OSMOTIC REGULATION IN MOSQUITO LARVAE: THE ROLE OF THE MALPIGHIAN TUBULES

By J. A. RAMSAY

From the Zoological Laboratory, University of Cambridge

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(With Three Text-figures)

INTRODUCTION

In a recent paper (Ramsay, 1950*a*) an investigation of the osmotic relations of the larvae of *Aedes* species was reported. Measurements of freezing-point depression were made on fluids collected from various parts of the body. In the case of *A. aegypti*, a fresh-water species, it was found that the fluid eliminated from the anus was strongly hypotonic to the haemolymph; the fluid entering the rectum from the intestine—presumably originating in the Malpighian tubules—was isotonic with the haemolymph. It was therefore concluded that the osmotic work required to produce a hyptonic 'urine' was carried out by the rectal epithelium and that the Malpighian tubules made no significant contribution to osmotic work. There is no reason to doubt this conclusion; but it is not to be taken to imply that the fluid excreted by the tubules is identical with the haemolymph in composition. The total concentration of the various osmotically active substances is virtually the same in both fluids, but their individual concentrations may be widely different. Evidence that the Malpighian tubules of *Limnophilus* larvae are concerned in ionic regulation has been presented by Boné & Koch (1942), who showed that the concentration of chloride in the intestinal fluid can be very different from that of the haemolymph.

It has not proved possible to collect intestinal fluid from a mosquito larva in quantity sufficient for the determination of chloride. But a method has recently been worked out whereby it is possible to determine sodium in small volumes of fluid, of the order of 10^{-3} cu.mm., and this has afforded a means of attacking the problem. The investigation now to be described is in the nature of an extension of the earlier work, and, to anticipate, it is demonstrated that although the fluid excreted by the Malpighian tubules has virtually the same osmotic pressure as the haemolymph, its sodium concentration can be very much lower.

MATERIAL AND METHODS

Larvae of *Aedes aegypti* were bred as before from eggs and were fed on a preparation of powdered dog biscuit. In the third instar they were removed to clean media and thereafter starved. The media used were distilled water, 0·75 and 1% NaCl. The distilled water was changed twice per week. The larvae were allowed to remain in the same medium for at least 5 days before being used for experiment.

Certain improvements were made in the method of collecting fluid from the intes-

tine. In the earlier work the larva was secured by two ligatures, one around the neck and the other around the respiratory siphon. Although there was good reason to believe that obstruction of the tracheal system was without effect on osmotic regulation in *Aedes* larvae, this feature of the technique was felt to be undesirable. It proved possible to construct a fine wire snare by which the base of the respiratory siphon could be held firmly but without constriction. With the larva secured in this way the spiracles were allowed to break the surface and could be observed to open and close in a natural manner.

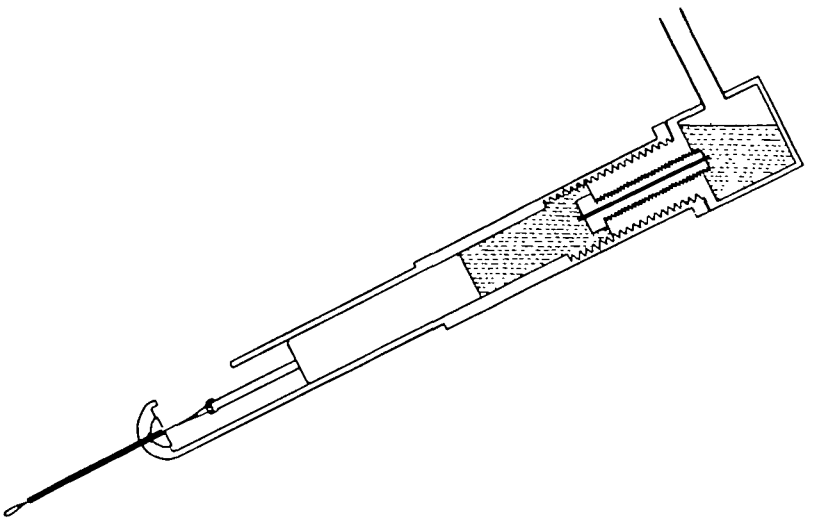

Fig. 1. For explanation see text.

