

EXCHANGES OF SODIUM AND POTASSIUM IN  
MOSQUITO LARVAE

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## INTRODUCTION

In an earlier paper (Ramsay, 1950) an account was given of osmotic regulation in mosquito larvae of the genus *Aedes*. In the case of the fresh-water species *A. aegypti* L. with which the present paper is concerned, the situation is as follows. The surface of the body is relatively impermeable to water except over the anal gills (Wigglesworth, 1933*a*). Water enters the body through these organs, which are also capable of secreting chloride into the haemolymph against a concentration gradient (Koch, 1938). A fluid, very slightly hypotonic to the haemolymph, is elaborated by the Malpighian tubules. Some of this fluid appears to pass forwards through the mid-gut, eventually to be reabsorbed into the haemolymph through the caeca; some of it passes down the intestine to the rectum where it becomes hypotonic to the haemolymph before being eliminated from the body.

In a subsequent paper (Ramsay, 1951) the part played by the Malpighian tubules was further studied, particularly in relation to the excretion of sodium. It was found that when the larvae were kept in tap water the concentration of sodium in the fluid from the Malpighian tubules was always less than that in the haemolymph, and that when the larvae were kept in various concentrations of NaCl (up to 1% NaCl, which was the maximum tolerated) the concentration of sodium in the tubule fluid, although increased, never exceeded that in the haemolymph. It thus appeared that when the external medium was poor in salts the Malpighian tubules could contribute to the work of salt retention by excreting a fluid containing less sodium than the haemolymph; but no evidence was found to suggest that they could excrete a fluid containing more sodium than the haemolymph under conditions in which sodium was present in excess.

At that time sodium was the only element whose concentration could be determined in the small volumes of fluid available. The technique of flame photometry has now been extended to make possible the simultaneous determination of sodium and potassium in samples of the order of  $10^{-3}$  mm.<sup>3</sup> in volume (Ramsay, Brown & Falloon, 1953). With this method the study of salt and water balance in mosquito larvae can be carried a stage further.

## MATERIAL AND METHODS

Larvae of *A. aegypti* were reared to the fourth instar as described in the earlier papers referred to above. They were then removed to clean tap water and kept in this medium for at least a week before being subjected to the various treatments which will be described in due course.

The methods used for collecting various fluids were substantially the same as already described in the earlier papers, but certain modifications were found to give better results.

The collection of representative samples of rectal fluid has always presented difficulties. It is possible to insert a pipette into the rectum via the anus and to collect the fluid which happens to be present at the time, but the amount thus available is usually inconveniently small. It is also possible to tie a cannula into the anal canal and to collect over a period, but this has the disadvantage that the fluid passes into the cannula almost as soon as it reaches the rectum from the intestine, and does not remain in contact with the rectal epithelium as long as it does in the normal animal. In the present work the rectal fluid was collected as it was voided through the anus. The larva was first washed quickly with distilled water and then carefully dried on cigarette paper; it was secured in the usual way by a snare around the respiratory siphon and by a ligature drawn tight around the neck, and was thus held down upon a hollow-ground glass slide. A small quantity of liquid paraffin was added, sufficient to cover the larva except for the opening of the respiratory siphon. A fine capillary pipette was then applied to the region of the anus, and any fluid emerging rose at once into the pipette.

For the collection of intestinal fluid the larva was secured as above and was immersed in the medium in which it had been kept prior to the experiment.

To collect midgut fluid a cannula was inserted through the mouth to about half way down the midgut. During collection the larva was kept in moist air, impaled upon the cannula. The insertion of the cannula is not difficult but the food and faeces in the gut often block it, so that the proportion of failures in this operation is relatively high.

Four determinations (of sodium and potassium simultaneously) were made on each sample and were compared with at least four determinations on a reference solution, the mean values being used for calculation. The standard error of figures given in the tables is believed to be of the order of  $\pm 2\%$  of the concentrations. The variation between samples of the same fluid taken from different animals was of a much greater order (standard deviation about  $\pm 10\%$  or greater) so that the errors of analysis do not enter significantly into any of the conclusions which are drawn. It is one of the shortcomings of the method that when the concentration of sodium exceeds that of potassium by a factor of 20 or more it is not possible to maintain the potential accuracy for both elements. This situation arises in the case of the haemolymph, and where relatively small differences in concentration were under study the two elements were determined separately (Tables 1 and 2).

