixation. In this way it was possible to compare the increase in diameter of different tracheae fairly accurately.

In some of these preparations the results may have been influenced by the failure of a cut trachea to separate completely from the wounded integument. To check this, operations were performed upon 3rd instar larvae and the 5th instar tracheae observed as before. The wound should have healed completely by the 4th instar. The variations in growth after two moults were often more striking and gave no cause to doubt the conclusions derived from earlier experiments.

Some of the experiments upon tracheal growth in *Rhodnius* were repeated upon the larva of *Calpodex ethlius* Stoll (Lepidoptera, Hesperidae), which deserves to be better known as an experimental insect. It occurs widely in the Caribbean, Central America, and the southern states of North America. The larvae are readily reared upon *Canna*, taking about 10 days to pass through 5 instars. The tracheae and all the body organs can be seen clearly through the thin colourless integument. The tracheae are not under tension as in fed *Rhodnius*, but they break fairly easily when pulled by a glass hook inserted through the integument. There are more body-wall muscles than in *Rhodnius*, so that any operation tends to result in some wounding. To reduce the complications of wounding upon tracheal growth, operations were performed upon 2nd instar larvae (they are then about 1 cm long by 0.12 cm in diameter) and the result observed in the 5th instar (about 8 cm long by 1 cm in diameter).

Neither *Rhodnius* nor *Calpodex* proved very suitable insects for investigating the extra-tracheal growth which results when the environmental oxygen tension is lowered, but the hypertrophy is readily stimulated in the larvae of *Tenebrio molitor* L. *Tenebrio* larvae were reared within a closed system of various circulating gas mixtures, the composition being determined with a gas analyser (Roughton and Scholander, 1943). Changes in the tracheal system were most easily detected after injecting cobalt naphthenate and developing the red compound with di-nitroso-resorcinol (Wigglesworth, 1954).

RESULTS

The role of oxygen tension

The experiments of Wigglesworth (1954), in which he observed increased tracheation in *Rhodnius* reared at reduced oxygen tensions, suggested that the oxygen tension within the system might play a part in determining tracheal growth. The hypertrophy was small compared with the normal variation in growth discussed under the next heading, even in oxygen tensions only just enough for survival (5%, 7.5%), but this did not exclude the possibility that even lower oxygen tensions inducing growth could be tolerated locally.

The lateral tracheae of a newly-fed 4th instar *Rhodnius* larva were cut upon each side of the right tergal trachea in abdominal segment three, together with the ventral trachea uniting it with the other side. The spiracle opening upon this now isolated system was occluded with soft wax. The effect of the oxygen shortage upon this section of trachea was observed at the next moult. The result varied in different preparations: in some the terminations had atrophied slightly and the main trachea showed signs of wounding, others appeared more normal, but none showed an increase in diameter greater than tracheae upon the control side of the animal.

Different combinations of spiracles were occluded in newly-fed 4th instar larvae to produce a varying degree of oxygen shortage. No effect could be detected upon the growth of the 5th instar tracheal system comparable to the normal variation discussed below. From these results it seemed unlikely that oxygen tension could act directly upon the tracheae to induce growth.

A varied oxygen tension had much more effect upon tracheal growth in *Calpodes* and *Tenebrio*. All the spiracles upon one side of some newly-moulted *Tenebrio* larvae were occluded with wax before they were allowed to feed and moult again. The tracheae upon the occluded side always showed much extra growth. In extreme cases the terminations became profusely tufted with fine tracheae and tracheoles with a corresponding increase in diameter of the main tracheae. Similar experiments were performed upon *Calpodes*. The results were not quite so obvious but occlusion always resulted in some hypertrophy.

A similar hypertrophy was observed in *Tenebrio* larvae reared in reduced oxygen tensions. Small larvae weighing 15–30 mg were kept in 10% oxygen in nitrogen at normal pressures for up to 40 days. Even after one moult extra tracheation could be seen in some larvae, and marked changes had occurred after three moults. The greatest hypertrophy occurred in the tracheae supplying the gut and muscles. The number and length of the finer tracheae increased and the main trunks were 2–3 times their normal diameter. A similar hypertrophy was obtained in larvae reared in air in large desiccators at half an atmosphere total pressure.

Tenebrio larvae were also reared in 50% oxygen in nitrogen. After three moults there had been a negligible growth of new tracheae and tracheoles. The tracheae had grown in length only and appeared very slender compared

with normal tracheae. Thus the metabolic activity concerned with determining tracheal growth is sensitive to oxygen at both high and low concentrations.

