THE EXCRETION AND STORAGE OF AMMONIA BY THE AQUATIC LARVA OF *SIALIS LUTARIA* (NEUROPTERA)

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INTRODUCTION

Delaunay (1931), in a review of the invertebrates, showed that an excellent correlation existed between the nature of the major nitrogenous component of the excreta and the nature of the environment, aquatic or terrestrial, in which an animal lived. Ammonia was shown to predominate in the excreta of aquatic species, urea or uric acid in the excreta of semi-terrestrial or terrestrial species. Delaunay put forward the view that the synthesis by these terrestrial forms of more complex molecules from ammonia was essentially a detoxication mechanism necessitated by a restricted water supply.

Although the insects are primarily a terrestrial group, representatives of a number of orders have become aquatic in one or more stages of their life histories. It is well known that those terrestrial species which have been examined, with the notable exception of blowfly larvae, excrete the bulk of their nitrogen in the form of uric acid (Wigglesworth, 1950). The possibility, however, that aquatic species might have reverted to ammonotelism does not seem to have been examined.

Preliminary tests were carried out on the excreta of a variety of aquatic insects. In all cases ammonia was found to be the major nitrogenous excretory product. An investigation into various aspects of the metabolism, toxicity and excretion of ammonia was then undertaken on the aquatic larva of *Sialis lutaria*. It is the purpose of the present communication to present some observations on the excretion and storage of ammonia in this species.

MATERIAL AND METHODS

Larvae of *Sialis* were obtained in abundance from ponds in the vicinity of Newcastle. After collection they were starved for a period of at least 1 week in slowly running tap water. During this period the bulk of the food residues in the gut was eliminated.

Ammonia was estimated by the ultra-micro diffusion method described by Shaw & Beadle (1949). Using N/100 acid and alkali, quantities of ammonia were estimated ranging from 0.1 to 1.0 μg. N with a standard deviation of 0.005 μg. N.

Total nitrogen was estimated by the ultra-micro Kjeldahl method of Shaw & Beadle (1949). Using N/20 acid and alkali, quantities of nitrogen, ranging from 0.5 to 5.0 μg., were estimated with a standard deviation of 0.04 μg. N.

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The procedure adopted for the extraction of ammonia from whole animals was as follows. A single larva was weighed then ground up for 3-4 min. in the bottom of a Pyrex centrifuge tube (1 x 7 cm.) containing glass powder and 0.5 or 1.0 ml. of a 10% solution of trichloroacetic acid. The tube was then centrifuged, and samples of the supernatant taken for analysis 30 min. later. The total volume of supernatant was obtained by combining the original volume of trichloroacetic acid with the water content of the larva. Desiccation experiments showed that the amount of water contained in a single larva was in the region of 80% of the original body weight. The liberation of ammonia which occurs when tissues are ground in water was found to be completely inhibited by trichloroacetic acid. Furthermore, suitable experiments showed that the extraction of ammonia was complete. The precaution of emptying the hindgut was always taken before extracting ammonia.

Larvae were weighed on a 500 mg. torsion balance.

For purposes of collecting either foregut fluid or hindgut fluid an animal was first narcotized in water through which CO₂ was gently bubbled (Beadle & Shaw, 1950) and then dried on filter-paper. The foregut fluid, generally brown in colour, was removed by inserting a glass capillary into the foregut through the mouth. In general, a sufficient amount was obtained to enable duplicate ammonia estimations to be made, each requiring in the order of 0.3 μl. The larva could be induced to eject the clear, colourless hindgut fluid by gently stimulating the hind region with a glass capillary when signs of recovery started to appear. As the fluid appeared, generally as a discrete drop, it was collected in the capillary. Generally sufficient was obtained to enable duplicate ammonia estimations to be made, each requiring in the order of 0.15-0.30 μl. Contamination of the clear midgut fluid with brown foregut fluid was never observed to occur.

The method of Beadle & Shaw (1950), with slight modification, was used for collecting haemolymph. First, the pronotum was made hydrofuge by application of wax made molten with a heated needle. A puncture was then made and the haemolymph extruded by gently squeezing the abdomen between forefinger and thumb. As the haemolymph appeared it was collected in a glass capillary. The amount of haemolymph removed generally varied from 10 to 20% of the original body weight.