The snare is illustrated in Fig. 1. It consists of a loop of tungsten wire, 0·002 in. thick, passed through a hypodermic needle. The ends of the loop are attached to a piston working in a cylinder which is filled with water and communicates with a second chamber through a fine glass capillary. A rubber tube connected to the second chamber is held in the operator's mouth. By blowing or sucking, the operator can extend or retract the loop, and the resistance offered by the capillary retards the movements of the piston so that accurate control is possible.

The neck of the larva was tightly ligatured as before. A glass cannula of about 100 μ diameter, mounted upon a micromanipulator, was inserted through the anus and rectum into the intestine as described in the earlier paper, and served to collect the droplets of fluid passing down the intestine. The larva, thus secured and cannulated, is illustrated in Fig. 2. It was also found possible, by stretching the larva, to straighten out the loop in the intestine sufficiently to allow the cannula to be passed right up to the pyloric chamber if necessary. Haemolymph was collected at the end of the experiment by drying the larva and puncturing it on a slide under liquid paraffin.

Although this technique is satisfactory in that the tracheal system can function normally and the anal gills are in free communication with the haemolymph, the disadvantage still remains that the midgut is open to the intestine and the fluid

collected is not necessarily derived from the Malpighian tubules alone. The forward bend taken by the Malpighian tubules after their origin from the pyloric chamber makes it impossible to close off the midgut by a ligature applied to the outside of