The ease with which changes in the tracheal system may be induced in *Tenebrio* and *Calpodes* may be correlated with the greater activity and muscle development and higher oxygen consumption than in *Rhodnius* (a *Tenebrio* larva weighing about 100 mg consumes 50–200 mm³ of oxygen per hour, while a resting *Rhodnius* larva weighing about 20 mg consumes only 1 to 3 mm³ per hour). If the tissues are responsible for stimulating tracheal growth, then the greater volume of respiring organs in *Tenebrio* and *Calpodes* might be expected to result in readily-stimulated tracheal hypertrophy.

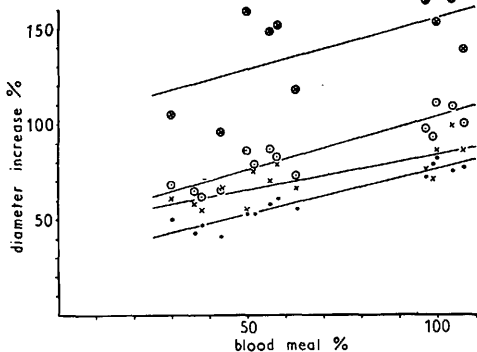


FIG. 2. The effect of feeding upon tracheal growth in *Rhodnius*. Ordinate: increase in diameter at the 4th to 5th moult. Abscissa: size of the blood meal as a percentage of the maximum possible meal.

- ⊗ tracheae to the testis.
- " " " gut.
- x main tergal tracheae in which the tracheae from the gut and tergites unite.
- tracheae to the tergites.

The relative stability of growth in *Rhodnius* tracheae suggested that they would be most suitable for investigating the co-ordination of growth between the tissues and the large tracheae.

The normal variation in growth

4th instar *Rhodnius* larvae were given blood meals of known, controlled size. The larvae were weighed, allowed to feed, and reweighed immediately they were detached from the host. The size of the blood meal as a percentage of the maximum possible feed was calculated from the initial linear dimensions of the larva, and its gain in weight compared with a similar standard set of measurements for gorged larvae. The diameters of the new 5th instar tracheae were compared with the old 4th instar tracheae just before moulting. The size of the blood meal as a percentage of the maximum possible feed is

used as the abscissa in fig. 2 plotted against the increase in diameter of the main tracheae supplying various organs.

There is considerable variation in growth both with the size of the blood meal and the organ supplied. This can be correlated with the formation of new terminal tracheae and tracheoles. There is a great growth of fine tracheae and tracheoles in the testis at this moult which is reflected in the increase in diameter of the main testis tracheae. Similarly the formation of new tracheoles on the tergal tracheae varies with the nutritional state. At the lower extreme,

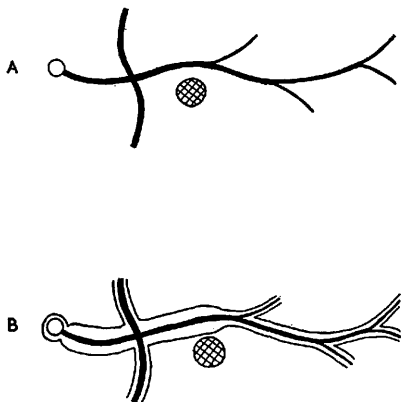


FIG. 3. Diagram to show the effect of implanting an organ with a high oxygen consumption below a tergite of a newly-fed 4th instar *Rhodnius* larva upon the increase in diameter of the 5th instar trachea. A, the tracheal supply in the 4th instar, showing the position of the implanted organ. B, the diameter of the new 5th instar trachea is indicated by a thin line round the old trachea. For clarity the new fine tracheae and tracheoles have not been represented.

when unfed larvae are induced to moult by grafting on to fed larvae, there is scarcely any growth of new tracheae and tracheoles and the existing tracheae only increase in diameter by about 35%. *Tenebrio* larvae moulting during starvation behave in the same way: no new tracheoles are formed and the existing tracheae increase in diameter by a smaller amount than normal. Presumably tracheal growth anticipates respiratory demand. Thus the proportions of the tracheal system are not maintained by uniform addition to the embryonic pattern; growth in a trachea can be related both to growth in the organ supplied and to the trachea which it joins on the way to the spiracle. For example, the tracheae to the gut increase in diameter more than the tergal tracheae, while the main trachea in which they unite increases intermediately.

These observations suggest that the dimensions of the tracheal system are

determined by the growth of the ultimate branches and they in turn by the tissues.

