THE ABSORPTION OF CHLORIDE IONS BY THE ANAL PAPILLAE OF DIPTERA LARVAE

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(With One Text-figure)

UNTIL recently the respiratory function of the anal papillae of Diptera larvae was unquestioned; they were generally called "anal gills". There has been, however, much discussion concerning the part played by these organs in the uptake of oxygen. Using Protozoa as indicators of oxygen tension, Fox (1920) proved that the uptake of oxygen was only slightly, if at all, higher in these organs than in the other parts of the skin. Wigglesworth (1933*a*) investigated, by the same method, the respiratory uptake in *Aedes* and obtained similar results.

Wigglesworth also showed that, in *Aedes*, the anal gills have a high permeability to water and that the animals absorb through them a fairly large amount of liquid: he, therefore, regards them as "water absorbing organs".

According to Harnisch (1934) the situation is exactly the opposite in *Chironomus*, viz. that the general body surface is relatively more permeable to water than are the anal gills.

This conclusion, however, is denied by Pagast (1936) after experimenting upon these larvae with hypertonic sugar solutions in combination with ligatures.

Most of the work of Pagast with *Aedes* confirms the results of Martini (1923) who showed that there is a relation between the size of these organs and the electrolyte content of the external medium. Pagast points out that the gills are probably permeable to sodium ions as well as to water, he agrees with Wigglesworth in regarding these structures as water-absorbing organs.

In previous papers, on the other hand, I (H. J. Koch, 1934*a*, *b*) put forward the hypothesis that the function of these and other organs in invertebrates might be analogous to that of the renal tubules of the vertebrate kidney. If so, they should absorb salts from the surrounding medium and secrete them into the general body cavity to compensate the loss of salts brought about by excretory processes.

As experiments in the Zoophysiological Laboratory of Copenhagen) showed that the skin of frogs and other fresh-water animals is able to take up chlorides from very dilute solutions, I was glad to have the opportunity of testing the localization of this function in invertebrates.

Frogs (Krogh, 1937) take up Cl from NaCl, absorbing both chloride and sodium ions; they absorb less chloride from KCl. From $CaCl_2$ they exchange chloride ions

for bicarbonate ions already present in the body; since calcium ions are not absorbed, it is concluded that the ion which is actively taken up by the skin is always the chloride ion. Frogs also actively absorb bromine ions; the iodide ion, however, penetrates only slowly by diffusion.

My experiments have shown firstly that Diptera larvae take up salts (as measured by the total chloride content of the larvae) from very dilute solutions and, secondly, that this active absorption takes place exclusively via the anal papillae. (See preliminary note, H. J. Koch and A. Krogh, 1936.)

MATERIAL AND GENERAL METHOD

Starving, nearly full-grown larvae in good condition were used. *Chironomus* material was brought by aeroplane from Belgium, while *Culex* material was collected during the months of May and June in small ponds in the neighbourhood of Copenhagen.

Chloride analyses of the whole larvae were carried out by means of Rehberg's method (1926) as modified by Schnohr (1934). This method is accurate to $\frac{1}{2}$ per cent of the total amount of chloride present. Before analysis, the larvae were dried on filter paper: for each sample 100–150 mg. of material were generally used. This is equivalent to 4–6 *Chironomus* larvae or 10–12 *Culex* larvae. The larvae were in all experiments first treated for a finite number of hours with distilled water continually renewed by means of a funnel with a lateral tube as shown in Fig. 1. The water was kept in motion and aerated by a stream of air bubbles.

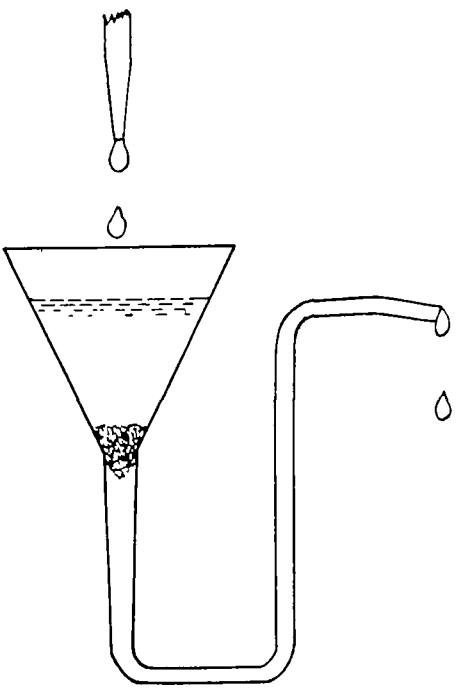

Fig. 1

The larvae were afterwards treated in the same way with chloride solution of a strength corresponding to 1/100 frog's Ringer. The Cl concentration of this solution is 0·039 mg. Cl per c.c. which is approximately the concentration in ordinary fresh water. The figures are expressed in milligrams Cl per gram animal.

