REGULATION OF THE HAEMOLYMPH IN THE SALINE WATER MOSQUITO LARVA AEDES DETRITUS EDW.

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(With Five Text-figures)

INTRODUCTION

Much work has been done with marine, brackish and fresh-water animals to discover the nature of the mechanism by which the composition and concentration of the body fluids can be maintained different from those of the external medium. Nothing however is known in this connexion of animals belonging to predominantly fresh-water groups which are able to live in waters of extremely high salinity. Such waters (coastal salt marshes, inland salt lakes and springs) vary to an extreme degree not only in salinity, which may reach saturation, but also in composition. One water may differ radically from another and may itself alter progressively as the result of the differential precipitation of salts during evaporation. Life under such conditions entails a greater degree of osmotic independence than in the other more constant aquatic environments.

In one case only, that of the brine shrimp Artemia salina, has the degree of osmotic independence actually been measured. Medwedewa (1927) measured the osmotic pressure of the body fluid by the Barger method and found that a rise in external osmotic pressure equivalent to 3.5 % NaCl is reflected in a rise of only 0.1 % NaCl in that of the body fluids. A similar conclusion by different methods was reached by Warren et al. (1938).

During a recent expedition to French North Africa, whose main object was an ecological study of some inland saline waters of the coastal and desert regions of Algeria, some experimental work on this problem was done in the University of Algiers. I wish to express my gratitude to Prof. M. Rose, Professor of Biology, and to his assistant, Mlle H. Hamon, through whose hospitality and assistance I was able to do this work.

Of several animals which might have been chosen for the purpose the larvae of the mosquito Aedes detritus had many advantages; they were found in large numbers,
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and could be transported and kept easily in the laboratory, but above all the osmotic regulatory mechanism and other physiological characteristics of the purely fresh-water species of the same genus, *A. aegypti*, have been investigated by Wigglesworth (1933a, b, c, 1938a). The methods used by him were therefore immediately applicable and a comparison between the two species was likely to prove of interest.

The main part of the work was done in Algiers, but it was extended at home on larvae of the same species sent from the Mosquito Control Institute at Hayling Island, Hants, for which I am much indebted to the Director, Mr J. F. Marshall.

**MATERIAL**

An account of the ecology and chemical characteristics of the Algerian saline waters will be published elsewhere. *Aedes detritus* were found in twelve different waters whose density, as measured by a hydrometer, ranged from 1007 to 1063. This range could be expressed as roughly equivalent to 32–286 % sea water or to 1·2–10·0 % NaCl. In the highest salinities (above 1050) no other mosquito larvae were found, in less saline waters (1010–1050) they were usually accompanied by another typical saline-water species, *A. caspius*. Below this range truly fresh-water species were often found also (*Culex laticinctus*, *C. tupuliformis*, *Theobaldia annulata*, *T. longiariolata*, *Anopheles multicolor*). The specimens of *Aedes detritus* used for the experiments in Algiers were obtained from a pool near the shore of the salt lake Dayet Morselli at Oran on the north coast (density 1019, i.e. equivalent to 3·0 % NaCl). They were transported and kept in the laboratory in this water. The larvae obtained from Hayling Island were collected from salt-marsh (i.e. sea-water) pools and sent in this water (1·8 % NaCl). It was found that these larvae could not be transferred so rapidly to water more saline than 3·5 % as could the Algerian larvae from more saline water. But once acclimatized to a given high salinity (e.g. 3·5 % NaCl) their subsequent behaviour when subjected to various salinities as regards tolerance and composition of haemolymph appeared identical in the two groups of larvae. It was therefore not considered necessary to distinguish between the figures obtained from the two groups. The stock cultures were kept well fed with powdered dog biscuit, but feeding was always stopped during the course of each experiment.

The only obvious structural feature of *A. detritus*, which distinguishes it from the fresh-water species *A. aegypti* and appears to be related to its ability to live in saline water, is the great reduction in size of the anal gills (Fig. 1). Wigglesworth (1938b) has shown that the gills of the fresh-water species function for the absorp-

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1 Cambournac (1937) records *A. detritus* and *A. caspius* from waters of chloride content ranging from 7 to 10 % NaCl in salt marshes on the Portuguese coast. Their recorded range in the Hayling Island salt marshes is from slightly saline water to 133 % sea water or 4·7 % NaCl (private communication from Mr J. F. Marshall).