THE EXCRETION OF AMMONIA

(a) Ammonia in the excreta

In order to establish the extent to which nitrogen was being excreted in the form of ammonia, duplicate total N and ammonia N analyses, each requiring 58 μl., were carried out on the water (0.5-0.75 ml.) in which a larva had been kept for a period of 2 days. The glass tube (2 x 7 cm.) containing water and larva was corked during the experimental period. Control experiments showed that there was no loss of ammonia during this period. Five experiments were performed with larvae varying in weight from 40 to 83 mg. The total N excreted averaged 11.0 (5.5-17.5) μg./100 mg. wet weight/24 hr. Of the total N excreted 63-97% (av. 86%) was found to be in the form of ammonia. The figures in brackets refer to the lowest and highest values respectively.
Confirmation that the volatile base estimated by diffusion analysis was in fact ammonia was obtained by comparing results obtained by diffusion analysis with results obtained by the well known Nessler colorimetric method. Six samples of excreta were subjected to analysis. The differences between the results obtained by the two methods were found, when subjected to the 't' test for significance, to be no greater than those which would be expected by chance. Trimethylamine, which has been found in the excreta of the marine teleost *Lophius piscatorius* (Grollman, 1929), is perhaps the only other volatile base likely to be met with in the excreta. In low concentration, however, trimethylamine develops neither colour nor precipitate with Nessler's reagent. The similarity of the results obtained by the two methods indicates, therefore, that the volatile nitrogenous base which is excreted is in fact ammonia.

(b) The site of ammonia excretion

The intestine of *Sialis* consists of a capacious foregut (generally containing a brown fluid), a very narrow midgut, and a hindgut, capable of distension, in which a clear colourless fluid accumulates, to be expelled periodically through the anus. The Malpighian tubules enter the intestine at the junction of midgut and hindgut. There seems little reason to doubt, although no experiments have been carried out to test this assumption, that the hindgut fluid is in fact urine having origin in the Malpighian tubules. In support of this contention, contamination of the clear, colourless hindgut fluid with brown foregut material has never been observed to occur.

Ammonia was never found in the foregut fluid (results of five separate estimations). On the other hand, the concentration of ammonia in the hindgut fluid was found to be very high—the results of five separate estimations, performed on the fluid removed from different larvae, ranged from 97 to 159 mg. ammonia N/100 ml. (average 136 mg. N/100 ml.). These results suggest that ammonia is being excreted by the Malpighian tubules. No attempt has yet been made, however, to establish the concentration of ammonia in the tubules.

Although it seemed unlikely, on the basis of the results just mentioned, that any excretion of ammonia across the general body surface was in fact occurring, the possibility was nevertheless checked. To test this possibility nine animals were treated in the following way. To prevent any elimination of fluid from the gut the mouth of each animal was blocked with wax and the region of the body just in front of the anus ligatured. Each animal was placed in 3 ml. of distilled water. At the end of 42 hr. the ammonia content of the water was determined. Ammonia was found to be absent in all but two cases, and then the quantity of ammonia found—less than one-tenth of that normally excreted by animals of similar weight—was very small, probably due to slight leakage of the highly ammoniacal hindgut fluid. It can be concluded, therefore, that ammonia is not excreted across the general body surface.
THE STORAGE OF AMMONIA

(a) The ammonia content of tissue fluids of normal larvae

Estimations have been made to establish to what extent the tissue fluids are being maintained ammonia free.

The total ammonia content of thirteen larvae was determined. In all cases the ammonia content was found to be very low, varying from 0.5 to 2.2 μg. N/100 mg. wet weight of tissue (average 1.0 μg. N).

Separate estimations were performed to determine the concentration of ammonia in the haemolymph. Analyses (duplicate) made within 1 min. after extraction and then 10 min. later (using 1.8 μl. for a single analysis) failed to detect any liberation of ammonia in the shed fluid. It is presumed, therefore, that the concentration of ammonia found in the shed haemolymph represents the actual concentration found in vivo.

Accurate measurements of the concentration of ammonia in the haemolymph were obtained by making duplicate estimations, each requiring 14 or 28 μl., on the combined fluid removed from a number of larvae. Care was taken to ensure that the whole process from extraction to analysis took no more than 10 min. Estimations were made on five such samples. The results obtained, varying from 0.37 to 0.76 mg. ammonia N/100 ml. (average 0.50 mg. N/100 ml.), demonstrated that ammonia was not completely removed from the haemolymph.