The branching pattern after artificially-stimulated terminal growth

Normal growth in the tracheal system is co-ordinated so that the cross-section of the diffusion path remains constant. It was of interest to see if artificially-stimulated terminal growth also induced the appropriate growth in the remainder of the system. Growth was stimulated on the 3rd abdominal tergite of one side by inserting a 4th instar ovary, testis, or corpus allatum in a newly-fed 4th instar larva after the manner of Wigglesworth (1954). At the next moult new 5th instar tracheae had formed to supply the implant. The increase in diameter of the main trachea over that upon the control side was detectable all the way back to the spiracle. Thus the proportions of the tracheal system as a whole are adjusted in response to new terminal growth. When the implant was inserted nearer to the spiracle only the region between it and the spiracle hypertrophied, although the terminal branches should have had a lower oxygen tension. This shows that oxygen tension has little direct effect upon the tracheae (fig. 3).

Tracheal extension and growth

The increase in diameter at moulting in many of the tracheae in *Rhodnius* is proportional to the size of the blood meal. After a large meal the tracheae are greatly stretched and conversely they are but little extended when the meal is small. From Wigglesworth's work (1937, 1940) on the multiplication of epidermal cells, it seemed possible that the growth of new cuticle might vary with the stretching of the trachea and the distance apart of the cells before moulting.

Fourth instar larvae were painted with celamel cement on the edge of the abdominal tergites of one side before feeding. Other larvae were painted in the same way when fully fed and shining with distension. In this way the lateral tracheae were kept in a permanently distorted position while those on the other (control) side were in their natural situation. The effect of this varied extension (0–50%) was observed on the growth of the 5th instar tracheae at the next moult. The increase in diameter bore no relation to the degree of extension.

Tracheae were also extended in another way. The lateral tracheae between spiracles 2–3 and 4–5 were cut and the ends drawn through the cuticle, stretching the lateral trachea between them by as much as 100% over the control side (fig. 4). No extra increase in diameter compared with the control side was detectable.

Thus, although extension may determine the future length of a trachea, the diameter is controlled in some other way.

The control of growth by the tissues

The simplest hypothesis to account for the co-ordination of tracheal growth,

such that the increase in cross-section of the main trachea is equal to the increase in cross-section of the terminal tracheae, is that tracheal growth is determined by the tissues.

To test this the tergal tracheae were cut in a segment upon one side of the abdomen in newly-fed 4th instar larvae. The two ends developed differently. At moulting the new trachea round the end with tissue-connexions increased normally or supernormally in diameter with a rounded end (figs. 5; 8, D, H).

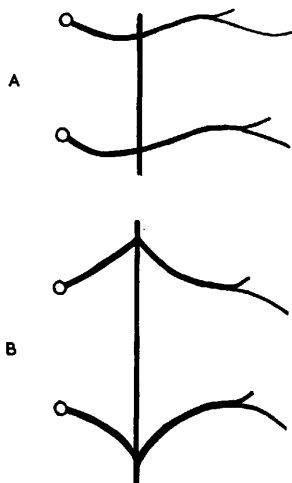


FIG. 4. Diagram showing the arrangement of the lateral trachea between abdominal spiracles 3 and 4 in a 4th instar *Rhodnius* larva. A, the natural position. B, the lateral trachea has been stretched by pulling the cut ends through epidermal wounds in neighbouring segments.

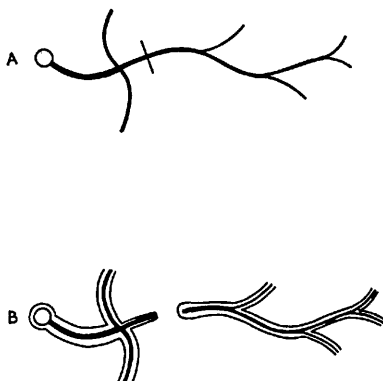


FIG. 5. Diagram to show the effect of cutting a tergal trachea in a 4th instar *Rhodnius* larva upon the growth of the new 5th instar tracheae. A, the tergal trachea in the 4th instar with the position of the cut indicated by a cross-line. B, the growth of the 5th instar trachea is represented by the new enveloping line. Compare with fig. 8, D, H.