2 I am indebted to Dr G. Senevet, Faculté de Médecine, Alger, for the identification of these species.

3 A total analysis of this and some other waters in which these larvae were found (to be published later) show that they are moderately well balanced from a physiological point of view, but the percentage concentration of Na is usually lower and that of Ca, Mg, and SO₄ higher than in sea water.
tion of water, and there is some evidence that the mechanism by which they can concentrate chloride from very dilute solutions is seated in these gills (Wigglesworth, 1938a; Koch, 1938). Neither of these functions is required in saline water, and the possession of large gills permeable to water would indeed prove a positive disadvantage under such conditions.

The main objects of the following experiments were: (a) to determine the degree to which the composition of the haemolymph can be regulated especially in hypertonic solutions, (b) to investigate the nature of the regulatory mechanism, and (c) to find whether the capacity to absorb and retain chloride in dilute solutions shown by the fresh-water species has been lost in A. detritus.

METHODS

Fourth instar larvae were dried on filter paper and the haemolymph was obtained by pricking the larvae on a freshly waxed micro-slide, the escaping fluid being sucked up with a waxed pipette.

Total osmotic pressure was estimated by Baldes' modification of the Hill
vapour method (Baldes, 1934) with NaCl solutions as standards. A Leeds-Northrup high-voltage sensitive galvanometer was used with a distance of 4 m. from mirror to scale. A movement of 0.5 mm. could be read on the scale which corresponded to a difference of 0.004% NaCl between the concentrations of standard and unknown, but repeated determinations with the same solutions gave results which were more erratic on some days than on others. Results accurate to the nearest 0.01% NaCl could, however, always be obtained, and they are recorded as such. Owing to the large range of osmotic pressures found under experimental conditions several standard NaCl solutions between 0.5 and 2.5% had to be used.

Chloride was determined by Wigglesworth's micro-adaptation of the Volhard titration method (Wigglesworth, 1937). By this, chloride can be estimated to the nearest 0.02% NaCl.

It was not possible to make more than one determination of total osmotic pressure or of chloride on the same larva. Each figure or point on a curve represents the concentration of the haemolymph of a single specimen.

Total osmotic pressure (of haemolymph and of electrolyte and non-electrolyte solutions) and chloride concentrations are expressed throughout as % NaCl, which denotes an osmotic pressure or chloride concentration equal to that of the given percentage solution of sodium chloride.

Ligaturing of larvae was effected by means of the finest possible strands drawn from the threads of a bolting silk net. These could be tied round the larva at any point, thus isolating one part of the body from another.

RELATION BETWEEN INTERNAL AND EXTERNAL CONCENTRATIONS

Larvae were acclimatized to artificial sea water in concentrations ranging from nil (distilled water) to 6.1% NaCl. They could be transferred directly without apparent harm from the natural medium or from 3.5% NaCl to distilled water and vice versa. For the range of concentrations lower than 3.5% NaCl they were placed in the required solutions and kept 24 hr. before extracting haemolymph. But in order to acclimatize them to higher concentrations (3.5–6.1%) it was necessary to proceed by stages of about 0.5%, keeping them in each solution for 12 hr. before changing to the next. They were then left for 24 hr. in the final solution. If the transfer to the higher concentrations was more rapid, the larvae became obviously unhealthy and many died. But once acclimatized they remained healthy in all these solutions, including distilled water, and some proceeded to pupate.

The relation between external and internal total osmotic pressure and chloride is shown in Fig. 2. The corresponding curves obtained by Wigglesworth from A. aegypti are plotted for comparison (Wigglesworth, 1938a). It is obvious that, whereas in dilute solutions both species can maintain their haemolymph hypertonic to the external medium, A. detritus alone is able to maintain an hypotonic haemolymph in solutions of osmotic pressure above about 0.8% NaCl. The same general conclusion is reached from the chloride curves, though A. aegypti shows a limited capacity to regulate chloride until the external concentration has reached about
1.6 % NaCl. From a closer study of the *A. detritus* curves the following facts will become apparent: (a) though both total osmotic pressure and chloride are powerfully regulated, there is nevertheless a progressive change in both, which is roughly proportional to the change in the external concentration, (b) in hypertonic solutions the progressive rise in total osmotic pressure is due mainly to rise of the chloride fraction, the non-chloride fraction remaining approximately constant, and (c) in hypotonic solutions, and particularly in distilled water, there is a disproportionate reduction of the chloride fraction, but the total osmotic pressure is regulated by a corresponding increase in the non-chloride fraction.