PARENTERAL UPTAKE OF CHLORIDE

If starving *Chironomus* or *Culex* larvae are washed with distilled water for a number of hours their chloride content decreases.

When animals treated in this way are afterwards washed with 1/100 frog's Ringer they take up chloride readily (see Table I). It will be noticed that the absorption is more rapid in *Culex* larvae.

Table I

Species	Series	No. of hours of treatment	No. of analyses	Range of figures	Mean
A Initial chloride content					
Chironomus	A_1	—	3	0·55–0·56	0·56
	B_1	—	5	0·51–0·60	0·55
Culex	I	—	2	1·00–0·98	0·99
B. Effect of treatment with distilled water					
Chironomus	A_1	48	2	0·33–0·36	0·35
	B_1	59	3	0·32–0·35	0·33
Culex	I	20	1	0·68	0·68
C. Effect of subsequent treatment with Ringer/100					
Chironomus	A_1	10½	3	0·56–0·61	0·59
	B_1	13¼	2	0·51–0·66	0·58
Culex	I	4	1	1·03	1·03

It is not difficult to prove that the rise of the chloride level in *Chironomus* is independent of any absorption by the gut. By making a ligature just behind the head in *Chironomus*, by means of a hair or a thin silk thread, the absorption of fluid through the mouth is prevented. Larvae treated in this way absorb chloride as fast as controls without ligatures (Table II).

Table II

Species	Series	No. of hours treated	No. of analyses	Range of figures	Mean
A. Initial chloride content after washing with distilled water					
Chironomus	H_1	54	3	0·27–0·29	0·28
	L_1	65	5	0·26–0·34	0·30
B. Ligature behind the head. Effect of treatment with Ringer/100					
Chironomus	H_1	19	3	0·41–0·44	0·42
	L_1	18½	1	0·65	0·65
C. Controls without ligature					
Chironomus	H_1	19	2	0·41–0·47	0·44
	L_1	18½	3	0·66–0·68	0·67

These experiments prove beyond doubt that *Chironomus* larvae are able to take up chloride otherwise than by absorption through the mouth. Alternative sites of absorption are (i) the general body surface or some part of it, (ii) the hind gut. The latter alternative need only be considered if the larvae were able to pump water into this part of their gut as is the case during the respiratory movements of the larvae of *Odonata*. No pumping movement has ever been observed by us in *Culex* or *Chironomus*, nor does it appear to be mentioned in the literature.

THE PARENTERAL UPTAKE OF CHLORINE BY SECRETORY ACTIVITY OF THE ANAL PAPILLAE

That the general body surface is not responsible for the chloride uptake is proved by experiments in which a ligature is made round the last body segment (the ligature being behind the ventral tubuli (gills), their possible action in salt uptake should be detectable).

The results of such an experiment was as follows (Table III).

Table III

Species	Series	No. of hours treated	No. of analyses	Range of figures	Mean
A. Initial chlorine content after washing with distilled water					
Chironomus	A_{II}	60	2	0·42–0·45	0·43
	B_I	67	3	0·31–0·39	0·34
B With ligature round last body segment. Effect of treatment with Ringer/100					
Chironomus	A_{II}	11	3	0 40–0·46	0·43
	B_I	10½	2	0 29–0 36	0 33
C. Controls without ligature					
Chironomus	A_{II}	11	3	0·62–0·77	0·71
	B_I	10½	2	0·47–0·53	0 50

This type of ligature prevents the manifestation of any activity on the part of the body surface lying behind the ligature and any possible uptake by the hind gut. As already pointed out, no observation supports the uptake by the hind gut.

It was therefore concluded that the anal papillae, showing a highly specific structure and properties are presumably responsible for the uptake of chloride.

I tried to obtain more definite indications about their function by destroying them.

Table IV

Species	Series	No. of hours treated	No. of analyses	Range of figures	Mean
A. Initial concentration of chloride after treatment with distilled water					
Chironomus	B_I	59	3	0·32–0·35	0·33
Culex	A_1	20	1	0·68	0·68
	B_2	10½	1	0 65	0·65
B. Effect of Ringer/100 after destruction of papillae with $AgNO_3$					
Chironomus	B_1	13¼	2	0 18–0·19	0·19
Culex	A_1	4	1	0·46	0·46
	B_2	3¼	1	0·35	0·35
C. Effect of Ringer/100 without destruction of papillae					
Chironomus	B_1	13¼	2	0·51–0·66	0·58
Culex	A_1	4	1	1·03	1·03
	B_2	No controls			

When the animals are treated with 0·2 per cent. $AgNO_3$ solution a precipitate appears in the anal papillae and at no other place (Gicklhorn & Keller, 1925; Koch, 1934*b*).