The end connected to the spiracle showed the smallest diameter-increase compatible with being formed round the old trachea, with a truncated end. The differences in growth cannot be attributed to wounding since they have had identical treatments. The two ends may have differed in the oxygen tension available to the tracheal cells and the tissues which they supplied, but when a branch of the isolated tergal trachea was also cut, it too failed to increase in diameter (fig. 6). The tergal trachea was also cut between the lateral trachea and the spiracle. Here the spiracle end tended to fail, while the length attached to the lateral trachea showed an increase in diameter, although both should have had an adequate oxygen supply (figs. 7; 8, B).

It has been shown that hypertrophy of the terminal tracheae results in an

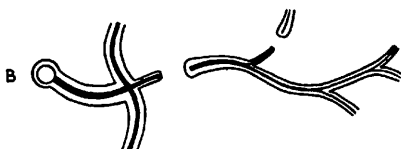


FIG. 6. Diagram summarizing the effect of cutting a tergal trachea in a 4th instar *Rhodnius* larva upon the growth of the new 5th instar tracheae. A, the tergal tracheae in the 4th instar with the position of the cuts indicated by cross-lines. B, the growth of the 5th instar trachea is represented by the new enveloping line.

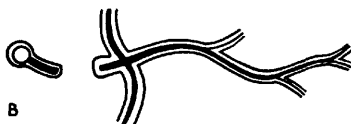


FIG. 7. Diagram summarizing the effect of cutting a tergal trachea in a 4th instar *Rhodnius* larva upon the growth of the new 5th instar tracheae. A, the tergal trachea in the 4th instar with the position of the cut indicated by a cross-line. B, the growth of the 5th instar trachea is represented by the new enveloping lines.

appropriate increase in diameter all the way to the spiracle. The converse is also true. The failure of a trachea cut without tissue-connexions induces a partial failure in the main branch, which it joins.

Tergal tracheae were also cut in 2nd instar *Calpodex* larvae. In the 5th instar the loose end leading to the spiracle had degenerated to a small conical



FIG. 8
M. LOCKE

appendage. The trachea isolated in the tissues did not grow normally either. There was a resemblance to the atrophied tracheae in the abdomen of *Rhodnius* after severe oxygen-lack due to occlusion of all the abdominal spiracles. Lack of oxygen might be expected to be more severe in the muscles of the body wall in *Calpodes*.

These experiments are consistent with growth being determined from the tracheal endings or the tissues.

The nodes and the control of growth on the lateral tracheae

The lateral tracheae are exceptions to the normal branching rule in that they link up different spiracles instead of taking part in the segmental diffusion path from spiracle to tissue (fig. 1, p. 374). It is therefore of particular interest to study the factors controlling their growth.

At each moult the remains of the old tracheae are drawn through the spiracles and shed with the exuvium. To allow this, the lateral tracheae break at the nodes, which are predetermined weak points having a characteristic structure. The cuticle at the nodes is rucked up in an irregular fashion, interrupting the regular array of helical or annular taenidia. The effects of cutting the lateral tracheae varied with the position of the cuts relative to the node. The lateral tracheae between spiracles 2 and 3 proved most useful for these experiments because they lacked tributaries. The new trachea on the cut end containing the node increased in diameter normally or almost normally in both directions from the node. The end without the node failed to increase in diameter by the amount expected from measurements upon the control

FIG. 8 (plate). Photomicrographs of tracheae from whole mounts of *Rhodnius* larvae. All have been fixed in aqueous Bouin and stained in Hansen's trioxyhaematein. A, B, C, D, G, and H are 4th instar larvae just before moulting to the 5th instar. E and F are from 5th instar larvae. A, a normal lateral trachea showing the maximum increase in diameter after a large blood meal.

B, trachea from the lateral trachea to the 3rd abdominal spiracle. The trachea was cut between the spiracle and the lateral trachea, together with the lateral trachea between spiracles 2 and 3, immediately after feeding. The large trachea, which has increased in diameter, is the branch isolated from the spiracle. The smaller cut end, which has failed to grow normally, is the lateral trachea isolated without a node. Compare with figs. 7 and 9.

C, the lateral trachea between abdominal spiracles 2 and 3. The trachea was cut immediately after feeding. The trachea has increased in diameter on both sides of the node. The nodes are marked with arrows. The old trachea has been displaced slightly away from the cut end. Compare with fig. 9.

D, trachea to the 3rd abdominal tergite. The trachea was cut immediately after feeding, leaving tissue-connexions but no outlet to a spiracle. The new trachea has increased in diameter round the cut end. Compare with fig. 5.

E, the lateral trachea between abdominal spiracles 2 and 3. The trachea was cut in the 3rd instar, isolating the end shown without a node. Compare its diameter with the trachea in F.