That no significant loss or gain of water occurs in any of these solutions is suggested by the fact that the larvae, so long as they are properly acclimatized, do not appear to alter in volume nor in the hydrostatic pressure of their haemolymph as judged by the ease with which it is obtained even from larvae taken from the highest concentrations. The curves in Fig. 2 also support this conclusion. If water were lost in hypertonic solutions it should be reflected in a rise in concentration of the non-chloride fraction. That this does not occur suggests that the rise in total osmotic pressure is due mainly to the penetration of salts and not to the removal of water under osmotic forces. The ability to regulate the non-chloride independently of the chloride fraction in hypotonic solutions was found also with *A. aegypti* (Wigglesworth, 1938a). It is worthy of note that the larvae of *A. detritus* are well able to live and develop in distilled water, though, as far as is known, they always breed in waters which are definitely more saline than ordinary fresh waters.

Fig. 2. Relation between total osmotic pressure and chloride concentration of the haemolymph of *A. detritus* larvae and those of the medium (continuous lines). Corresponding curves from *A. aegypti* (dotted) taken from Wigglesworth (1938a). The diagonal straight line represents haemolymph and medium as continuously isotonic.
THE EFFECT OF PURE SALT SOLUTIONS

The larvae were kept previously in sea water (3.5% NaCl) for 24 hr. and were then transferred to isotonic solutions of NaCl, KCl, MgCl₂, and CaCl₂. The results of this experiment are tabulated in Table II. In NaCl they were perfectly normal in every way and some eventually pupated; the total osmotic pressure and chloride were maintained at the level characteristic of larvae kept in sea water (3.5% NaCl). In the other three chlorides muscular activity eventually stops; KCl is more effective in this respect than MgCl₂ or CaCl₂. When all movement has ceased and even (in KCl) the heart beat has stopped, the haemolymph is still considerably hypotonic to the external medium. In this respect also KCl is more detrimental than the others. This result is not necessarily due to the maintenance intact of the osmo-regulatory mechanism in spite of complete cessation of muscular movement. It is more reasonably explained as the result of stoppage of salt penetration from outside. The main site of salt exchange is the gut and not the body surface (see next section), and cessation of muscular movement might be expected to stop active uptake of saline water into the gut and thus reduce the rate of salt penetration.

The above experiments show at least that the cations in question with the exception of Na⁺ can enter the larva and cause an upset in the normal ionic balance, and a consequent stoppage of body and heart muscles. It is to be presumed that Na⁺ also enters from NaCl solutions though there is no proof that it does so, but it is only to be expected that an increase in concentration of Na⁺ ions in the haemolymph would be less harmful than a corresponding increase in that of the other cations.

THE EFFECT OF NON-ELECTROLYTES

After acclimatization to sea water (3.5% NaCl) larvae were subjected to isotonic solutions of sucrose and glycerol. The results (Fig. 3) were unexpected and of considerable interest.

In sucrose there was obvious shrinkage and a progressive decrease in activity. After 24 hr. the larvae were apparently dead. The haemolymph was by then isotonic with the external medium (3.5%). Shrinkage was so great that several larvae had to be used in order to get sufficient fluid for the final reading. In spite of the increase in total osmotic pressure the chloride concentration fell rapidly to the value characteristic of larvae kept in distilled water. Salts had therefore freely leaked away, and the rise in total osmotic pressure must have been due to concentration of the non-chloride fraction resulting from extraction of water under osmotic forces.

The visible effect of glycerol was remarkably different from that of sucrose. Little or no shrinkage was detectable, and the larvae behaved normally and many proceeded to pupation. But the total osmotic pressure of the haemolymph rose, though less rapidly than in sucrose, towards a value little below that of the external medium, and the chloride concentration fell as in sucrose. This presumably shows that the larvae are freely permeable to glycerol and not to the larger sucrose mole-
cule. It is surprising that the normal functions are not upset by the presence of glycerol in the haemolymph. They are still able to retain the necessary residuum of salts as when in distilled water.

It is important to determine the region of the body in which the exchange of substances takes place between haemolymph and external environment.

For this purpose the above glycerol experiments were repeated, but with larvae previously ligatured (a) between the head and thorax and between the 8th and 9th segments, (b) between the head and thorax alone, and (c) between the 8th and 9th segments alone. The results are recorded in Table I. If these figures are compared with the curves in Fig. 3, it is evident that stoppage of both ends of the gut inhibits the uptake of glycerol and loss of salts during the first 24 hr. Though a ligature between the head and thorax alone is equally effective, the inhibition appears to be rather less marked when the ligature is made between the 8th and 9th segments. This is presumably due to the fact that the medium is taken in by the mouth and that this is prevented by a ligature at the front end, but stoppage of the hind end of the gut may still permit of some intake, though considerably less than when the gut is unconstricted. The main region in which there is an exchange of substances between the haemolymph and external medium is therefore the gut. The experiment also disposes of the possibility that any exchange occurs through the anal gills, which in the larvae ligatured between the head and thorax alone (Table I b) are in free communication with the haemolymph. Examination of the gills themselves also supports this conclusion (see p. 356).
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Table I

Larvae previously acclimatized to sea water (3.5% NaCl) were ligatured and transferred for 1 hr. to isotonic glycerol. Total osmotic pressure and chloride concentration of the haemolymph are expressed as % NaCl.