Concentrations are expressed in mm./l. or m.equiv./l. Since in earlier work concentrations were expressed as % NaCl it may be helpful to state the relation  $1\% \text{ NaCl} \equiv 171 \text{ mm./l. NaCl}$ .

Where any doubt appeared to exist about the reality of differences between one fluid and another the 't' test for significance was applied, and the values of *P* were obtained from the tables of Fisher & Yates (1938). The usual conventions are adopted, i.e.  $P < 0.05$  is significant,  $P < 0.01$  is highly significant.

RESULTS

(1) *Sodium and potassium content of whole larvae.* Fifty larvae were taken from the stock in tap water, washed and dried with cigarette paper and then weighed in a platinum crucible. They were then dried and ashed at 600° C. and the ash was extracted with dilute HCl. Determination of sodium and potassium in this extract showed that these elements were present in whole larvae in the following amounts:

Sodium 0.066 m.equiv. per gram wet weight.  
 Potassium 0.033 m.equiv. per gram wet weight.

In a later experiment larvae from another culture were used. They were first placed for 5 days in a slow current of distilled water and then divided into two lots, one being placed in NaCl, 17 mm./l., and the other in KCl, 17 mm./l. After 5 days in these solutions fifty larvae were taken from each, washed quickly in distilled water, dried and analysed as above. The results are given below:

	Medium, NaCl, 17 mm./l. (m.equiv./g.)	Medium, KCl, 17 mm./l. (m.equiv./g.)
Sodium	0.080	0.074
Potassium	0.027	0.038

These changes in the sodium and potassium content of the larvae very clearly reflect the changes in the external medium, though in very much less degree. This is as one might expect, and by themselves these figures tell us very little. When they are considered in relation to similar figures for the haemolymph further conclusions can be drawn.

(2) *Sodium and potassium concentrations in the haemolymph.* Larvae were taken from stock and placed in a slow current of distilled water for 7 days. At the end of this time haemolymph was collected from six larvae and the remainder were divided between the following four solutions: NaCl, 85 and 1.7 mm./l.; KCl, 85 and 1.7 mm./l. After 5 days in these solutions six larvae were taken from each and the haemolymph collected. The results of the analysis are presented in Table 1.

Table 1. *Effects of different external media upon the composition of the haemolymph*

(Concentrations in m.equiv./l.)

Distilled water			NaCl, 85 mm./l.		NaCl, 1.7 mm./l.		KCl, 85 mm./l.		KCl, 1.7 mm./l.					
ial	Na	K	Serial	Na	K	Serial	Na	K	Serial	Na	K	Serial	Na	K
1	89	4.1	7	120	—	13	96	—	19	—	4.4	25	—	4.4
2	89	3.1	8	115	—	14	90	—	20	—	5.3	26	—	3.9
3	75	3.0	9	112	—	15	107	—	21	—	6.9	27	—	4.1
4	91	3.5	10	100	—	16	103	—	22	—	5.0	28	—	4.6
5	85	2.4	11	115	—	17	100	—	23	—	5.9	29	—	4.1
6	93	2.7	12	119	—	18	106	—	24	—	6.5	30	—	4.0
v.	87	3.1	—	113	—	—	100	—	—	—	5.7	—	—	4.2

The figures in Table 1 show that the sodium and potassium concentrations in the haemolymph remain relatively constant in the face of considerable changes in the external medium. At the same time the small differences observed, e.g. in the sodium concentrations 113–100 and 100–87 m.equiv./l., are real ( $P < 0.01$ ), and therefore the internal medium does in fact follow the changes in the external medium, albeit to a limited extent.