F, the same preparation as E, showing the cut end isolated with a node. The node is close to the junction with the tergal trachea and the 3rd and 4th instar cut ends have broken there and remain in the stump, marked by arrows.

G, the same preparation as C, but showing the cut end without a node, which has failed to increase normally in diameter. Compare with fig. 9.

H, the same preparation as D but showing the cut end without tissue-connexions but with an outlet to the spiracle. The cut end has increased negligibly in diameter. Compare with fig. 5.

side (figs. 9; 8, C, G, E, F). There can be no possibility of this growth being proportional to oxygen tension since both ends have a spiracular connexion. When the cut was made through the node, damaging it, both ends tended to fail. When the lateral tracheae had tributary tracheae as in segments 3-4 and 5-6, even a nodeless section increased in diameter, provided that it had a small tributary. It was difficult to establish any quantitative relationship; even a

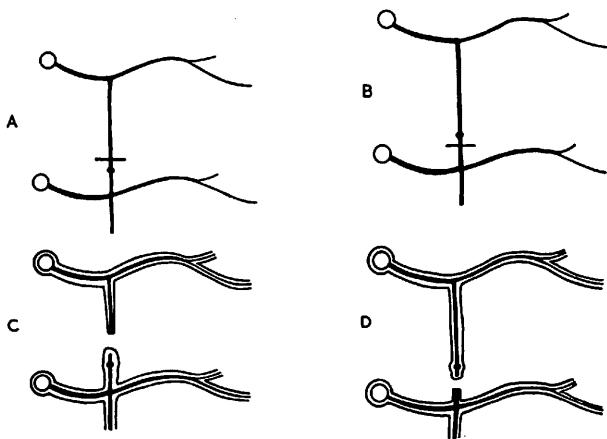


FIG. 9. Diagram showing how cutting the lateral trachea in 4th instar *Rhodnius* affects the growth of the new 5th instar trachea differently according to the position of the cut relative to the node. A, B, the lateral tracheae between abdominal spiracles 2 and 3 with the position of the cuts marked by cross-lines. The nodes are represented by a thickening. C, D, the growth of the 5th instar tracheae is represented by the new enveloping line.

small tributary was enough to allow some growth between it and the junction with the tergal trachea. These results suggest that the diameter of a lateral trachea is determined from the node in the same way that the diameter of a tergal trachea is determined from the tissue-endings.

This interpretation of the part played by the node is also suggested by the marked changes in tracheal diameter which may occur on each side of it. The lateral tracheae pass forward from the abdomen to the thorax (fig. 1). There is an abrupt change in diameter at a node between abdominal segments 1 and 2, the anterior trachea being excessively slender. This could result from differential growth controlled through the node.

In the abdomen of *Rhodnius* the spiracles upon each side are connected by a ventral trachea with a node in the midline. The ventral tracheae lie between longitudinal segmental muscles and the epidermis, and after cutting they do not separate easily from the wound of entry. The results are therefore more difficult to interpret and less reliable than those from the lateral tracheae. To

allow for the wounding the operations were performed upon 3rd instar larvae and the tracheae observed in the 5th instar. Cuts were made upon one side of the node. The loose ends in the 5th instar atrophied when they were without a node.

This experiment sometimes occurs naturally. The ventral tracheae on the first abdominal segment often remain in two unattached halves, perhaps because there was a break in an earlier instar but more probably as a result of the failure of the embryonic tracheal cells to unite in the midline. When this happens there is no trace of an irregularly buckled cuticle at the tip, but the tracheae continue to grow normally at each moult. Thus the irregularly buckled cuticle is only a secondary effect of the union of tracheal cells.

The larvae of *Calpodes* were not entirely satisfactory for confirming these results. Cut tracheae showed far more plasticity in their response, perhaps because of the proximity of tissues with a high respiratory demand. Many cut tracheae failed to separate from the wounded region and after 2 or 3 moults new terminal tracheae and tracheoles had arisen. When the cut section of lateral trachea was short and without a node, and when it did not redevelop tissue-connexions, its growth was very limited. In the longer ends with or without nodes there was often at least normal growth, but this could be attributed to the new tracheae and tracheoles which almost always redeveloped.