(b) The effect of preventing excretion on the ammonia content of the body

In the hope of obtaining some answer to the question of whether larval tissues are capable of storing ammonia the following experiment was performed.

An animal was narcotized with CO₂ and then dried on filter-paper. The mouth was blocked (to prevent drinking) by the application of molten wax and a ligature applied round the abdomen (the site of ligature varied for reasons given below). The region posterior to the ligature was now cut off and the wound waxed over. The preparation was kept in an approximate isotonic solution of dextrose (6.2 g./100 ml. changed every day) for 5 days at room temperature. At the end of this period analyses were performed to establish the concentration of ammonia in the haemolymph and the total ammonia content of the body portion. The volume of haemolymph extracted was determined by weighing the preparation before and after extraction. The total ammonia content of the extruded haemolymph was combined with the total ammonia content of the body portion to give the true ammonia content of the body portion.

Two groups, each of six animals, were prepared. In the first group the ligature was applied between the fourth and fifth abdominal segments. This ligature lies in front of the point of entry of the Malpighian tubules into the gut. Thus there can be no question of urine passing into the gut. Animals of the second group were ligatured between the thorax and abdomen. These preparations are of interest because the Malpighian tubules do not extend further forwards than the junction between thorax and abdomen. Thus such preparations are devoid of Malpighian tubule tissue.
All preparations were alive at the end of 5 days. It has been found, however, that 'head-thorax' preparations rarely live much longer. The other type of preparation may live, and appear healthy, for as long as 3 weeks.

In the case of those preparations retaining the anterior portion of the abdomen the concentration of ammonia in the haemolymph varied from 0.0 to 0.9 mg. N/100 ml. (average 0.6 mg. N/100 ml.) and the total ammonia content of the body varied from 1.2 to 1.8 μg. N/100 mg. wet weight of tissue (average 1.4 μg. N/100 ml.).

In the case of those preparations ('head-thorax') devoid of Malpighian tubule tissue the concentration of ammonia in the haemolymph varied from 0.0 to 1.4 mg. N/100 ml. (average 0.6 mg. N/100 ml.) and the total ammonia content of the body varied from 0.6 to 1.7 μg. N/100 mg. wet weight of tissue (average 1.1 μg. N/100 mg.).

The values obtained in the case of both types of preparation are of the same order as those values obtained on normal larvae. These results would suggest, therefore, that larval tissues have the capacity to 'store' appreciable quantities of ammonia. Furthermore, although the Malpighian tubules themselves may be able to 'store' ammonia, this property is also found in non-Malpighian tubule tissue. The alternative possibility that deamination may have stopped does not seem feasible.

On the basis that the daily ammonia output of larvae averages 10 μg. N/100 mg. wet weight/24 hr. during starvation it can be calculated that the experimental larvae have 'stored' in the region of 50 μg. N/100 mg. wet weight of tissue in 5 days.

(c) The disappearance of ammonia in larval tissue

Support for the view that larval tissues are capable of 'storing' ammonia was obtained in the following way. The mouth of a larva was blocked with wax to prevent drinking and then the ammonia content raised by immersion for some time in a 30 mg. N/100 ml. solution of ammonia. This method of raising the ammonia content is discussed in greater detail below. A ligature was now made between the thorax and abdomen. Haemolymph was removed from the abdomen and the concentration of ammonia determined. The abdomen was now cut off and discarded. After waxing the site of amputation the anterior portion ('head-thorax') was placed in 1.0 ml. N/1000 HCl (to trap any ammonia which diffused out) for a period of 7-8 hr. At the end of this period both the ammonia content of the HCl and the anterior portion of the larva were determined and the results combined. Comparison was now made with the amount of ammonia calculated to be present in the anterior portion when first placed in dilute acid. This calculation was made on the basis of previous experiments which involved determination of the concentration of ammonia in the haemolymph removed from the abdomen followed by almost simultaneous determination of the total ammonia content of the anterior body portion. The results obtained (Table 1) show, as might be expected, that the total ammonia content of the anterior body portion is closely related to the concentration of ammonia in the haemolymph. On the average, the figure expressing the total ammonia content of the anterior body portion (μg. N/100 mg. wet weight) was found to be 10% greater than the figure obtained for expressing the concentration of ammonia in the haemolymph (mg. ammonia N/100 ml.).
Excretion and storage of ammonia by larva of Sialis lutaria

