THE ADAPTATION OF MOSQUITO LARVAE TO SALT WATER

By V. B. WIGGLESWORTH, M.A., M.D.

(From the Department of Entomology, London School of Hygiene and Tropical Medicine.)

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(With Two Text-figures.)

It is well known that the larvae of certain mosquitoes can thrive both in fresh water and in water of a high degree of salinity, while the larvae of other mosquitoes are quickly killed by salt water; and that such differences may exist even between local races of the same species (Evans, 1931). It is evident that we have here to deal with a physiological adaptation, and it was the object of the present work to discover what the nature of this adaptation might be.

The physiological properties of larvae of the yellow-fever mosquito (Aedes (Stegomyia) argenteus Poir), which were accustomed to salt water, have been compared with those same properties in normal larvae. It so happens that this particular larva is unable to tolerate very salt water; but it is probable that the relatively small degree of resistance which it can acquire is of the same kind as that occurring in more tolerant species, and therefore the mechanism of this resistance may throw light upon the general problem.

LETHAL CONCENTRATION OF SALT FOR LARVAE REARED IN FRESH WATER.

Sodium chloride. Fourth stage larvae (i.e. larvae in their final instar), reared in fresh water, were transferred to sodium chloride solutions of various strengths at 24° C. They are not affected by solutions of 0.9 per cent. and less. In 1.0 per cent. solutions many larvae die within about a week (although a few may survive permanently), and the mortality increases with rising concentration. At 1.1 and 1.2 per cent. the larvae nearly all die in 4-7 days. At 1.3 per cent. they die within 72 hours, and at 1.4 per cent. within 48 hours. Individual variations are considerable.

Artificial sea water1. Experiments were carried out under the same conditions using diluted sea water in place of sodium chloride. The concentration of sea water may be conveniently expressed in terms of those solutions of sodium chloride which have the same osmotic pressure, this being calculated approximately on the assumption that all the salts are completely ionised. These equivalent concentrations are shown throughout in inverted commas.

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1 The artificial sea water used had the following composition: NaCl, 2.83 per cent.; KCl, 0.076 per cent.; MgCl₂, 0.501 per cent.; CaCl₂, 0.122 per cent. This has an osmotic pressure approximately equal to 3.5 per cent. sodium chloride. No bicarbonate was added to this mixture which was therefore more acid than natural sea water.
It is found that the larvae are unaffected by a concentration of "1·1 per cent."; at "1·2 per cent." there is a considerable mortality in the course of a week, but many of the larvae survive. At "1·3 per cent." very few survive; at "1·4 per cent." they are nearly all dead in a week; at "1·5 per cent." all die within 4 days, and at "1·6 per cent." in about 48 hours. There is much individual variation (for example, very rarely a larva may survive transference to "1·4 per cent." sea water, and live apparently indefinitely), but it is evident that sea water is considerably less toxic than pure sodium chloride, 1·2 per cent. NaCl being about equally toxic with "1·5 per cent." sea water.

EFFECT OF SALTS ON THE GROWTH OF NEWLY HATCHED LARVAE.

One of the difficulties in studying the effect of the composition of the water on the growth of mosquito larvae is the indirect effect which this composition may have by its influence on the micro-organisms upon which the larvae feed. In the present work this difficulty has been avoided by feeding the larvae on dried and finely powdered serum (Loeffler's dried serum) upon which they thrive extremely well. An equal amount of this (as measured on the point of a knife) is added to equal (10 c.c.) volumes of the fluid to be tested, and an equal number (about half a dozen) newly hatched larvae placed in each.

Sodium chloride. A series of concentrations of sodium chloride ranging from distilled water to 3 per cent. was employed, being kept at 24° C. The larvae grow rapidly in pure water, being all at the fourth or final instar in 5 days. Growth is very slightly delayed below 0·8 per cent. NaCl. There is greater inhibition at 0·9 per cent., larvae being at the second or third instar in 5 days. At 1·0 per cent. there is often considerable mortality and the growth of the survivors is extremely slow, there being almost no growth, and larvae still in the first instar at the end of 5 days. At 1·1 per cent. the larvae fail to grow, and die in about 48 hours. These are all dead in about 2 hours in 1·5 per cent., and in about 1 hour at 2·0 per cent. and over.