<table>
<thead>
<tr>
<th>Ligatures</th>
<th>Total osmotic pressure</th>
<th>Chloride</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Head-thorax and VIII-IX</td>
<td>1.31, 1.14, 1.32</td>
<td>0.45, 0.45, 0.48</td>
<td></td>
</tr>
<tr>
<td>(b) Head-thorax</td>
<td>1.16, 1.12, 1.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) VIII-IX</td>
<td>1.68, 1.44, 1.69</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table II

<table>
<thead>
<tr>
<th>Solution = 3.5% NaCl</th>
<th>Time in hours</th>
<th>Total osmotic pressure = % NaCl</th>
<th>CI content = % NaCl</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>24</td>
<td>1.24, 1.29, 1.47</td>
<td>0.67, 0.65, 0.69</td>
<td>Normal. Some pupate</td>
</tr>
<tr>
<td></td>
<td>83</td>
<td>-</td>
<td>-</td>
<td>Motionless but heart beating</td>
</tr>
<tr>
<td>KCl</td>
<td>3</td>
<td>1.28, 1.54, 2.85</td>
<td>-</td>
<td>Heart stopped</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MgCl₂</td>
<td>8</td>
<td>1.13, 1.26</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.37, 1.18</td>
<td>-</td>
<td>Motionless but heart beating</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.37, 1.18</td>
<td>-</td>
<td>Motionless but heart beating</td>
</tr>
</tbody>
</table>

Table III

Larvae were transferred from sea water (1.8% NaCl) to distilled water and the chloride concentration of the haemolymph (expressed as % NaCl) was measured at intervals during 96 hr. After 42 hr. some of these were transferred to three hypotonic solutions containing a progressively larger amount of NaCl, and the chloride concentration of the haemolymph was subsequently compared with that of the larvae remaining in distilled water.

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>Distilled water (0.003% NaCl)</th>
<th>Tap water (0.006% NaCl)</th>
<th>0.006% NaCl</th>
<th>0.04% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>0.37</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>42</td>
<td>0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>66</td>
<td>0.10</td>
<td>0.10</td>
<td>0.08</td>
<td>0.20</td>
</tr>
<tr>
<td>72</td>
<td>-</td>
<td>0.08</td>
<td>0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>96</td>
<td>0.04</td>
<td>0.10</td>
<td>0.10</td>
<td>0.25</td>
</tr>
</tbody>
</table>
THE REGULATORY MECHANISM

The experiments so far detailed suggest that the gut of these larvae is permeable to water, to chloride, to the cations of sea water and to non-electrolyte molecules as large as glycerol, but not as large as sucrose. If we can conclude from this that the gut offers a free passage to all the ions of sea water, the curves in Fig. 2 are more easily interpreted, and the tentative conclusion already reached when discussing them is further corroborated. Adaptation to high salinities is apparently not a question of controlling or compensating for extraction of water by osmotic forces. It is rather the penetration of salts due to the concentration gradient which has to be counteracted in some way in order that a relatively constant haemolymph may be maintained.

The most obvious hypothesis on which to work is that salts are being continually excreted, and the Malpighian tubes might be suspected of doing this. In order to test this hypothesis larvae acclimatized to sea water (3.5 % NaCl) were ligatured in appropriate positions and returned to the same solution. After a certain interval the total osmotic pressure and chloride concentration of the haemolymph were measured. The results are tabulated in Fig. 4. This is in reality a table in which the columns of figures are plotted as points on a vertical scale, the vertical line to the left of each group of points represents the spread and the horizontal line the mean of each group. It can be seen at once that a ligature between the 5th and 6th segments (i.e. in front of the Malpighian tubes, see Fig. 1) results in a definite rise in both total osmotic pressure and chloride, and that this effect is more marked after 24 than 15 hr. The increase in total osmotic pressure is obviously due in the main to an increase in the chloride fraction, and thus to the accumulation of salts in the haemolymph. No such effect is produced by a ligature between the head and thorax. In order to test whether this change is due to stoppage of the tracheal system, which necessarily results from a ligature between the 5th and 6th segments, the siphon was ligatured at its base in another experiment. This also produced no effect.