Since the 'whole' larvae considered in the previous subsection came from a different culture and were not exposed to the same solutions as the 'haemolymph' larvae now under discussion, some caution is necessary when comparing the figures. Nevertheless, it seems clear that the sodium:potassium ratio in the whole larva is about 2.5:1, whereas in the haemolymph it is about 25:1. If we assume that the haemolymph is some 50% of the wet weight the change in the sodium content of the whole larvae (as between different external media) is very nearly accounted for by the change in the concentration of sodium in the haemolymph. Presumably most of the sodium is in the haemolymph and not in the tissues. But the change in the potassium content of the whole larva is very much greater than can be accounted for by the change in the haemolymph. Presumably most of the potassium is held in the tissues, and furthermore the amount so held is dependent upon the availability of potassium in the external medium. At first sight it is surprising, in view of the very low concentration of potassium in the haemolymph, that these larvae can survive some 3 weeks or longer in a current of distilled water without losing all their potassium; but it seems likely that the tissues constitute a reserve from which the low potassium concentration of the haemolymph can be maintained for a long time.

(3) *Uptake by the anal gills.* In the experiment described in § (2) above, all the larvae were exposed to distilled water before being placed in salt solutions; the figures in Table 1 can therefore be used to show that the larvae are able to take up sodium and potassium from the external medium even when the concentrations of these elements in the external medium are lower than in the haemolymph. The demonstration by Koch (1938) that chloride is taken up from the medium by the anal gills and secreted into the haemolymph against the concentration gradient makes it seem highly probable that these same organs are also concerned in the active uptake of cations.

As Wigglesworth (1933*a*) has shown, it is possible to destroy the anal gills by exposing the larvae to 5% NaCl for 2–3 min. Gill-less larvae were prepared in this way and, together with an equal number of normal larvae from the same culture, were placed in a slow current of distilled water. After 5 days some (both gill-less and normal) were transferred to KCl, 1.7 mm./l.; after 8 days others were transferred to NaCl, 85 mm./l. Haemolymph was collected after 12–14 hr. exposure to these solutions. This short exposure was suggested by the work of Wigglesworth (1938), who found that after transference from distilled water to salt solutions the larvae showed an initial period of over-compensation during which abnormally high values of chloride concentration were found in the haemolymph; it was hoped that over-compensation would exaggerate any differences which might exist between gill-less larvae and normal larvae.

The results of these experiments are summarized in Table 2, and the differences as between gill-less and normal larvae are highly significant. They support the view that the anal gills are able to take up sodium and potassium from the external medium and secrete them into the haemolymph against the concentration gradient.

Table 2. *Effects of removal of the anal gills upon the uptake of sodium and potassium from dilute external media*

(Concentrations of sodium and potassium in the haemolymph, m.equiv./l.)

Distilled water → NaCl 85 mm./l.				Distilled water → KCl 1.7 mm./l.			
Normal		Without gills		Normal		Without gills	
Serial	Na	Serial	Na	Serial	K	Serial	K
31	131	37	85	43	7.8	49	4.2
32	129	38	112	44	5.6	50	4.7
33	103	39	117	45	8.0	51	5.6
34	116	40	99	46	6.2	52	3.4
35	129	41	90	47	5.3	53	4.1
36	110	42	78	48	5.7	54	3.8
Av.	120	—	97	—	6.4	—	4.3

(4) *Excretion by the Malpighian tubules.* Reasons have been given (Ramsay, 1951) for believing that the fluid which passes down the intestine is mainly derived from the Malpighian tubules. Collections have been made of this fluid from larvae kept in a current of distilled water (5 days): in NaCl, 85 mm./l. (10 days); and in KCl, 85 mm./l. (8 days). All these larvae were taken from the same culture. The results of the analysis are presented in Table 3.

Table 3. *Effects of different external media upon the composition of the intestinal fluid*

(Concentrations in m.equiv./l.)