The same results are obtained if the eggs are allowed to hatch in these solutions. Thus they hatch normally in 1·2 per cent. NaCl; but the larvae all die within 2 days.

Artificial sea water. Larvae reared under the same conditions in artificial sea water at various dilutions develop at about the normal rate up to a concentration of "1·1 per cent." (in terms of the concentration of NaCl having the same osmotic pressure). At "1·2 per cent." growth is slightly inhibited. At "1·3 per cent." many of the larvae die in a few days and the growth of the survivors is very slow. At "1·4 per cent." all die in about 48 hours; and at "1·5 per cent." most are dead within 24 hours.

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Sodium chloride. Newly hatched larvae were placed in a weighed amount of 0·9 per cent. NaCl in an open vessel with a supply of dried and powdered serum for food, and the concentration of salt allowed to increase slowly by evaporation, the mixture being stirred each day. The specific gravity of the initial fluid being known, the concentration at any stage can be calculated from the weight.
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The larvae do not become very resistant to sodium chloride; and if this is allowed to reach a concentration of about 1-3 per cent. during 10 days, most of the larvae are dead or moribund. But if the evaporation is arrested when the concentration has reached 1-0 or 1-1 per cent., or if the larvae are kept in 1-0 per cent. NaCl from the outset, it can always be noted that for some days they show practically no growth; but when growth has once begun it proceeds fairly rapidly. This suggests that some sort of adaptation is occurring, and it will be shown later that such is the case.

Sea water. More striking adaptation can be obtained with artificial sea water. The experiments have been carried out as with sodium chloride, the larvae being started off in sea water diluted three times. Under these conditions, some of the larvae will survive until the water has reached a concentration of about "2-2 per cent." in 3 weeks. If kept at this concentration, however, they die in a few days.

On the other hand, if the evaporation is arrested when the concentration is about "1-9 per cent." sea water, the larvae will survive for at least a week. These larvae grow extremely slowly and it is doubtful if they would ever reach maturity; but there is no doubt that they have become adapted to the sea water, for we have seen that larvae reared in fresh water die within 48 hours in sea water of this concentration. In sea water equivalent to "1-5 per cent." NaCl the adapted larvae have remained alive for 3 or 4 weeks.

CHARACTERS OF LARVAE ACCUSTOMED TO SALT WATER.

General appearance. Larvae accustomed to 1 per cent. NaCl show no obvious difference from larvae in fresh water. On the other hand, larvae in sea water equivalent to "1-75 per cent." NaCl have the body considerably shrunken, so that the indentations between the abdominal segments are more pronounced than usual, and the head sinks into the thorax. This shrunken appearance becomes more marked the greater the concentration of the salt.

Intestine. The gut often contains less solid matter and more fluid than in fresh water, both inside and outside the peritrophic membrane. The cells of the caeca and the mid-gut appear normal.

Anal gills. The anal gills may sometimes be slightly deformed; but usually they are of the normal size and shape. The cells may be perfectly normal or just a little thickened (Fig. 1 A and A'). More often, however, they have the appearance shown in Fig. 1 B and B', where the cells are greatly thickened so that they extend far into the lumen of the gill. The cytoplasm, which contains large vacuoles, is separated from the cuticle but bound to this by conspicuous strands. Sometimes one or more of the gills are normal and the others have the cells swollen in this manner. (The normal structure and properties of the anal gills have already been described (Wigglesworth, 1932 a).)

Malpighian tubes. The cells of the Malpighian tubes are usually less granular than normal, both in larvae reared in 1 per cent. NaCl and in diluted sea water. Sometimes they contain in the lumen a considerable amount of solid matter,
presumably uric acid, since it has the same appearance as the uric acid in the Malpighian tubes of the adult. (The Malpighian tubes of larvae in fresh water never contain any solid matter.)