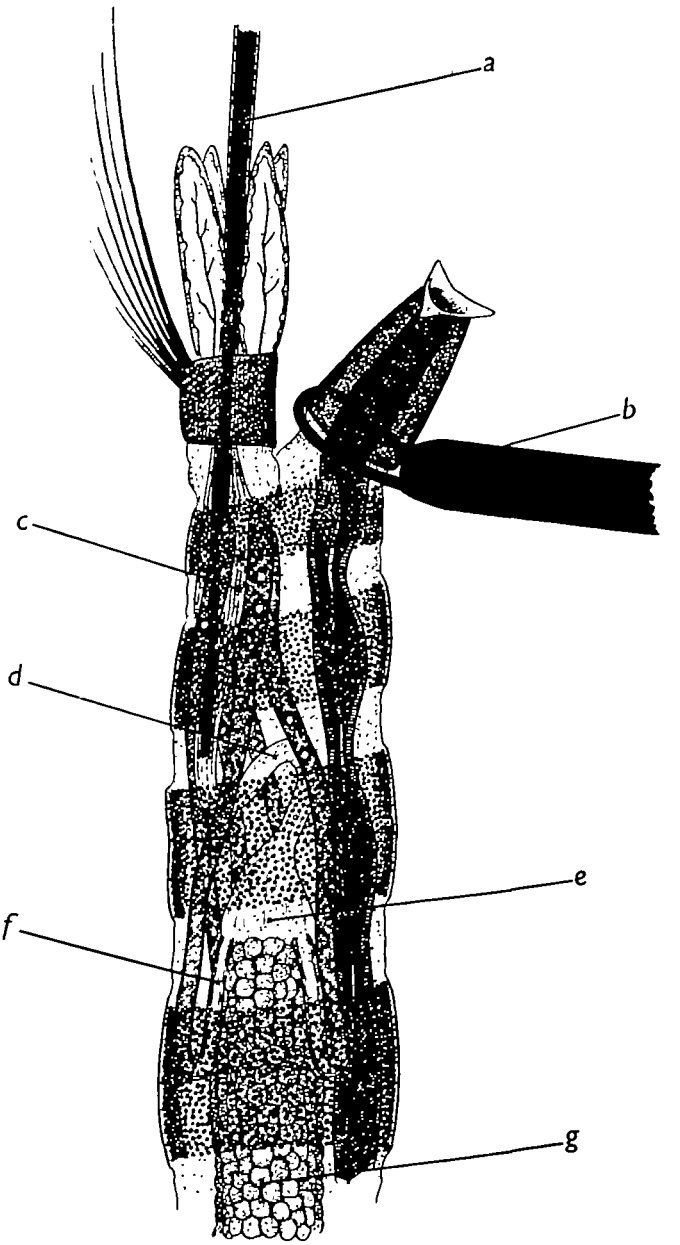

Fig. 2. Collection of intestinal fluid. *a*, cannula filled with liquid paraffin (stained with Sudan III) and inserted into posterior part of intestine; *b*, snare holding base of respiratory siphon; *c*, rectum with distal regions of Malpighian tubules applied to it; *d*, loop of intestine; *e*, pyloric chamber; *f*, proximal region of Malpighian tubule; *g*, midgut.

the larva. Attempts to obstruct the midgut just anterior to the pyloric chamber with droplets of liquid paraffin or bubbles of air were unsuccessful. Some experiments were therefore carried out on preparations of the Malpighian tubules removed from the animal, in which circumstances isolation of the midgut was possible.

The larva was placed on a slide under liquid paraffin. The body was first cut through between thorax and abdomen and then torn apart just anterior to the respiratory siphon. In this way the gut and Malpighian tubules were drawn out of the remains of the body. A fine silk ligature was then tied around the midgut just anterior to the pyloric chamber, and a cannula was inserted into the pyloric chamber via the intestine. In such a preparation the tracheal system was of course destroyed, and the question arose whether a sufficient supply of oxygen reached the tubules where they lay in a drop of haemolymph under a thin layer of liquid paraffin. It was not possible to make any direct measurement of the oxygen tension in the haemolymph, but an indication was obtained by adding a little ox blood to the preparation; spectroscopic examination showed that the haemoglobin remained well oxygenated for an indefinite period. This point having been established, the addition of ox blood was of course avoided in experiments in which fluids were taken for analysis.

Collections were also made directly from the Malpighian tubules removed from the body either (*a*) distally, from a point about one-quarter of the way down the tubule from its blind end, or (*b*) proximally, from a point just before the opening of the tubule into the pyloric chamber. The technique was very much the same as that employed on the earthworm nephridium (Ramsay, 1949*b*). The operation was carried out on a slide to which a cover-glass had been fixed to provide a 'step', and the tubule was kept in a drop of haemolymph under liquid paraffin. In (*a*) a fine silk ligature was tied around the tubule at the appropriate distance from the blind end, and the knot was held against the 'step' by a needle (in this case, of tungsten) while the collecting pipette was inserted. After the insertion a small droplet of liquid paraffin was extruded from the pipette to seal off the rest of the tubule from the puncture, and the point of the pipette was then thrust farther in so as to lie in the lumen beyond the droplet (see Fig. 3). In (*b*) no ligature was applied. The intestine was held down over the 'step' by the needle, the pipette was thrust through the wall of the pyloric chamber and worked into the tubule through the natural opening; the droplet of liquid paraffin was then extruded as before. These operations were carried out under the high-power binocular. The quantities of fluid collected were $2–3 \times 10^{-3}$ cu.mm.

After collection the samples of intestinal fluid and haemolymph were transferred to watch-glasses varnished internally with Bakelite 'Damarda' lacquer and filled with liquid paraffin; the lacquer, being hydrofuge, prevents the watery droplets from spreading over the surface of the watch-glass. The much smaller samples taken directly from the Malpighian tubules were retained in the collecting pipette and transferred directly to other pipettes. Measurements of osmotic pressure were carried out by the freezing-point method (Ramsay, 1949*a*) and measurements of sodium by an adaptation of flame photometry (Ramsay, 1950*b*; Ramsay, Falloon & Machin, 1951). The freezing-point measurements were carried out in duplicate and

the sodium measurements in triplicate. Both osmotic pressure and sodium concentration are expressed in terms of the equivalent concentration of NaCl per cent. The figures for osmotic pressure (based on duplicates) are believed to have a probable error of $< \pm 0{\cdot}01\%$ NaCl, and those for sodium (based on triplicates) to have a probable error of $< \pm 0{\cdot}02\%$ NaCl. The 't' test for significance has been used, and the values of 'P' are taken from Fisher & Yates (1938). $P < 0{\cdot}05$ is considered to be significant and $P < 0{\cdot}01$ to be highly significant. Table 1 gives the protocol of a single experiment.