If the result of a ligature between the 5th and 6th segments is due to the disconnexion of the Malpighian tubes from the rest of the body, it might be expected that a ligature behind the tubes would be ineffective. This was tested by a ligature between the 8th and 9th segments, which, however, produced a definite rise in osmotic pressure and chloride concentration (Fig. 4). This result might be considered as invalidating the hypothesis that the Malpighian tubes excrete excess of salts, but it is possible that a ligature behind the entrance of the tubes into the gut might hinder the excretory activity of the tubes as the result of accumulation of salts in that part of the gut. There is some indication (Fig. 4) that after 24 hr. the rise in concentration is less in those larvae ligatured behind than in those ligatured in front of the Malpighian tubes. This perhaps suggests that the tubes are capable of some limited activity when the hind end of the gut is closed.

On the above hypothesis the accumulation of glycerol in the haemolymph (Fig. 3) must be due to the inability of the larvae to excrete this substance sufficiently rapidly. Why then is the rise in total osmotic pressure of unligatured larvae
in glycerol, in spite of considerable loss of salts, more rapid than in larvae ligatured in front of the Malpighian tubes kept in sea water of the same osmotic pressure (compare Figs. 3 and 4, 24 hr.)? In answer to this it must be pointed out that a ligature round the body stops the gut, and it has already been shown that the prevention of a free passage through the gut inhibits the rate of penetration of substances from the external medium (p. 352). Unfortunately, no means has yet been discovered of occluding the Malpighian tubes without the gut. The method used by Hurst with blow-fly larvae by which the tubes are broken by external pressure with a blunt needle (personal communication from Mr H. Hurst) was tried but without success. The external pigmentation makes it very difficult to see clearly the course of the tubes, and they lie so close to the gut that it does not seem possible to break them by this method without damaging the gut also. By pressing larvae under a cover-slip the Malpighian tubes can be squeezed out together with some of the haemolymph, in which they are then immersed. When examined under a microscope the tubes of larvae from distilled water were not obviously different from those of larvae acclimatized to sea water (3·5 % NaCl). There was in fact no evidence from their appearance that they were excreting less fluid when the larvae were in sea water.

**ABSORPTION AND RETENTION OF CHLORIDE**

The ability to absorb chloride from dilute solutions was tested by the experiment recorded in Table III. Larvae were transferred direct from their natural medium of sea water (1·8 % NaCl) to distilled water and thereafter starved. After
42 hr. the chloride concentration of the haemolymph was approaching the low value which was roughly maintained for the duration of the experiment (96 hr.). At this point (42 hr.) some were removed and placed in solutions of varying chloride content, but in every case lower than that of the haemolymph after 42 hr. in distilled water. In solutions containing up to 0·006 % NaCl there was no significant increase in the chloride concentration of the haemolymph. In this they are therefore distinguishable from *A. aegypti*, which, in tap water (0·006 % NaCl), can increase its chloride concentration from c. 0·20 to 0·35 % NaCl in 24 hr. (Wigglesworth, 1938 a). But in 0·04 % NaCl, which is only a little below the chloride concentration of the haemolymph after 42 hr. in distilled water, it is obvious that *A. detritus* can concentrate chloride from the external medium (Table III). *A. detritus* can therefore retain chloride as efficiently as *A. aegypti*, but the capacity to absorb chloride from dilute solutions, though still evident, is considerably reduced.

**THE ANAL GILLS**

The anal gills of *A. detritus*, as already mentioned, are very small as compared with those of fresh-water species (Fig. 1). They are not flattened leaf-like structures like those of *A. aegypti*, but are circular in cross-section (Fig. 5 c). For this reason it is more difficult to define their internal structure under a microscope. But it was possible to see something of the internal protoplasmic lining. In intact larvae this lining was always very thick and vacuolated, but individuals varied enormously in the number and size of the vacuoles. There were faint signs of striations towards the outer edge (Fig. 5). No significant difference could be seen between gills of larvae accustomed to distilled water, sea water (3·5 % NaCl), 3·5 % NaCl, and glycerol (3·5 % NaCl), though, as has been shown already, these conditions produce considerable changes in the osmotic pressure of the haemolymph. Wigglesworth showed that hypertonic solutions of NaCl penetrate the gill surface very rapidly and cause marked swelling of the cells of the protoplasmic lining in *A. aegypti* (Wigglesworth, 1933 a). The appearance of this lining even in distilled water was quite different from that of *A. aegypti* under the same conditions, in which it forms a well-defined thin non-vacuolated layer. But it was somewhat similar to that of the majority of *A. aegypti* reared in sea water (1·5 % NaCl), which caused it to become thicker and vacuolated (Wigglesworth, 1933 b).