**Osmotic pressure of body fluid.** The tracheoles in the head of larvae reared in 1 per cent. NaCl or "1.75 per cent." sea water contain fluid as in the normal larva. Now it has been shown (Wigglesworth, 1930) that this fluid is removed from the tracheoles if the osmotic pressure of the haemolymph is increased, e.g. if the head is punctured in 1 per cent. NaCl. The fact that the tracheoles contain a normal amount of fluid in larvae in salt water suggests that the osmotic pressure of the body fluids has not been increased; and this conclusion is supported by the fact that on puncturing the head of the larva in the fluid in which it has been reared (1 per cent.

![Figure 1](image_url)

**Physiological properties of larvae accustomed to salt water as compared with larvae in fresh water.**

**Anal gills.** It is evident that the properties of the anal gills must be considerably changed in larvae accustomed to salt water. For, as already described (Wigglesworth, 1932a), if larvae from fresh water are immersed in 1 per cent. NaCl or "1.4 per cent." sea water, the cells of the gills swell up enormously. This swelling is resisted by the inherent elasticity of the cells, due at least in part to the vertical filaments which tie the cells to the cuticle. In larvae from salt water these filaments have become conspicuous strands. This suggests that the essential change in the gills of larvae accustomed to salt water is the increased strength of these elastic structures.
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If this be so, the cells of the gills should resist swelling when the concentration of the medium is still further increased. This is found to be the case. When larvae from 1 per cent. NaCl are exposed to 2 per cent. NaCl, there may be a little more swelling of the cells, but there is never the extreme distension seen when larvae from fresh water are immersed in 1 per cent. NaCl.\(^1\)

Furthermore, if the permeability of the various elements of the gills of larvae in salt water is the same as that already described in fresh water (Wigglesworth, 1932\(^a\)), the elastic strands in the cells must be subject to a constant strain, and therefore on placing the larvae in fresh water the cells should contract. This also is found to be the case. Fig. 1B shows the appearance of the gills in 50 per cent. sea water; Fig. 1C the same gills a few minutes after transference to fresh water. Conversely, the existence of this strain is further evidence that the blood of the larva is not in osmotic equilibrium with the outer fluid.

**Intestine.** There is a distinct change in the properties of the epithelium of the mid-gut. This may be readily demonstrated by placing under a supported coverslip some 1 per cent. NaCl or "1.5 per cent." sea water containing larvae accustomed thereto, introducing some larvae from fresh water, and adding a little solid trypan blue.

All the larvae take in the solid particles of dye very actively and for some time there is no difference between them. But at the end of about 2 hours the caeca of the "fresh-water larvae" are seen to be distended with a dilute solution of the dye (Fig. 2A), whereas in the "salt water larvae" the caeca are contracted and contain solid dye (Fig. 2B). It is evident that, as in the normal larvae already described (Wigglesworth, 1932\(^b\)), the larvae accustomed to salt water can absorb the fluid rapidly from the caeca; whereas larvae from fresh water are unable to do so. Enough salt water is, presumably, taken in with the solid particles of dye to affect the epithelial cells.

At the end of 24 hours the gut of the "salt-water larvae" contains a solid column of dye inside the peritrophic membrane, and solid masses of dye in the caeca, the gut epithelium being quite normal in appearance (Fig. 2D); whereas in the "fresh-water larvae" the gut contains very little solid matter within the peritrophic membrane, and none in the caeca; it is often greatly distended with fluid so that the cells are flattened and, in many places, tend to round themselves off and become separated from the epithelium (Fig. 2C). This happens in both 1 per cent. NaCl and "1.5-1.75 per cent." sea water.

Further, no trypan blue or indigo carmine is absorbed by the "salt-water larvae," whereas in the "fresh-water larvae" it is not uncommon to find some trypan blue in the pericardial cells or some solid indigo carmine in the lumen of the Malpighian tubes, showing that the permeability of the gut wall is abnormally increased by salt water in larvae not accustomed to it.

Thus, in larvae not accustomed to salt water the latter appears to act in the manner

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\(^1\) On the other hand, it is perhaps doubtful whether these "skeletal" elements in the cells can really resist the very great osmotic forces involved. Perhaps the more important changes in the cells are those affecting the cohesion of the colloids in the presence of salts, i.e. changes in the invisible elastic elements.
of a saline cathartic, which increases the fluid contents of the gut. Also, the substance which binds the cells together and to the basement membrane seems to be dissolved. In the "adapted" larvae the cells are not detached from the gut wall and the fluid is absorbed, although, as already pointed out, there may be rather more fluid than in larvae in fresh water.