The precipitation of the silver salt kills the cells, affording an easy method for the differential destruction of the anal papillae.

In larvae treated in this way the chlorine level falls rapidly and death follows after two or three days. It was found that such larvae were unable to take up chloride.

This is shown clearly by the following figures for *Culex* and *Chironomus* (Table IV):

These experiments do not definitely prove that the chloride uptake is brought about by the activity of the anal gills: it may be objected that by treating the animals with $AgNO_3$ not only the anal gills are put out of action, but also other cells or organs which are really responsible for the chloride uptake. This objection has much less weight in an experiment in which the anal papillae of *Culex* larvae were destroyed by means of immersion for 5 min. in 20 per cent NaCl solution. This method has been used by Wigglesworth (1933*b*) and by Pagast for making gill-less larvae, which continue to live normally but grow more slowly.

The result of this experiment was as follows (Table V):

Table V

Species	Series	No. of hours treated	No. of analyses	Range of figures	Mean
A. Previous treatment: distilled water					
Culex	A_1	20	1	0·68	0·68
	B_{1a}	65	2	0·88–0·97	0·93
	B_{1b}	103	1	0·62	0·62
B. After destruction of anal papillae with NaCl 5 per cent and subsequent treatment with Ringer/100					
Culex	A_1	4	1	0·49	0·49
	B_{1a}	5	3	0·70–0·77	0·74
	B_{1b}	5	2	0·27–0·38	0·33
C. Controls with pupillae in Ringer/100					
Culex	A_1	4	1	1·03	1·03
	B_{1a}	No controls	—	—	—
	B_{1b}	No controls	—	—	—

The possibility of a distant poisoning action of a chemical nature was eliminated in experiments in which the anal papillae are destroyed by heat. It is not very difficult to provoke the coagulation of the protoplasm in the cells of the anal gills of *Chironomus* larvae by means of a hot needle: this alteration is visible owing to a change of colour.

The result (Table VI) in respect to the function of the anal gills is the same as that found in larvae treated with $AgNO_3$.

Since a destruction of the anal papillae, either by chemical procedure or by heat is always coupled with loss of salt, it is conceivable that an uptake of salt may

occur, but owing to an enormously increased condition of permeability salts are washed out (with the inflowing water continually eliminated) or lost, at such a rate that a rise of the chloride level cannot be detected.

Table VI

Species	Series	No. of hours treated	No. of analyses	Range of figures	Mean
A. Chloride content after treatment with distilled water					
Chironomus	B_1	Unwashed	5	0·51–0·60	0·55
	H_3	65	4	0·27–0·36	0·32
B. Effect of treatment with Ringer/100 on animals with 4 burned anal papillae					
Chironomus	B_1	21½	2*	—	—
	H_3	22¾	3	0·11–0·23	0·17
C. Control with normal gills in Ringer/100					
Chironomus	B_1	No control	—	—	—
	H_3	22¾	4	0·62–0·73	0·68

* No chloride detectible.

Such a possibility is completely eliminated, or at least reduced to a minimum, by making a ligature round the papillae and afterwards destroying them with $AgNO_3$. (A ligature alone cannot ensure the complete elimination of gill function, because with such ligatures, one can never be sure that a small part of the papillae at the base will not remain in communication with the body cavity and so maintain activity.) For such purposes two ligatures were made round the gills with very thin silk threads, binding together the two of each side; the larvae were afterwards treated for 2 min. with 0·2 per cent $AgNO_3$ solution. This experiment gave the following results (Table VII): showing that uptake of salt does not, in fact, occur.

Table VII

Species	Series	No. of hours treated	No. of analyses	Range of figures	Mean
A. Initial chlorine content after treatment with distilled water					
Chironomus	O_1	60	3	0·27–0·31	0·29
B. Effect of treatment with Ringer/100 after ligature round 4 gills and subsequent treatment with $AgNO_3$					
Chironomus	O_1	22½	2	0·16–0·24	0·20
C Control normal treated with Ringer/100					
Chironomus	O_1	22½	3	0·52–0·61	0·55

In the animals of this experiment the eventual uptake of water into the rectum could continue, yet the result shows, however, that there was no uptake of chloride.