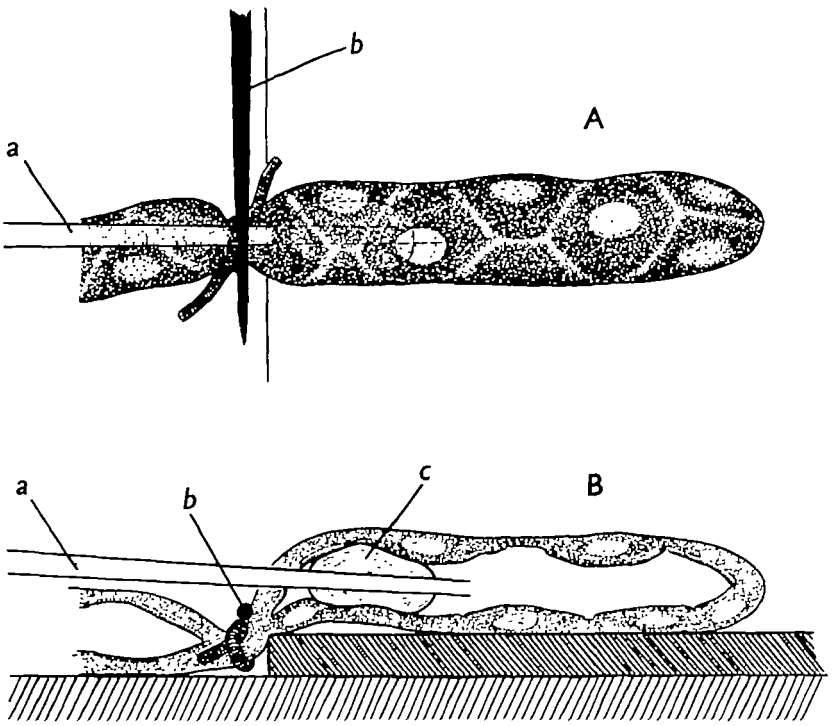

Fig. 3. Collection of fluid from distal region of Malpighian tubule. A, from above, as seen by operator; B, as imagined in vertical section. *a*, collecting pipette; *b*, tungsten needle; *c*, droplet of liquid paraffin.

RESULTS

Part I. Work was begun on the collection of intestinal fluid from larvae reared in distilled water and the results are presented in Table 2. The osmotic pressure of the intestinal fluid is seen to be a little lower than that of the haemolymph; the sodium concentration is very much lower, the difference being 0·37% NaCl, nearly 60% of the sodium concentration of the haemolymph.

It therefore appears that, although the power of the tubule to set up differences in osmotic pressure is negligible, a substantial difference in the concentration of sodium can be brought about. In reaching this conclusion, however, the assumption is made that the fluid collected from the intestine originates in the Malpighian tubules; the possibility that the concentration difference is brought about by the cells of the

midgut cannot wholly be excluded. In order to put this to the test collections were made from the intestine in preparations having the midgut isolated by a ligature as

Table 1. *Serial 7*

Flame photometer

	Deflexion		Deflexion
1. 0·5 % NaCl	192	7. Intestinal fluid	99
2. 0·5 % NaCl	189	8. Haemolymph	248
3. 0·5 % NaCl	198	9. Intestinal fluid	100
4. Haemolymph	246	10. 0·5 % NaCl	188
5. Intestinal fluid	96	11. 0·5 % NaCl	190
6. Haemolymph	247	12. 0·5 % NaCl	190

	Average deflexion	Concentration	
		Calibration curve	% NaCl
0·5 % NaCl	191	12·5	0·50
Haemolymph	247	17·5	0·70
Intestinal fluid	98	5·85	0·23

Freezing-point

(Distilled water: freezing-point, 2·990° C.; 1·00 % NaCl ≡ △0·600° C.)