Wounding and tracheal growth

Many of the early experiments gave inconsistent results which it was believed might be due to wounding. The effects of wounding upon the lateral tracheae were observed in *Rhodnius*. The node upon the lateral trachea between spiracles 2 and 3 usually lies very close to the more posterior spiracle, so that a cut soon after feeding isolated a long anterior end which later showed little increase in diameter. When this loose end was attached to the wounded epidermis by pulling the tip through the cuticle, the new trachea increased in diameter. This was not due merely to the attachment to the epidermis. When the operation was performed on 3rd instar larvae the trachea showed little increase in diameter at the 4th to 5th moult although the 4th instar trachea was attached to the cuticle. The wounded region must have provided something normally produced by the node. When a cut trachea complete with node was attached to the wounded epidermis, the new trachea increased in diameter rather more than normally. The wound accentuated the normal effect of the node alone. Epidermal wounds are regions of great cellular activity with mitoses and cuticle secretion which normally only occur at moulting. It is therefore not surprising that wounding should stimulate tracheal growth. The variation in growth of isolated cut ends may be attributable to differences in the injury to the tracheal cells which must arise during an operation.

The polarity of the tracheal system with respect to the control of growth

It has been shown that large tracheae only increase normally in diameter when they are connected with an organizing centre—a node, an epidermal

wound, or tracheal terminations in the tissues. This control must involve transport of the stimulus along the intervening tracheae.

When the lateral trachea between abdominal spiracles 2 and 3 is cut soon after feeding, the long anterior length lacks a node and fails to increase normally in diameter. The trachea itself is capable of growing without a node since it does so when attached to a wound at its tip. It is not obvious why it should fail, since it is attached at its other end to the tergal trachea and tissue-connexions which grow normally. If the stimulus for growth in diameter moves from the tracheal endings to the spiracles by any process similar to diffusion it should be possible to alter its course to pass along the lateral trachea by severing its connexion with the spiracle.

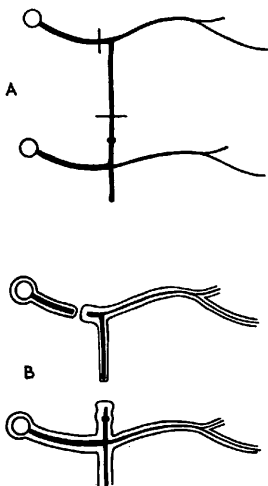


FIG. 10. Diagram of the cuts and growth of *Rhodnius* tracheae described in the text. A, the 4th instar lateral trachea between abdominal spiracles 2 and 3 with the position of the cuts indicated by cross lines. B, the growth of the new 5th instar trachea is represented by the enveloping line.

tracheal system. The experiment described above suggests that movement of the stimulus causing increase in diameter can only take place in one direction. This is not due to a simple concentration gradient but is an intrinsic property of the trachea.

The time at which tracheal growth is determined

Most tracheae are almost cylindrical between branches but there may be a slight tendency to taper towards a node. This becomes more pronounced after most operations. When a cut lateral trachea is without a node and is relatively short the effect is least noticeable. It is most obvious in tracheae attached to wounds. The trachea tapers towards the wound when no node is

The anterior section of the lateral trachea between abdominal spiracles 2 and 3 was isolated without a node soon after feeding. At the same time the connexion with the spiracle was cut as close to the junction with the tergal and lateral tracheae as possible. This did not cause complete isolation from the rest of the tracheal system, for the tergal trachea connects by a slender branch to a thoracic spiracle. The lateral trachea still failed to increase in diameter (fig. 10).

It might have been expected that the 'strength' of the stimulus for increase in diameter from the tergal tracheal endings could induce a similar change to that brought about by a small wound, but there is a difference in the point of application of the stimulus. This leads to a concept of polarity of growth in the

present and away from it when there is a node; that is to say, the direction of taper agrees with the direction of polarity mentioned above.

Each stage of growth in the tracheal system begins at the spiracles and moves inwards towards the tissues. When the new cuticle is expanding, the lateral tracheae are about half a day behind the spiracles, and the tracheal terminations are about one day further in arrears. Presumably the time at which the future diameter is fully determined follows the same sequence. A wound stimulus to tracheal growth may also have to follow this timing.

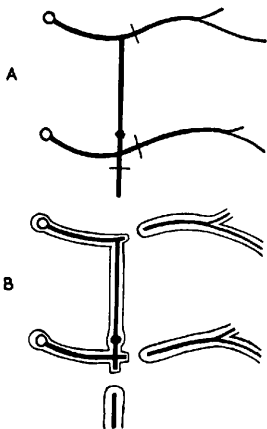


FIG. 11. Diagram of the cuts and resulting growth in *Rhodnius* tracheae described in the text. A, the 4th instar lateral trachea between abdominal spiracles 2 and 3; the positions of the cuts are marked by cross-lines. B, the growth of the new 5th instar trachea is represented by the enveloping lines. Growth of the lateral trachea is unaffected by isolation from tissue-connexions.