Acid (N/50 HCl) and alkali (N/20 NaOH) produced very marked effects on the protoplasmic lining noticeable within a few minutes when applied externally to intact larvae of *A. aegypti* (Wigglesworth, 1933 b). *A. detritus* larvae accustomed to distilled water were subjected to the same treatment, but no change could be observed in the gill lining in either acid or alkali over a period of 1 hr. The subsequent mortality was higher in the acid than in the alkali, but after 48 hr. two of twelve larvae in the acid and four of seven larvae in the alkali were still alive and apparently normal. There was still one larva alive in the alkali after 6 days. On the other hand, ten larvae subjected to N/20 KOH were all dead after 20 hr., though in these also no change could be observed in the gill lining during the first hour.
These observations, besides showing the extraordinary resistance of the larvae to abnormal conditions, suggest very strongly that the gill surface is extremely impermeable and thus differs completely from that of \textit{A. aegypti}. Comparison of the external cuticle of the gills in the two species showed that in \textit{A. detritus} it is unmistakably thicker.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{fig5.png}
\caption{Structure of the anal gills of intact \textit{A. detritus} larvae under \( \times \) 10 objective lens. Extent of air in tracheoles indicated by continuous lines. \( a \), acclimatized to sea water (3\% NaCl) immediately after placing under coverslip; \( b \), ditto, 5 min. later; \( c \), ditto, seen from distal end; \( d \), acclimatized to glycerol (3\% NaCl) immediately after placing under cover-slip. Structure of gills and position of air essentially similar after acclimatizing to distilled water and to 3\% NaCl.}
\end{figure}

\textbf{THE EXTENT OF FLUID IN THE TRACHEOLES}

The amount of fluid in the tracheole endings was variable in larvae examined immediately after placing under a cover-slip. This applied both to the tracheoles in the gills and to those in the head. But in larvae accustomed for 24 hr. to distilled water, sea water (3\% NaCl), glycerol (3\% NaCl), or 3\% NaCl there was no significant difference in this respect, and in all cases a considerable extent of the distal tracheoles contained fluid. This was soon extracted and replaced by air if the larvae were left under the cover-slip (Fig. 5\( b \)). This also occurs with \textit{A. aegypti}, and Wigglesworth interpreted it as due to a rise in osmotic pressure of the haemolymph resulting from asphyxiation, a rise which he was able to demonstrate (Wigglesworth, 1938a). But in \textit{A. detritus} considerable increase in the osmotic pressure of the haemolymph is induced by transferring larvae from distilled water to sea water.
(3.5 % NaCl) or to glycerol (3.5 % NaCl) (Figs. 1, 3). But in neither case is fluid extracted from the tracheoles, though repeated observations were made during the first few hours as well as (as reported above) at the end of 24 hr. These observations will be considered again in the discussion in relation to theories to account for the absorption of the tracheole fluid.

DISCUSSION

The surface of the trout egg is impermeable to both salts and water though oxygen and carbon dioxide can pass through readily (Gray, 1932; Krogh & Ussing, 1937). This appears to be the condition of the body surface of the mosquito larva, but adaptation to media differing greatly in osmotic pressure from that of the body fluids cannot be effected solely by these means, since interchange of substances between internal and external environments must necessarily occur via the gut. The above experiments in fact show that many of the solute molecules normally present in the environment can penetrate rapidly via the gut, which therefore does not constitute a semipermeable membrane across which osmotic forces are at work. The regulatory mechanism is one which must compensate for or control the inward diffusion of salts. There is no evidence to show whether the amount of water entering the gut can be controlled or whether there is any active mechanism in the gut surface regulating inward diffusion of ions. But the experiments recorded in Fig. 4 show that part at least of the mechanism is an active excretion of excess salt by some organ situated in the last three segments of the body. That this organ is the Malpighian tube system is indicated but is not conclusively proved. Regulation in hypertonic solutions therefore resembles that of the euryhaline and stenohaline marine teleost fish which absorb sea water through the gut and excrete excess of salt through the gills (Smith, 1932; Keys, 1933). The fish, however, are continuously dependent upon the environment for their supply of water which is continuously lost to the external seawater. The water content of the blood of the eel in sea water is rapidly reduced by blocking the mouth (Keys, 1933). That this does not occur with the larva of A. detritus when ligatured between head and thorax (Fig. 4) is obviously attributable to the impermeable nature of the body surface. Since there is no evidence of water loss from unligatured larvae in hypertonic saline, it is not necessary to postulate an active resorption of water by part of the alimentary canal, which was suggested by Wigglesworth as part of the mechanism by which larvae of A. aegypti can become adapted to saline water (1.75 % NaCl) (Wigglesworth, 1933c). He has since, however, shown that they do not in fact maintain an hypotonic haemolymph in saline water (Wigglesworth, 1938a). In A. detritus uptake of water is not a necessary part of the regulatory mechanism except in so far as it may serve as a vehicle for the removal of excess salt.