	Freezing-point	△° C.	% NaCl	
Haemolymph	2·550	0·440	0·900	av. 0·908
Haemolymph	2·540	0·450	0·916	
Intestinal fluid	2·540	0·450	0·916	av. 0·916
Intestinal fluid	2·540	0·450	0·916	

	Osmotic pressure (% NaCl)	Sodium concentration (% NaCl)
Haemolymph	0·91	0·70
Intestinal fluid	0·92	0·23

Table 2. *Larvae from distilled water*

Serial	Osmotic pressure as % NaCl			Na as % NaCl		
	Haemo-lymph	Intestinal fluid	Diff.	Haemo-lymph	Intestinal fluid	Diff.
			+ −			+ −
1	0·83	0·82	0·01	0·52	0·25	0·27
2	0·85	0·81	0·04	0·65	0·37	0·28
3	0·75	0·63	0·12	0·65	0·31	0·34
4	0·68	0·60	0·08	0·65	0·18	0·47
5	0·72	0·65	0·07	0·69	0·25	0·44
6	0·70	0·65	0·05	0·62	0·22	0·40
			Av. −0·065 $P < 0·01$			Av. −0·37 $P < 0·01$

described in the preceding section. The results of these experiments are given in Table 3, and it can be seen that they are in conformity with the results given in

Table 2. We are therefore at liberty to believe that differences in sodium concentration between intestinal fluid and haemolymph are attributable to the activity of the Malpighian tubules.

The Malpighian tubules therefore play an active part in the retention of sodium in the haemolymph of larvae which are reared in distilled water; it now remains to

Table 3. *Larvae from distilled water (preparation)*

Serial	Osmotic pressure as % NaCl			Na as % NaCl		
	Haemo-lymph	Intestinal fluid	Diff.	Haemo-lymph	Intestinal fluid	Diff.
			+ −			+ −
7	0·91	0·92	0·01	0·70	0·23	0·47
8	0·82	0·85	0·03	0·75	0·18	0·57
9	1·13	1·05	0·08	0·78	0·38	0·40
10	0·72	0·65	0·07	0·51	0·27	0·24
			Av. −0·027			Av. −0·42
			P=0·05–0·04			P <0·01

discover whether their activity can be adapted to assist in getting rid of sodium under conditions in which this ion tends to accumulate in the haemolymph. The work of Boné and Koch on *Limnophilus* suggests that in this animal the fluid excreted by the tubules has a lower chloride concentration than the haemolymph when the external medium is poor in chloride, and a higher concentration when the external medium is rich in chloride. It would be of interest to discover a comparable relationship in the sodium balance of *Aedes*.

Table 4. *Larvae from* 0·75% NaCl

Serial	Osmotic pressure as % NaCl			Na as % NaCl		
	Haemo-lymph	Intestinal fluid	Diff.	Haemo-lymph	Intestinal fluid	Diff.
			+ −			+ −
11	0·95	0·90	0·05	0·77	0·80	0·03
12	0·92	0·83	0·09	0·81	0·65	0·16
13	1·14	1·01	0·13	1·10	0·69	0·41
14	0·96	0·91	0·05	0·85	0·60	0·25
15	0·98	0·90	0·08	0·94	0·59	0·35
16	0·95	0·92	0·03	0·98	0·38	0·60
			Av. −0·072			Av. −0·29
			P <0·01			P=0·05–0·01

Experiments were next carried out on larvae adapted to 0·75% NaCl. The results of these experiments, given in Table 4, show, first, that the sodium concentration of the haemolymph is greater than in larvae adapted to distilled water and, secondly, that the sodium concentration of the intestinal fluid is still significantly lower than that of the haemolymph. But the difference in sodium concentration is now smaller, both in the absolute sense (0·29% NaCl as compared with 0·37% NaCl) and in the relative sense (32% of the sodium concentration of the haemolymph as compared with 59%).