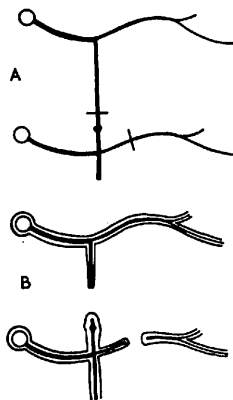


FIG. 12. Diagram of the cuts and resulting growth of *Rhodnius* tracheae described in the text. A, the 4th instar lateral trachea between abdominal spiracles 2 and 3; the positions of the cuts are marked by cross-lines and the node by a swelling. B, the growth of the new 5th instar trachea is represented by the enveloping lines.

A wound acting at the tip of a cut lateral trachea without a node might then be expected to determine the tracheal diameter farthest from the wound first, at a time when the wound is most active, and the trachea closest to the wound last, when the wound reaction has almost ceased. Similarly with the polarity reversed, only the tip next to the wound would hypertrophy.

As a result of the polarity—the property by which tracheae respond to a stimulus to growth only upon the spiracle side of the stimulus—it has been postulated that tracheal growth is controlled from the nodes and tissues. It is in accord with this hypothesis that growth should be completed first farthest from the source of control.

The control of the nodes by a blood factor

Growth of the tracheal system is related to tissue growth and the size of the blood meal (fig. 2). The links between feeding and tissue growth are unknown. Perhaps certain metabolites are essential and are transported both by the blood and from cell to cell. Now the control of growth on the lateral tracheae by the nodes is presumably brought about in the same way as the control of growth in the rest of the tracheal system by the tissue-endings. But the nodes are isolated, suspended in haemolymph; they can only receive information about the size of the blood meal by way of the blood or by way of the tracheal epithelium. If the nodes are influenced by way of the tracheal epithelium it might be expected that the pathway would be from the terminations in the tissues.

The node with lateral trachea between abdominal segments 2 and 3 was isolated from tissue-connexions immediately after feeding, as in fig. 11. The new 5th instar lateral trachea showed normal growth. A lateral trachea was also isolated in another way, as in fig. 12. The end of the tergal trachea cut from its tissue-connexions showed a more or less complete failure but this did not influence the growth of the lateral tracheae with nodes. In earlier experiments (fig. 7), it had been noticed that growth of the lateral trachea was unaffected by cutting its connexion with a spiracle. Thus the variation in growth with feeding shown by the lateral tracheae is probably mediated through the blood.

The tissues as a stimulus for tracheole formation

When the lateral abdominal tracheae in *Rhodnius* were cut and allowed to remain free in the haemocoel, no new fine tracheae or tracheoles developed to re-establish contact with the tissues. But when the tracheae were cut and attached to epidermal wounds in the 3rd instar, terminal tracheoles quite commonly developed by the 5th instar. In the caterpillars of *Calpodex*, operations upon the tracheae rarely resulted in complete separation of the cut end from the tissues, and it was difficult to prevent the formation of quite extensive tissue-connexions after two or three moults. In *Tenebrio* larvae the lateral tracheae are normally devoid of side branches, but stimulation by occluding the spiracles sometimes produced numerous tracheoles, many of which supplied their own tracheal epithelium. Thus under the stress of oxygen-lack tracheal cells not normally destined to become tracheoles may yet do so. This occurred most frequently in lateral tracheae close to muscles. These observations suggest that contact with the tissues is necessary for tracheal cells to differentiate into tracheoles.

DISCUSSION

The tracheal epithelium is composed of cells of one sort only, and the cuticle which they secrete has a simple annular or helical pattern which has been attributed to the operation of simple physical forces. The only differentiation which a tracheal cell may undergo is in the formation of tracheoles, which

only occurs in certain regions and may be treated as a separate problem. A trachea may grow in diameter or in length, both of which may be measured numerically. Growth in length is mainly the result of extension in the preceding instar, but growth in diameter displays several features of interest. It may be considered as a particularly simple example of a general problem. The progress of determination in an egg is progressive in time in three dimensions. Surface cuticular patterns are a problem in two dimensions, but tracheal determination is in one dimension only. Discussion at present is necessarily speculative, but it seems that as a result of the simplicity of tracheal growth further study could lead to an answer to the problem of defining determination in less abstract terms.