It is interesting to see that the larvae of A. detritus can live in hypotonic solutions including distilled water as well as the purely fresh-water species, though they have been recorded only from saline waters. This naturally entails the power to retain salts, which has been shown to be as effective as in A. aegypti (Table III). The power to absorb chloride against a steep concentration gradient is characteristic of
many fresh-water animals, and it has been suggested that it may be of some functional significance in maintaining the salt content of the blood in water of very low salinity (Krogh, 1937, 1938). It would, however, become of significance only if food were lacking or contained too little salt to be absorbed through the gut. In *A. detritus* chloride absorption is obviously not even part of the mechanism by which it regulates its body fluid in very dilute solutions, since it can absorb chloride only from solutions approaching the concentration of the haemolymph (Table III).

It was suggested by Koch (1938), and was to some extent supported by Wigglesworth (1938a), that the chloride absorbing mechanism in fresh-water larvae is seated in the anal gills. Breeding larvae of *A. aegypti* and *Culex pipiens* in slightly saline water (up to 0.9% NaCl) results in a marked reduction in size of the anal gills especially in *Culex* (Wigglesworth, 1938a), and there is some relation between the size of the gills of different species and the nature of their usual habitats, in that the species with the largest gills breed in small temporary collections of rain water which might be expected to be of very low salinity (Hopkins, 1936, 1938). On the other hand, there is no conclusive and direct evidence that the gills function in this way, and Wigglesworth (1938a) found that larvae of *C. pipiens* whose gills had been reduced to an extreme degree of breeding in 0.9% NaCl were yet able to absorb chloride as effectively as larvae with full-sized gills. The observations on *Aedes detritus* contribute something to this controversy in that there is little doubt that the gills are impermeable to salts. It is therefore very unlikely that in this species the chloride-absorbing mechanism is situated in these organs.

In considering the remarkable capacity of the larvae of *A. detritus* to live in abnormal environments (e.g. distilled water, sea water (10% NaCl), pure NaCl and glycerol solutions, and for some days in N/20 NaOH), it must be remembered that their resistance depends not only upon the power of regulating the concentration of the haemolymph. They are in fact unharmed by a considerable alteration in both concentration and composition of the haemolymph as is well demonstrated by the action of glycerol (3.5% NaCl). In this the concentration of the haemolymph is roughly trebled and must contain some quantity of glycerol. The tissues are therefore extremely resistant to changes in the concentration and composition of the body fluid, and this resistance must be regarded as part of the adaptive mechanism. In connexion with resistance to alkali it is worth recalling that larvae of *A. natronius* were found in an extremely alkaline crater lake in Uganda whose water had a salinity equivalent to 3.9% NaCl, and alkalinity of 0.7N and a pH of over 10.5 (Beadle, 1932; Hopkins, 1936).

Wigglesworth (1930) developed a theory of tracheal respiration based mainly upon experiments with *A. aegypti* larvae, the essence of which is contained in the following quotation: "If it be assumed that the terminal portions of the tracheal tubes are bounded by a semipermeable membrane, then liquid will be drawn up the tubes by capillarity until its progress is arrested by the osmotic pressure of the tissue fluids. During activity lactic acid and, probably, other substances will be produced, the osmotic pressure will rise, liquid will be absorbed and air will extend down the tubes towards the active tissues." This theory was propounded in view of the fact
that the following conditions result in the absorption of tracheole fluid: (a) asphyxiation under a cover-slip (confirmed above with *A. detritus*), supposedly due to the accumulation of unoxidized metabolites and consequent rise in osmotic pressure, (b) injection of haemolymph of asphyxiated larvae into normal resting larvae, and (c) similar injections of hypertonic NaCl and potassium lactate. Wigglesworth later confirmed that asphyxiation does in fact result in a rise of haemolymph osmotic pressure (Wigglesworth, 1938a), but at the same time discovered a serious objection to the theory in its original form. The haemolymph osmotic pressure of *A. aegypti* larvae adapted to 1-2 % NaCl is even higher than that of asphyxiated larvae in distilled water, but there is no significant alteration in the extent of the fluid on the tracheoles. If, however, salt-adapted larvae are asphyxiated, the fluid is absorbed and the osmotic pressure of the haemolymph rises still further. Wigglesworth was therefore driven to postulate some additional factor such as the power of “imbibition” of the protoplastic lining of the tracheole walls, which might undergo some adaptive change.