Table 5. *Larvae from* 1% NaCl

Serial	Osmotic pressure as % NaCl			Na as % NaCl		
	Haemo-lymph	Intestinal fluid	Diff.	Haemo-lymph	Intestinal fluid	Diff.
			+ −			+ −
17	1·15	1·23	0·08	1·33	1·36	0·03
18	1·16	1·12	0·04	1·15	1·14	0·01
19	1·54	1·50	0·04	1·07	1·02	0·05
20	1·41	1·26	0·15	1·36	1·39	0·03
21	1·33	1·20	0·13	1·31	1·19	0·12
22	1·13	1·08	0·05	1·11	1·08	0·03
			Av. −0·05 P=0·2–0·1			Av. −0·02 P=0·4–0·3

Further to test this point the experiments were repeated on larvae adapted to 1% NaCl. This external concentration is definitely harmful as judged by increased death-rate, but the larvae taken for use were active and appeared healthy. Table 5 gives the results of this series of experiments, and it can be seen that the sodium concentration of the intestinal fluid now closely approaches that of the haemolymph but certainly does not exceed it. It may therefore be said that the sodium-retaining activity of the tubule is capable of adaptation in respect of the availability of this ion, but not to the extent of an actual reversal of direction as is the case with chloride in *Limnophilus*.

It will be observed that in certain cases, e.g. serials 16, 17, 18, 20, the sodium concentration (as % NaCl) is greater than the osmotic pressure (as % NaCl) of the same fluid. There is, of course, no anomaly in this; it may mean either that some of the sodium is not in free solution, or, more probably, that sodium ions are in electrical balance with polyvalent anions.

Part II. In the vertebrate kidney it has long been known that the primary process of urine formation is the separation of an ultrafiltrate of the plasma in Bowman's capsule, and that the composition of this ultrafiltrate is modified during its subsequent passage through the tubule. Evidence has accumulated to show that an analogous process occurs in the excretory organs of many invertebrates (Picken, 1936, 1937; Krogh, 1938). The analogy is less easy to trace in the case of the Malpighian tubules of insects. In cryptonephric insects, such as the mealworm, it has been suggested that a process of ultrafiltration occurs in the modified distal portions of the tubules which are applied to the rectum (Poll, 1934; Patton & Craig, 1939). On the other hand, in their more usual arrangement the Malpighian tubules have been compared with the aglomerular nephrons of certain fishes (Boné & Koch). Even if there is no primary process of ultrafiltration it does not necessarily follow that urine of constant composition is uniformly excreted over the whole length of the tubule. Wigglesworth (1931) has described two regions in the Malpighian tubules of *Rhodnius*, of different histological appearance and with different physiological properties. In the distal region of the tubule the cells contain granules and the striated border next to the lumen is of the 'wabensaum' type; in the proximal region there are no granules and the striated border is of the 'börstensaum' type. Fluid is secreted into the

lumen in the distal region and resorbed in the proximal region, and there is evidence that both these processes may be selective in respect of dissolved substances.

In *Rhodnius* the two regions of the tubule are sharply discontinuous; in *Aedes* there is a gradual transition in histological appearance around the bend of the forwardly directed loop. In the distal region, which is of slightly greater diameter, the tubule appears opaque owing to the presence of granules; in the proximal region, just before the opening into the pyloric chamber, the cytoplasm becomes clear and transparent. Pagast (1936) has shown that the density of the granules (vacuoles, in his account) is related to the external medium in which the larvae are kept, the density being greatest in distilled water and least in solutions of NaCl. The presence of these granules makes it very difficult to study the striated border. In the course of the present work an examination was made of the tubules of larvae reared in different media. The tubules were examined fresh, in droplets of haemolymph under liquid paraffin, and were compared with tubules of *Rhodnius* similarly mounted. In the *Aedes* tubule the striated border at the proximal end is easily seen; it is usually sharply demarcated from the lumen by a clear edge, and while in some tubules there may be a slight fraying of this edge there is no unmistakable 'börstensaum' as in *Rhodnius*. Over the distal region the striated border is more difficult to observe, but it appears to be demarcated from the lumen by a sharp and slightly scalloped edge, characteristic of 'wabensaum'. The striated border is on the whole thinner in *Aedes* than in *Rhodnius*, but this may be related to the apparently greater distension of the tubules in *Aedes*. Wigglesworth (personal communication) examined the tubules of *Aedes* but was not able to recognize two distinct regions as in *Rhodnius* nor to decide that the tubule of *Aedes* could be identified with one or other of the two regions of the tubule of *Rhodnius*. Other descriptions of the Malpighian tubules of mosquitoes (Roubaud, 1923; Missiroli, 1925, 1927; de Boissezon, 1930) are mostly incomplete and generally based on fixed material. Lecaillon (1899) described 'prolongements ciliformes' of the living cells of the midgut and Malpighian tubules in *Culex pipiens*, but this observation does not seem to have been confirmed. A difference in function between the two ends of the tubule in *Aedes* could hardly be argued on the histological evidence.