The tracheae are parallel-sided tubes which branch in such a way that the total cross-sectional area of the diffusion path remains constant. Growth in the tracheal system varies with feeding and the organ supplied and occurs by the addition of a variable number of fine branches with tracheoles, the old tracheae increasing in diameter by an amount sufficient to maintain this branching pattern. Several processes can be distinguished which result in the maintenance of the branching pattern—the control of growth by the nodes and tissues, the polarity of the tracheae with respect to the operation of this control, and the timing of determination.

When epithelial continuity in the tracheal system is lost by cutting, the growth of only one of the ends is affected. The trachea on the spiracle side of the cut requires something from the tissue side in order to grow normally. It seems reasonable to equate this with the control of growth which results in the total cross-section remaining constant at different levels of branching. This implies that the region in which the diameter is being determined is under continuous control from the nodes or tissues. The mechanism of control is obscure. Lack of control does not completely prevent growth, it only reduces the diameter of the new trachea. Thus whether co-ordinated in their growth or not, tracheae must moult with the rest of the animal. The control is quantitative. The increase in diameter is precisely related to the growth of new tracheae formed later.

The polarity of this control seems to be one of the most important properties of the tracheal system. It is self-evident in the tree-like branching of tracheae, but this is not invariable; for in some tracheae, particularly the lateral tracheae of *Dixippus*, for example, tributary tracheae may make T-junctions.

Determination begins at the spiracles and moves in towards the source of control. It is tempting to suppose that it follows the completion of determination in the neighbouring cuticle. The timing of determination is unaffected by breaking the epithelial continuity, unlike the stimulus for the control of increase in diameter (fig. 13). Presumably, therefore, the time at which tracheal growth is determined is an inherent property of the trachea. This suggests that the polarity of a trachea is a result of the timing of determination. If the above hypotheses are correct, the stimulus from the tissues resulting in the control of growth is a constant one to produce parallel-sided tracheae in

a normal tracheal system. This contrasts with the stimulus to tracheal growth from epidermal wounds, which dies away with time, and conical tracheae result. This suggests how bulbous tracheae and air-sacs may arise: the stimulus from the controlling end is at first low, later it rises to a peak and dies away again.

It seems very probable that tracheole-formation is directly stimulated by the tissues. This would account for the specific main pattern in a tracheal system,

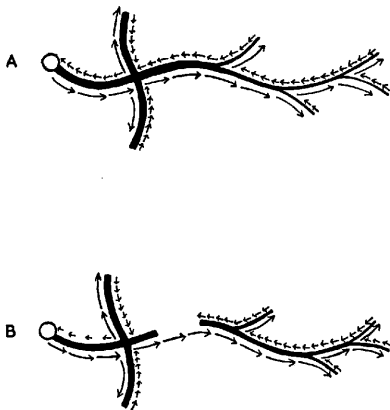


FIG. 13. Diagram to illustrate how the quantitative control of tracheal growth is dependent upon the continuity of the tracheal epithelium in contrast to the timing of determination, which is an intrinsic property of the trachea. The small arrows represent the quantitative control and the large arrows the progress of determination with time. A, in a normal trachea. B, in a cut trachea.

determined by the embryonic arrangement and the distribution of organs, and the minor variation, the result of local tissue requirements. Albrecht (1953), for example, describing the locust, says: 'In most cases, it seems as though structures requiring an especially large oxygen supply achieve this by enlargement and modification of the nearest part of the tracheal system, irrespective of the morphological relations of the primitive system. . . . Thus there is superimposed on the primitive tracheal metamerism a kind of functional specialisation.'

The effects of oxygen tension have proved to be a side issue to the main factors controlling tracheal growth. Some process related to tracheal growth must be sensitive to oxygen, but the stability of growth in *Rhodnius* under different oxygen tensions suggests that the relation is not a very direct one. Even in *Tenebrio* and *Calpodes*, oxygen tension probably plays little part in determining normal tracheal growth.

It must be realized that a first study of determination, even in a system as

simple as the tracheal system, can do little but redescribe the problem in more familiar terms. The problem will be taken a stage further in a future paper upon the part played by the tracheal cells.

I am very grateful to Professor Wigglesworth for supervising this work, which I began while holding an Agricultural Research Council award at Cambridge, where I profited from discussions with Dr. Beament and other members of the Department. I also thank Mr. G. L. Underwood and Dr. A. F. W. Hughes for kindly criticizing the manuscript.

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