![Fig. 2. A, larva, reared in fresh water, after immersion for 2 hours in 1 per cent. NaCl with solid trypan blue. B, larva, reared in 1 per cent. NaCl, after 2 hours in the same mixture. C, larva, reared in fresh water, after 16 hours in the mixture. D, larva, reared in 1 per cent. NaCl, after 16 hours. (From camera lucida drawings.)](image)

**UPTAKE AND EXCRETION OF FLUID BY LARVAE IN SALT WATER.**

In the preceding paper (Wigglesworth, 1932b) it has been shown that in fresh water the larvae absorb water by the anal gills and excrete it again through the Malpighian tubes. It was suggested that this uptake of water was a purely osmotic phenomenon. If so, it should not occur in hypertonic salt water.

This question has been tested in the same way as in the normal larvae (by ligaturing the body with a fine hair at the fifth abdominal segment so as not to obstruct the Malpighian tubes), and it has been found that in such larvae there is practically no discharge of fluid from the anus in the course of 1 or 2 hours. This is the case both in larvae adapted to 1 per cent. NaCl or "1·5 per cent." sea water.

This result agrees with the idea that the transference of water through the gills is due to osmosis alone. If this is so, since the medium is hypertonic to the blood, the larva must be losing water continuously by osmosis. It has not been possible to obtain direct evidence of such loss, but that it occurs is suggested by the fact that larvae reared in diluted sea water begin to shrink when the concentration reaches about "1·5 per cent." and by the time death occurs they are extremely contracted. This is not the case with larvae transferred directly from fresh water to such solutions; for it has been shown that the salt water can enter the gills of these, dissolving
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the cell contents and forcing away the inner membrane (Wigglesworth, 1932a); consequently there is no removal of water by osmosis and the larva dies in 24-48 hours without showing any shrinkage.

There is no active circulation of blood within the gills, and therefore the fluid they contain will tend to stagnate and come into osmotic equilibrium with the hypertonic medium outside, so that any exosmosis of fluid that occurs will be slow. But if water is being lost, even slowly, from the gills, the question arises as to how the larva maintains its body volume in hypertonic salt water without coming into osmotic equilibrium. It is clear that some part of the alimentary or excretory system must be doing work against osmotic pressure. Several possibilities exist: (i) the larva may be swallowing the hypertonic salt water and absorbing from it a fluid more dilute than the blood, (ii) the Malpighian tubes may be excreting hypertonic urine, (iii) water or a dilute solution may be reabsorbed in the rectum.

It is possible that all these factors contribute in varying degree, though how much each contributes has not been determined. The absorption of a dilute fluid from the gut has no special evidence to support it; but the fact that solid matter (uric acid) may sometimes occur in the Malpighian tubes strongly suggests that the urine is more concentrated than the blood, and certain observations in larvae in salt water have proved conclusively that reabsorption of fluid does occur in the rectum. Thus, after ligaturing the body just in front of the Malpighian tubes, there is often no excretion of fluid from the anus, and yet small drops of fluid may be carried down the hind-gut at regular intervals. For example, in one larva accustomed to 1 per cent. NaCl which was observed for an hour, fourteen small drops passed down the hind-gut at 4-min. intervals. During this same period only three minute drops were discharged from the anus, yet the rectum did not become distended. It is evident that fluid must have been reabsorbed in the rectum, and it is possible that the re-absorption may be exaggerated in these larvae accustomed to salt water.

EFFECT OF FRESH WATER ON LARVAE REARED IN SALT WATER.

If larvae accustomed to 45 per cent. sea water are returned to fresh water, the body volume usually increases, and the larva becomes abnormally swollen; but this effect is only temporary (or sometimes absent altogether), and within an hour or two the normal form is recovered and the larva shows no ill effects.

This abnormal swelling may be demonstrated more clearly if the larva is ligatured just in front of the Malpighian tubes, so as to restrict the part of the body into which the fluid enters. As described in the preceding paper, the "fresh-water larva" treated in this way shows no change in volume, the fluid absorbed by the gills being eliminated through the anus by the Malpighian tubes. But in the "salt-water larva" the Malpighian tubes seem unable to cope with the fluid that is taken in, and the body becomes excessively swollen; and when the swelling has reached a certain degree, the Malpighian tubes apparently cease to function, for no more fluid is passed down the hind-gut.