It is, therefore, possible to conclude that the anal papillae are the only organs responsible for the uptake of chloride from the surrounding medium.

This conclusion is strengthened by an experiment in which the uptake continues—but is of course slower—in animals in which the function of 2 of the 4 gills is prevented by a ligature.

This is shown by the following figures (Table VIII):

Table VIII

Species	Series	No. of hours treated	No. of analyses	Range of figures	Mean
A. Initial chlorine content after treatment with distilled water					
Chironomus	L_1	65	5	0·26–0·34	0·30
B. Effect of treatment with Ringer/100 after making a ligature round 2 gills					
Chironomus	L_1	18½	3	0·44–0 67	0·56
C. Control without ligature					
Chironomus	L_1	18½	3	0·66–0·68	0·67

Since, at the beginning of all these experiments, the concentration of Cl in the animals was nearly ten times as high as in the outside medium, it becomes clear that this absorption is an active process involving the expenditure of energy.

UPTAKE OF THE OTHER IONS

It has long been known that the renal tubules of vertebrates cannot distinguish between Cl and Br, the same fact was found for the uptake of salts by the frog's skin (A. Krogh). The salt-absorbing mechanism of *Chironomus* shows the same peculiarity as proved by this experiment (Table IX). The uptake of NaBr, however, is slower than the uptake of NaCl.

Table IX

Species	Series	No of hours treated	No. of analyses	Range of figures	Mean
A. Initial chloride concentration after washing with distilled water					
Chironomus	*F*	65	2	0·27–0·29	0·28
B. Effect of treatment with NaBr Mol 0·00111					
Chironomus	*F*	48	3	0·38–0·47	0·42*
C. Control treated with NaCl Mol. 0 00111					
Chironomus	*F*	48	3	0·56–0·70	0·64

The figures give Cl + Br calculated as Cl.

FUNCTION OF THE ANAL PAPILLAE AND OSMO-REGULATION

Since these organs absorb chlorides from solutions as dilute as ordinary fresh water it is evident that they are concerned in osmo-regulation.

The amount of salt absorbed from the outside medium is equal to that which is lost through other parts of the body.

Part of the salt loss probably occurs in the urine. A comparison of the salt loss in pure distilled water and distilled water +3 per cent glucose suggests, however, that the loss through the urine is small. With the glucose solution the urine flow must be reduced on account of the reduction of osmotic inflow of water. The result was as follows (Table X):

Table X

Species	Series	No of hours treated	No of analyses	Range of figures	Mean
A. Initial chloride content					
Chironomus	Q_1	—	4	0·47–0·56	0 53
B. Treated with pure distilled water					
Chironomus	Q_1	23	3	0·41–0·46	0·44
C. Treated with distilled water + 3 per cent glucose					
Chironomus	Q_1	23	3	0·42–0·45	0·43

As there is no significant difference in salt loss in the two cases it is probable that the main loss of salt occurs through the whole integument.

That there is a salt loss and an important one through the body surface, and that this loss is compensated by the activity of the anal papillae is shown clearly by the following experiment (Table XI).

Table XI

Species	Series	No. of hours treated	No. of analyses	Range of figures	Mean
A. Initial concentration					
Chironomus	*P*	—	3	3·81–4·50	4·15
B. Treated with tap water + ½ glucose					
(*a*) Normal animals					
Chironomus	*P*	16	2	4·19–5·83	5·01
(*b*) With ligature round last body segment					
Chironomus	*P*	16	2	2·73–2·92	2·82

Animals of the same stock were kept in tap water; some animals were completely normal, others had a ligature round the last body segment (this ligature prevents both salt uptake and urine loss). The chlorine is calculated on a basis of dry weight of the animals, because the ligatured animals swelled somewhat.

This experiment proves that the integument in this fresh water species at least is permeable to salts, and that the anal papillae play an important part in maintaining the steady state of the salt content of the body fluid.

It seems probable that the difference in size of the anal papillae of larvae from different biotopes or reared in different salt solutions (Martini and Pagast) is a functional adaptation to salt absorption from these media.

SUMMARY

By micro-chloride determinations combined with other observations, it is shown that *Chironomus* and *Culex* larvae are able to take up chloride parenterally from solutions with a chloride content corresponding to that of ordinary fresh water. This active absorption takes place exclusively in the anal papillae (anal gills). Since, in *Chironomus*, salt diffuses continually through the whole body surface, these organs, by their salt-absorbing function, play an important part in maintaining the salt content of the body fluid.

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