The observations on *A. detritus* present precisely the same difficulties but in a much more exaggerated form. Transfer from distilled water to sea water (3·5 % NaCl) causes a much greater rise in osmotic pressure than can ever be produced in *A. aegypti* without killing them, and in glycerol (3·5 % NaCl) it is about trebled, but the extent of the fluid in the tracheoles is not thereby altered. Yet a few minutes’ asphyxiation under a cover-slip induces rapid absorption of fluid. If, according to Wigglesworth’s original theory, the tracheole walls are semipermeable, then in the glycerol-adapted larvae some extremely powerful adaptive mechanism must come into operation, whereby a difference in osmotic pressure equivalent to c. 3 % NaCl is maintained between the two sides of the walls, though a subsequent small rise in haemolymph osmotic pressure due to asphyxiation is sufficient to withdraw the fluid. It seems in fact impossible to maintain the conception of semipermeable tracheole walls in view of these facts. The phenomenon of adaptation to conditions which result in a large increase in haemolymph osmotic pressure makes it probable that the tracheole fluid is isotonic with the haemolymph and that osmotic forces acting across a semipermeable membrane are not responsible for the movements of the tracheole fluid. The necessary force must presumably reside in the substance of the protoplastic lining, perhaps in the swelling properties of its colloidal constituents. That such “imbibitional” properties are profoundly influenced by changes in the concentration of the non-colloidal solutes is well known. Wigglesworth (1938a) showed with *A. aegypti* that a rapid increase in the salt concentration of the medium causes swelling of the cells lining the anal gills, but that in larvae accustomed gradually to saline water some adaptive change occurs in these cells whereby the swelling is inhibited, this change being accompanied by a visible alteration in structure. If similar properties were possessed by the protoplastic lining of the tracheoles, they might account for the observed phenomena of fluid absorption and adaptation. On the other hand, from observations on the removal of fluid from the entire tracheal system after the hatching of larvae of *A. aegypti* Wigglesworth (1938b) suggested that the process may involve an active secretory mechanism,
possibly under the control of the nervous system. Further research on this problem is obviously needed, and A. detritus might prove a very suitable material in view of the fact that the concentration and composition of the body fluids can be altered so radically without damage to the larva.

SUMMARY

1. The larvae of the mosquito Aedes detritus have been reported only from definitely saline waters. They have been found in water of salinity equivalent to c. 10 % NaCl.

2. In the laboratory they were acclimatized with ease to distilled water, sea water (7 % NaCl), 3.5 % NaCl, and glycerol (3.5 % NaCl). They also show considerable resistance to N/20 NaOH, but less to N/20 KOH and N/50 HCl. They are unable to live permanently in solutions of the chlorides of potassium, calcium and magnesium of osmotic pressure equivalent to 3.5 % NaCl.

3. In sea water of varying salinity they can regulate both the total osmotic pressure and chloride content of the haemolymph. A rise from nil to 6.0 % NaCl in the osmotic pressure of the medium is reflected in an increase of from c. 0.8 % to 1.4 % NaCl in that of the haemolymph.

4. In hypotonic solutions and distilled water much chloride is lost, but this is compensated by an increase in the non-chloride fraction. In hypertonic sea water the rise in osmotic pressure is due to increase in the chloride fraction, the non-chloride fraction remaining constant.

5. From this and from experiments with non-electrolytes it is concluded that the larva is permeable to salts and to molecules as large as glycerol, and that the regulatory mechanism in hypertonic saline is concerned with compensation rather for penetration of salts than for loss of water by osmosis.

6. Ligature experiments suggest that this mechanism is the excretion of salt by the Malpighian tubes, but further proof is required.

7. Salt exchange with the environment takes place via the gut, the body surface being impermeable to salts and water.

8. The larvae are able to concentrate chloride from hypotonic solutions but not as effectively as fresh-water species and only when the chloride content of the medium is a little below that of the haemolymph.

9. The anal gills, as in all salt-water species, are very small and appear to be impermeable to salts and water. It is therefore concluded that they are not the seat of the chloride-absorbing mechanism.

10. The osmotic pressure of the haemolymph is trebled by treatment with glycerol (3.5 % NaCl), which must be mainly the result of penetration of glycerol. The larva will however live normally in this, and an important factor in the resistance to abnormal media is therefore the adaptability of the tissues to changes in the concentration and composition of the haemolymph.
II. The increase in the osmotic pressure of the haemolymph induced by hypertonic sea water and glycerol does not alter the amount of fluid in the tracheoles. This is discussed in relation to the possible mechanism for the absorption of the tracheole fluid.

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REFERENCES


—— (1938). *Nature, Lond.,* 734, 482.


—— (1933b). *J. exp. Biol.* 10, 16.
—— (1933c). *J. exp. Biol.* 10, 27.