1 The fourth possibility, namely an extrarenal excretion of a concentrated salt solution such as occurs in marine bony fishes (Smith, 1932) seems highly improbable in the mosquito larva.
From this experiment it appears that the gills of the "salt-water larva" allow water to diffuse into them more rapidly than those of the normal larva. It was shown in the previous paper that, in the normal larva, water enters through the gills very slowly; but it could not be decided whether this was due to the cells being only slightly permeable, or whether they contain a mechanism which actively resists the osmotic inflow of water (such as that pictured by Adolph (1930) in the skin of the frog). So here it is uncertain whether the gills of the "salt-water larva" have become more permeable to water, or whether the hypothetical mechanism which normally excludes water has failed to develop.

But there seems to be another factor which leads to this abnormal swelling. In the preceding section of this paper a larva was described, which in 1 per cent. NaCl continued to pass drops of fluid down the hind-gut at intervals of about 4 min. and this fluid was mostly reabsorbed in the rectum. Now when this larva was immersed in tap water the excretion of fluid by the Malpighian tubes increased greatly, and drops of about the same size as before were passed down the hind-gut, on an average, every 2 min. for half an hour, and yet only three very small drops were discharged from the anus. The fluid did not accumulate in the rectum; it had clearly been reabsorbed into the body, which became excessively distended behind the ligature.

This observation suggests that an increased activity on the part of the rectum in reabsorbing fluid may be a factor both in the adaptation of the larva to salt water and in the excessive swelling which occurs when such larvae are ligatured and restored to fresh water.

It may be remarked here that there is much variation in the response of individual larvae. In some the Malpighian tubes cease to secrete very soon after the ligatured larva has been transferred to fresh water; in others a considerable amount of fluid is discharged from the anus, so that the amount absorbed in the rectum cannot be determined. In larvae whose Malpighian tubes contain solid matter (see p. 29) this is quickly washed out when they are transferred to fresh water.

DISCUSSION.

The general question of the relation of the body fluids of aquatic animals to their environment has been fully discussed in several recent reviews (Schlieper, 1930; Adolph, 1930; Pantin, 1931), so that an elaborate discussion is not necessary in this place.

It appears from the results described in this paper that the mosquito larva is homoisosmotic in both fresh water and salt water, though it cannot be said within what limits the osmotic pressure is kept. This result was to be expected in an animal derived from terrestrial ancestors; and it agrees with the few observations that have been made on other insects (e.g. *Dytiscus*, by Bachman (1912)).

The mosquito larva employed (*Aedes argenteus*) is not able to withstand very high concentrations of salt, being limited in pure sodium chloride to about 1.0-1.1 per cent. and in sea water to about 50 per cent. (isotonic with 1.75 per cent. NaCl). These results agree approximately with those of Macfie (1921) and of certain
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Brazilian observers (quoted by Howard, Dyar and Knab 1912). It may be noted here that some mosquito larvae can resist far higher concentrations of salt. Thus W. T. de Vogel (1907) found that *Anopheles rossi* (or *ludlowi*) would breed in sea water which had been concentrated to one-third its normal volume, and R. Vogel (1927) records larvae of *Aedes zammittii* Theo, in pools of concentrated sea water with crusts of salt on the surface. Other examples are quoted by Balfour (1921). Similar differences in resistance to salt water are familiar enough among fresh water fishes (Schlieper, 1930).

The results agree with those of previous writers (Ostwald, 1905, 1907; Koidsumi, 1931) in showing that the toxic effects of the hypertonic salt solutions are not due to osmotic pressure alone, as originally suggested by Paul Bert (1871), but that the salt itself has a destructive action on the living tissues. This is evidenced by the fact that pure sodium chloride kills larvae at a lower concentration than does diluted sea water. The latter is a balanced solution (in the sense of Loeb 1900); but, as Loeb and Wasteneys (1915) point out, it ceases to be a balanced solution above a certain concentration; and thus it is that when larvae from fresh water are placed in sea water, isotonic with 1.75 per cent NaCl, they show the same disruptive effects in the epithelium of the gut as they do in 1.2 per cent. of pure NaCl. It would be interesting to repeat these experiments on species with greater powers of resistance to salt water.