Table 1. Results of experiment to determine the relationship between the concentration of ammonia in the haemolymph and the total ammonia content of the anterior portion (head-thorax) of larvae of Sialis

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of ammonia in haemolymph (mg. NH₄-N/100 ml.)</td>
<td>3.2</td>
<td>4.7</td>
<td>6.0</td>
<td>6.8</td>
<td>7.3</td>
<td>7.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Weight of head-thorax (mg.)</td>
<td>43</td>
<td>48</td>
<td>55</td>
<td>52</td>
<td>39</td>
<td>33</td>
<td>47</td>
</tr>
<tr>
<td>Ammonia content of head-thorax (µg. NH₄-N/N100 mg.)</td>
<td>2.4</td>
<td>4.0</td>
<td>7.0</td>
<td>7.4</td>
<td>6.4</td>
<td>10.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Table 2. Results of experiment comparing the ammonia recovered from the head-thorax at the end of 7–8 hr. immersion in N/1000 HCl with that calculated to be present before immersion

<table>
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<tr>
<th>Time of events</th>
<th>Exp. no.</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>Av.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before placing in N/1000 HCl</td>
<td>Concentration of ammonia in haemolymph (mg. NH₄-N/100 ml.)</td>
<td>11.5</td>
<td>11.5</td>
<td>12.0</td>
<td>14.4</td>
<td>15.5</td>
<td>17.0</td>
<td>9.7</td>
</tr>
<tr>
<td>Weight of head-thorax (mg.)</td>
<td>51</td>
<td>52</td>
<td>40</td>
<td>31</td>
<td>42</td>
<td>40</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Ammonia content of head-thorax (µg. NH₄-N/N100 mg.)</td>
<td>11.2</td>
<td>13.4</td>
<td>13.0</td>
<td>16.0</td>
<td>16.0</td>
<td>22.0</td>
<td>10.7</td>
<td></td>
</tr>
</tbody>
</table>

The experiment was performed on six larvae (Table 2). In order to confirm the results obtained the same experiment was performed on another six larvae, but instead of determining the total ammonia content of the anterior body portion at the end of 7–8 hr. the concentration of ammonia in the haemolymph was determined. Comparison was then made with the concentration which was present after immersion in the ammonia solution. The results are presented in Table 3.

The preparations remained alive and active during the experimental period.

The results obtained show that a mechanism exists in the tissues of larvae of Sialis for removing ammonia in chemical combination. Thus the ammonia recovered at the end of 7–8 hr. averaged only 26% of that calculated to be present after immersion in the ammonia solution.
Table 3. Results of experiments comparing the ammonia recovered from the head-thorax (calculated) at the end of 7–8 hr. immersion in N/1000 HCl with that calculated to be present before immersion

<table>
<thead>
<tr>
<th>Time of events</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Av.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before placing in N/1000 HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of ammonia in haemolymph (mg. NH₃-N/100 ml.)</td>
<td>5.8</td>
<td>8.2</td>
<td>5.6</td>
<td>14.8</td>
<td>12.6</td>
<td>11.8</td>
<td>—</td>
</tr>
<tr>
<td>Weight of head-thorax (mg.)</td>
<td>42</td>
<td>48</td>
<td>43</td>
<td>52</td>
<td>37</td>
<td>50</td>
<td>—</td>
</tr>
<tr>
<td>Ammonia content of head-thorax (µg. NH₃-N/100 mg. calculated)</td>
<td>6.4</td>
<td>9.0</td>
<td>6.2</td>
<td>16.3</td>
<td>13.9</td>
<td>13.0</td>
<td>—</td>
</tr>
</tbody>
</table>

| After 7–8 hr. in N/1000 HCl |    |    |    |    |    |    |     |
| Concentration of ammonia in haemolymph (mg. NH₃-N/100 ml.) | 1.1 | 3.0 | 1.8 | 3.1 | 2.2 | 1.2 | —   |
| Ammonia content of head-thorax (µg. NH₃-N/100 mg. calculated) | 1.2 | 3.3 | 2.0 | 3.4 | 2.4 | 1.3 | —   |
| Ammonia content of HCl (µg. NH₃-N) | 0.6 | 0.4 | 0.4 | 0.4 | 0.4 | 0.3 | —   |
| Combined results for ammonia content of head-thorax and HCl (µg. NH₃-N/100 mg.) | 1.8 | 3.7 | 2.4 | 3.8 | 2.8 | 1.6 | —   |
| % ammonia recovered | 28 | 41 | 39 | 23 | 20 | 13 | 27 |