In the present attempt to obtain physiological evidence larvae adapted to distilled water were used. The collections of fluid from distal and proximal regions were made as already described and were analysed for sodium only. The results are presented in Table 6. As far as the proximal region of the tubule is concerned little comment is required; there is a clear difference in sodium concentration between tubule fluid and haemolymph, of the same order as that given in Table 2. The fluid collected from the distal region shows a similar difference, but it is smaller and the variance is much greater. In one case, serial 25, the tubule fluid is identical with the haemolymph.

It is not particularly difficult to thrust the pipette into the lumen of the tubule, even at the distal end, but once inside the point is almost completely obscured by the granules. If penetration has been successful, the droplet of liquid paraffin can be seen to occupy the lumen of the tubule as it is extruded from the pipette. But it is then necessary to thrust the pipette farther in, so that its point lies beyond the drop-

Table 6. *Larvae from distilled water. Collection from Malpighian tubules direct*

Serial	Na as % NaCl					
	Distal region			Proximal region		
	Haemo-lymph	Tubule fluid	Diff.	Haemo-lymph	Tubule fluid	Diff.
			+ −			+ −
23	0·39	0·22	0·17	—	—	—
24	0·38	0·29	0·09	—	—	—
25	0·37	0·37	(0·00)	—	—	—
26	—	—	—	0·40	0·13	0·27
27	—	—	—	0·44	0·15	0·29
28	—	—	—	0·54	0·11	0·43
29	0·40	0·10	0·30	—	—	—
30	0·48	0·14	0·34	—	—	—
31	0·45	0·17	0·28	—	—	—
32	0·51	0·25	0·26	—	—	—
33	—	—	—	0·46	0·15	0·31
34	—	—	—	0·49	0·22	0·27
35	—	—	—	0·48	0·12	0·36
			Av. −0·24			Av. −0·32
			P=0·2–0·1			

let, and this movement has to be made 'blind'. Further, the walls of the Malpighian tubule do not seem to have the same self-sealing properties as are found in the annelid nephridium—which is one reason for sealing with a droplet of liquid paraffin behind the point of the pipette. It is therefore possible that the second thrust of the pipette may tear the wall of the tubule and allow the haemolymph to enter; it is even possible that the point of the pipette may pass right out of the tubule by penetrating the wall on the under side without being observed by the operator. This is very probably what happened in serial 25, and the smaller differences of serials 23 and 24 may possibly be the result of damage to the walls and some entry of haemolymph into the lumen.

In analysing these results serial 25 has been disregarded. The mean difference between tubule fluid and haemolymph is 0·24% NaCl for the distal region and 0·32% NaCl for the proximal region, and the difference between these two figures is not statistically significant.

This result is somewhat unsatisfactory. But if the variance associated with the distal region of the tubule is really due to faulty operative technique as has been supposed, and if one sees no reasonable prospect of eliminating such faults, then it is pointless to accumulate further data. One can only conclude that the experiments have failed to demonstrate a difference in sodium concentration between the two ends of the tubule; but one may add that if such a difference does exist it is unlikely to be greater than 0·1% NaCl.

DISCUSSION

This investigation was undertaken primarily to find the answer to one simple question. It had been shown earlier that the intestinal fluid (presumed to be derived from the Malpighian tubules) was isotonic with the haemolymph. Can the tubule separate a fluid containing more or less sodium than the haemolymph, or is it

non-selective as Patton & Craig concluded in the case of the tubules of the mealworm? The answer to this question is that the tubule can separate a fluid containing less sodium.