It was found by Martini (1923) that the gills of certain mosquito larvae reared in water containing salt were shorter than those of larvae from fresh water, and Vogel (1927) noted that in larvae occurring naturally in concentrated sea water the gills are absent or reduced to minute swellings. Since it has been shown (Wigglesworth, 1932b) that the anal gills are the only part of the body surface which is readily permeable to water, the resistance of the larvae to high osmotic pressures will obviously be favoured by their absence, and it has been seen (Wigglesworth, 1932b) that the anal gills are in fact readily caused to slough off by immersing the larvae for a short period in salt water. Some experiments were performed to see if larvae of *Aedes argenteus* deprived of their gills in this way were more resistant to salt water. They showed no increased resistance, which serves to confirm the conclusion that these larvae are not killed by the osmotic pressure but by the toxic action of the salt water, acting probably by way of the intestine.

It may be recalled that Schlieper (1930) suggests that the reduction of anal gills in mosquito larvae from salt water is associated with the diminished oxygen requirements in an animal which no longer has to maintain an osmotic pressure difference between its body fluids and the outer medium. But the explanation given above is more feasible. Martini (1923) himself was inclined to seek some adaptive significance for the shorter anal gills of larvae reared in salt water, but he does not disregard the possibility of this phenomenon being a direct effect of the salt on the cell colloids.

The other results recorded in this paper serve to show what a complex series of changes are involved in the adaptation of the larvae to salt water. These changes may be divided (rather artificially perhaps) into (i) changes in the physiological
activity of the organs, and (ii) changes in the colloidal substance of the surface membranes.

Under (i) may be mentioned the ability of the mid-gut to absorb fluid from the salt gut contents. There are several other possible changes, such as the excretion of a more concentrated urine by the Malpighian tubes, an increase in the activity of reabsorption in the rectum and, possibly, physiological changes in the cells of the anal gills. But these have not been demonstrated with any certainty.

Under (ii) may be included the changes in the cells of the anal gills, where the elastic filaments which hold the cells to the cuticle become much denser, and able to resist swelling and dispersion in the salt solution, and the changes in the epithelial cells of the gut (or perhaps in their intercellular matrix) so that they are no longer detached from one another, or from the basement membrane, by the salt water. It is possible that in these two cases the essential change in the colloids concerned is of the same nature, and it is probably because this change can only be effected very slowly that adaptation to salt water is possible only if the concentration is increased very gradually. It is well known that the stability of the intercellular matrices, as well as the surface membranes of the cells, is very different in different salts (Lillie, 1921; Gray, 1926), and this no doubt explains why the larva can become adapted to sea water more readily than to pure sodium chloride.

SUMMARY.

Larvae of *Aedes argenteus* reared in fresh water are killed by 1·1 per cent. NaCl or by “sea water”¹ isotonic with 1·3–1·4 per cent. NaCl. Newly hatched larvae are killed by 1·1 per cent. NaCl or “sea water” equivalent to 1·3 per cent. NaCl.

By gradually increasing the concentration, larvae can be made resistant to 1·1 per cent. NaCl and to “sea water” equivalent to 1·75 per cent. NaCl (50 per cent. sea water).

The nature of the physiological adaptation in these larvae has been studied and the following conclusions reached:

1. The elastic strands in the cells of the gills become exaggerated, and these cells resist swelling in hypertonic salt solutions.

2. There are changes in the epithelium of the mid-gut so that: (a) the cells are no longer caused to swell up and become detached from the basement membrane;

³ An artificial solution was used, in which the balance of metallic ions was approximately that of sea water.
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and (b) the mid-gut and caeca can absorb the salt fluid and so avoid the excessive distension which occurs in unadapted larvae.

3. It is possible that the Malpighian tubes excrete a more concentrated urine and that the reabsorptive activity of the rectum is increased.

The mosquito larva appears to be homoiosmotic in both fresh water and in hypertonic salt water.

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