Distilled water			NaCl, 85 mm./l.			KCl, 85 mm./l.		
Serial	Na	K	Serial	Na	K	Serial	Na	K
55	23	81	63	101	73	67	31	128
56	26	87	64	72	92	68	14	140
57	18	106	65	66	94	69	26	151
58	16	104	66	45	101	70	22	134
59	13	69	—	—	—	—	—	—
60	31	80	—	—	—	—	—	—
61	40	77	—	—	—	—	—	—
62	28	102	—	—	—	—	—	—
Av.	24	88	—	71	90	—	23	138

It has already been shown that sodium and potassium enter the body from the external medium. They leave the body by way of the Malpighian tubules, from

which the intestinal fluid is derived. It would therefore be natural to expect that the concentration of sodium (or potassium) in the intestinal fluid would increase or decrease conformably with increase or decrease of the sodium concentration in the external medium. This is seen to be the case. If we compare the intestinal fluid of the 'NaCl larvae' with that of the 'distilled water larvae' we find that the sodium concentration is greater while the potassium concentration is the same; and if we make the comparison between the 'KCl larvae' and the 'distilled water larvae' we find conversely that the potassium concentration is greater while the sodium concentration is the same. What is unexpected is to find that in the intestinal fluid the potassium concentration is always greater than the sodium concentration, irrespective of the composition of the external medium. This is true not only of the average figures but also of the individual figures, with the single exception of serial 63, Table 3. Furthermore, in the earlier work (see Introduction), it was found for sodium that the concentration in the intestinal fluid *never* exceeded the concentration in the haemolymph; we now find for potassium that the concentration in the intestinal fluid *always* exceeds the concentration in the haemolymph. This matter receives further consideration in the Discussion.

(5) *Midgut fluid.* This is a convenient point, before discussing the composition of the midgut fluid, at which to describe certain observations which relate to the further course of the fluid which passes from the Malpighian tubules into the gut. The Malpighian tubules open into the gut in the region of the pyloric chamber, at the junction of midgut and intestine. From the pyloric chamber droplets of fluid can be seen to pass backwards, by peristalsis, down the intestine. The addition of non-diffusible dyes, such as trypan blue, to the gut contents indicates that there is a movement of fluid forwards from the pyloric chamber through the midgut to the caeca, and through the walls of the caeca to the haemolymph. So much is already known from the observations of Wigglesworth (1933*a*), confirmed by the writer (Ramsay, 1950). In the absence of any evidence to the contrary, it is natural to suppose that the fluid originating in the Malpighian tubules contributes to these two movements; but we have no idea of how the total flow is divided between the two channels and how the division is regulated.

For purposes of inserting a cannula through the anus into the intestine it is helpful to stretch the body of the larva. A chance observation suggested that the amount of intestinal fluid collected was greater if the body was kept stretched than if it was allowed to relax to a more normal length. This observation seemed worth following up; but since a stretching force applied between the respiratory siphon and the neck is not one which is normally encountered by the larva, it was decided to stretch the body by the more natural method of increasing the volume of the haemolymph.

For these experiments larvae were taken direct from the stock in tap water. The haemolymph of five larvae was collected and mixed in a cannula which was then tied into the body of a sixth larva just behind the thorax. By applying slight pressure or suction the body of the larva could be stretched or caused to collapse. The droplets passing down the intestine were counted over periods of 10 min. in the normal

condition and in the stretched condition; unfortunately in the collapsed condition it was not possible to see the intestine:

	Preparation A (droplets)	Preparation B (droplets)
Stretched	10	12
Normal	0	0
Stretched	13	12

It seems that we have here a simple nervous mechanism by which the body volume is regulated. The peristaltic waves passing down the intestine are initiated and probably coordinated by the central nervous system—at least, that is a reasonable inference from the fact that no peristalsis is observed when the intestine is removed from the body and its connexions with the central nervous system are severed. Increase in the volume of the haemolymph stretches the body, and this appears to be the stimulus which initiates peristalsis; fluid passes down to the rectum and is voided through the anus. If the volume of the haemolymph is less than normal and the body wall is not under tension, peristalsis fails to occur and the fluid from the Malpighian tubules passes forwards through the midgut and is not lost to the body.