Arising out of this part of the investigation there are two matters which should not pass unremarked. First, it has been shown that if the midgut is ligatured off the composition of the intestinal fluid is not significantly altered (compare Tables 2 and 3). This might mean that the midgut and the tubules produce fluids of identical composition which then pass down the intestine; but it is very much more likely to mean that the normal flow of intestinal fluid is derived almost exclusively from the tubules—as has often been supposed, though upon very little evidence. Secondly, in the earlier paper the intestinal fluid was stated to be isotonic with the haemolymph; to be precise, the intestinal fluid was found to be hypotonic to the extent of 0·04% NaCl, but this difference was not statistically significant. In the present work the intestinal fluid has been found to be hypotonic to the extent of 0·065% NaCl, and the difference is statistically highly significant. 0·065% NaCl corresponds to less than 10% of the osmotic pressure of the haemolymph, and whatever it may be statistically, this small difference in osmotic pressure does not appear to be of any great physiological significance.

The scope of the investigation was then enlarged to embrace an extension of the original problem. If the tubule is capable of separating a fluid containing less sodium than the haemolymph, is this activity capable of modification under different physiological conditions? The answer to this question is that under conditions in which the sodium concentration of the haemolymph is high the sodium concentration of the tubule fluid approximates to that of the haemolymph; but it does not exceed that of the haemolymph as found by Boné & Koch for chloride in *Limnophilus*. From the figures published by Boné & Koch it might be objected that the relation which they found between the chloride concentration of the 'urine' and that of the external medium might largely be accounted for if the animal swallowed the external medium in considerable quantity and passed it through the midgut to the intestine. In a personal communication Prof. Koch informs me that in the course of the work on *Limnophilus* a certain number of experiments were made in which a ligature was applied to the neck of the larva, and this did not appear to reduce the 'urine' flow; it is therefore likely that the objection is met and that the relation is a real one. In the present state of knowledge we have no reason to suppose that the conclusions of Boné & Koch on *Limnophilus* are incompatible with the conclusions reached in the present work on *Aedes*. Nor is it impossible that the Malpighian tubules of *Corethra* eliminate a hypertonic fluid as is supposed by Schaller (1949); but it is to be noted that this contention is not supported by any analysis of the fluid.

Lastly, an approach was made towards a new and wider problem—the localization of function in Malpighian tubules. The results of this investigation on the tubules of *Aedes* were negative; but at the same time there was very little reason to have expected otherwise. The Malpighian tubules of *Aedes* are short and relatively simple; and when one considers the variety of form presented by the tubules of other insects,

not only in themselves but in their relations with other organs of the body, one cannot but feel that if an investigation of this wider problem is to make useful progress very careful consideration ought first to be given to the choice of material. It does not seem likely that such choice would fall upon mosquito larvae.

SUMMARY

1. The part played by the Malpighian tubules in the salt and water balance of *Aedes aegypti* larvae has been studied; the intestinal fluid and haemolymph have been compared in respect of freezing-point depression and sodium concentration.

2. It appears highly probable that the fluid passing down the intestine is derived from the Malpighian tubules with little or no contribution from the midgut.

3. When the larvae are kept in fresh water the intestinal fluid is very slightly hypotonic to the haemolymph (not isotonic as previously reported), but its sodium concentration is only about one-half that of the haemolymph.

4. When the larvae are kept in solutions of NaCl the difference in sodium concentration between intestinal fluid and haemolymph decreases. In an external medium of 1% NaCl the difference is abolished.

5. There is thus evidence that when the external medium is poor in salts the Malpighian tubules can contribute to the work of salt retention by excreting a fluid containing less sodium than the haemolymph; but there is no evidence that under any conditions they can excrete a fluid containing more sodium than the haemolymph.

6. Evidence of a decrease in the sodium concentration of the tubule fluid from distal region to proximal region is not statistically significant.

Once again it is a pleasure to thank Dr Wigglesworth for reading the typescript of this paper and for much valuable discussion.

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