It is of some slight interest to know how far the sodium and potassium concentrations of the tubule fluid are maintained during passage through the midgut. Collections were made from the middle region of the midgut, from five larvae taken directly from the stock in tap water and placed in a medium of NaCl, 85 mM./l., KCl, 4.3 mM./l.; this fairly saline medium was used since in saline media there is greater evacuation of the solid contents of the midgut (Wigglesworth, 1933*b*). The results of the analyses are presented in Table 4, from which it can be seen that even at this short distance from the pyloric chamber there is a substantial return towards the sodium:potassium ratio characteristic of the haemolymph.

Table 4. *Composition of midgut fluid in external medium: NaCl, 85 mM./l.; KCl, 4.3 mM./l.*  
(Concentrations in m.equiv./l.)

Serial	Na	K
71	171	24
72	174	44
73	230	31
74	131	18
75	117	16
Av.	165	26

(6) *Rectal fluid.* Collections of rectal fluid were made from the same groups of larvae as were used for collections of intestinal fluid. By the time the larvae were used for experiment they had remained in their various media for some 3 weeks. The analyses are presented in Table 5.

Table 5. *Effects of different external media upon the composition of the rectal fluid*

(Concentrations in m.equiv./l.)

Distilled water			NaCl, 85 mm./l.			KCl, 85 mm./l.		
Serial	Na	K	Serial	Na	K	Serial	Na	K
76	1	5	80	81	22	84	22	74
77	15	16	81	117	7	85	19	77
78	1	43	82	87	24	86	11	92
79	0	36	83	113	18	87	2.5	114
Av.	4	25	—	100	18	—	14	90

Taking first the 'NaCl larvae', and comparing them with the 'distilled water larvae', we find that the sodium concentration of the rectal fluid is greater while the potassium concentration is not significantly different. Taking the 'KCl larvae' we find, conversely, that the sodium concentration is virtually the same while the potassium concentration is greater. We can now compare the figures in Tables 3 and 5, and from them see how the composition of the intestinal fluid is altered during its passage through rectum.\*

(i) In the 'distilled water larvae' both sodium and potassium are absorbed in the rectum ( $P < 0.01$ ).

(ii) In the 'NaCl larvae' potassium is absorbed in the rectum ( $P < 0.01$ ) but the concentration of sodium is not significantly changed.

(iii) In the 'KCl larvae' potassium is absorbed in the rectum ( $P < 0.01$ ), but only to a limited extent, while the concentration of sodium is not significantly changed.

This last conclusion is unexpected. We know from the 'distilled water larvae' that both sodium and potassium can be absorbed in the rectum, the former against the concentration gradient. In the 'NaCl larvae' it is reasonable to expect that this absorption of sodium will be held in abeyance since the element is entering the body all the time and must be got rid of. But in the 'KCl larvae' one would have expected that sodium would be absorbed and that all the potassium would be got rid of.

The difficulties of obtaining representative samples of rectal fluid have been touched upon in the Introduction; in brief, what they amount to is that under the conditions of the experiment the fluid is voided prematurely and is not kept in contact with the rectal epithelium for as long as it is normally. One cannot believe that the figures for the rectal fluid of 'distilled water larvae' are representative of the normal composition of the fluid which leaves the anus; for if the larva can live for 3 weeks in a current of distilled water—as these larvae had—and if the concentration of potassium in the fluid voided from the rectum is 25 m.equiv./l., then a simple calculation shows that all the potassium in the body would be lost if the

\* There is no direct evidence to show whether the changes which occur in the rectum are brought about by absorption or by secretion, but there is some indirect evidence in favour of the absorption theory and this is accepted as a working hypothesis for the present.



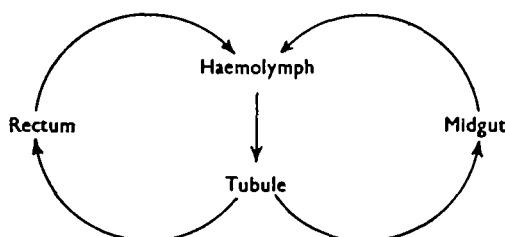
Rate of elimination was as much as 0.05 mm.<sup>3</sup>/hr. The rate of collection of rectal fluid during the present experiments was undoubtedly very much greater than this, and it seems likely that the fluid did not remain in the rectum long enough for absorption to run its full course. The concentration of sodium in the intestinal fluid of the 'KCl larvae' is in any case already somewhat low, and if it has not been possible to demonstrate a further reduction of concentration in the rectum, this failure is probably attributable to the technical imperfections of the experiment. The question of the retention of potassium by the 'KCl larvae' is discussed in the next section.

There is no doubt that the uptake of sodium in the rectum takes place against the concentration gradient; in the case of potassium the movement is from high concentration to low concentration and there is no need to postulate any process of active transport.

#### DISCUSSION

The results of the experiments described in this paper have been discussed as they were presented and there is not a great deal to be added now. In outline, the sodium and potassium exchanges of the larva are straightforward. When the larva is in tap water, sodium (and potassium if it is present) is taken up by the anal gills and secreted into the haemolymph. Both sodium and potassium are present in the fluid which leaves the Malpighian tubules, and both elements are absorbed from this fluid before it leaves the rectum. When there is excess of sodium in the external medium the net inward flux is increased, the concentration of sodium in the tubule fluid rises and the absorption of sodium in the rectum is held in abeyance so that the sodium escapes from the body. The same terms can also be used to describe the movements of potassium when this element is present in excess.

But much is thereby left unsaid. It is not clear why the concentration of potassium in the tubule fluid is always greater than that of sodium even when there is no inward flux of potassium, and it is not clear why there is considerable absorption of potassium in the rectum under conditions which call for the elimination of this element. What is clear is that quite irrespective of the medium in which the larva finds itself there is a circulation of potassium within the body:



Without measurements of electrical potential to support the measurements of concentration it is not possible to establish that the movement of an ion against a concentration gradient is due to the active transport of that ion. No measurements

of potential difference across the wall of the Malpighian tubule have yet been made. If, therefore, it is now suggested that the circulation of potassium is driven by some sort of 'potassium pump' operating in the Malpighian tubules, this will be understood to be no more than a tentative working hypothesis.

A similar state of affairs has been found in the Malpighian tubules of *Rhodnius* (Ramsay, 1952). In *Rhodnius*, as in *Aedes*, there is very much more sodium than potassium in the haemolymph. In the upper portion of the tubule in *Rhodnius* the fluid contains very much more potassium and less sodium than the haemolymph; in the lower portion the concentration of potassium falls and that of sodium rises so that the composition of the fluid alters towards that of the haemolymph. There is, therefore, a circulation of potassium in *Rhodnius*, comparable with that in *Aedes*.

Since the circulation of potassium in *Aedes* is not obviously adaptive in relation to ion exchanges with the external medium, and since a comparable circulation is found in the unrelated genus *Rhodnius*, one is tempted to see it as something which is fundamental to the process of urine formation in Malpighian tubules. At the moment this can be no more than speculation; but it will certainly be interesting to see how far a high concentration of potassium is demonstrable in the Malpighian tubules of other insects.

#### SUMMARY

1. The movements of sodium and potassium through the body and their exchanges between the body and the external medium have been studied in the larva of *Aedes aegypti* L.
2. Briefly, both elements enter the body via the anal gills, are excreted by the Malpighian tubules and can be absorbed from the tubule fluid in the rectum. When either element is present in the medium to excess its concentration in the tubule fluid is increased and the extent of its absorption in the rectum is decreased.
3. Under all conditions the concentration of potassium in the tubule fluid is greater than its concentration in the haemolymph, and the concentration of sodium is less. There is a circulation of potassium from haemolymph to tubule, to rectum or midgut and so back to haemolymph.
4. The composition of the haemolymph in respect of sodium and potassium is remarkably constant in the face of changes in the medium.
5. The volume of the larva appears to be regulated by a nervous mechanism. If the volume is increased peristaltic waves pass down the intestine more frequently and more fluid is voided through the anus.

I wish to thank Dr V. B. Wigglesworth for reading this paper in typescript and for his observations upon it.

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