(d) The effect of the penetration of ammonia

The method employed for raising the concentration of ammonia in the tissue fluids warrants some comment. It is generally supposed that ammonia is a highly toxic compound. Only in the case of birds and mammals, however, has a clear-cut demonstration been obtained that ammonia is in fact highly toxic (Sumner, 1937). A concentration in the blood of 5 mg. ammonia N/100 ml. was found to be lethal. On the other hand, the haemolymph of larvae of the blowfly Lucilia serica may contain as much as 20 mg. ammonia N/100 ml. in normal circumstances (Lennox, 1941). The effect of raising the concentration of ammonia in the body fluids of larvae of Sialis by placing the larvae in a solution of dilute ammonia has therefore been examined.

A curve for the ‘penetration’ of ammonia (Fig. 1) was obtained by placing a number of larvae in a 20 mg. N/100 ml. solution of ammonia (in the experiments just described a 30 mg. N/100 ml. solution was employed to increase the rate of penetration). In order to prevent the possibility that drinking might occur the mouths of the larvae were blocked with wax. At intervals up to 3 hr. larvae were removed and analyses performed to determine the concentration of ammonia in the haemolymph. The effect on the behaviour of the animal was noted.

No toxic symptoms were apparent until the concentration of ammonia in the haemolymph had been raised to a level in the region of 7.0 mg. N/100 ml. When this concentration had been reached the larvae showed a tendency to lie upside down, legs out-stretched. Normal movements were still possible, however. Progressive deterioration set in as the concentration was increased. Larvae with a concentration of ammonia in the haemolymph in the region of 15 mg. N/100 ml. remained
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motionless, upside down, only showing signs of activity, in the form of twitchings of legs and abdomen, when mechanically stimulated. Larvae with a concentration of ammonia in the haemolymph in the region of 20 mg. N/100 ml. did not even respond to mechanical stimulation.

If left in the solution of ammonia death occurred. Larvae returned to water, even those with a concentration of ammonia in the haemolymph of 20 mg. N/100 ml. recovered gradually over a period of hours. Whether a complete return to normality was obtained, however, is not known.

The effect of increasing the internal concentration of ammonia on the pH of the haemolymph was also considered. The pH was determined colorimetrically using the B.D.H. Capillator. After addition of the indicator (phenol red) the end of the capillary, into which the combined haemolymph and indicator had been taken, was waxed over and the protein precipitate centrifuged down. Compensation was made for the pale yellow colour of the blood. Measurements were obtained on the haemolymph of normal larvae (the haemolymph of a single larva sufficing for one measurement) and then on the haemolymph of larvae showing marked symptoms of ammonia poisoning.

The pH of the haemolymph of seven normal larvae was found to vary from 7-0 to 7-4 (average 7-2). Values of 7-2-7-5 were obtained by Beadle & Beadle (1949). These authors measured the pH of the haemolymph of larvae of Sialis by means of micro-glass capillary electrodes without exposing the haemolymph to air.
The pH of the haemolymph of seven larvae with a concentration of ammonia in the haemolymph in the order of 10–15 mg N/100 ml. was found to vary from 7.2 to 7.5 (average 7.4). Most of these values lie below the upper pH value (7.4) found in normal blood. It does not seem possible, therefore, to correlate toxic symptoms with an increase in alkalinity of the haemolymph. The possibility remains, however, that the peripheral nervous system might have been affected by local pH changes. Further investigation is therefore required before any definite conclusions can be made on the extent to which ammonia is toxic to larvae of *Sialis*. What seems certain, however, is that it is less toxic to *Sialis* than it is to mammals and birds.

**DISCUSSION**

It has been shown that ammonia, amounting to about 90% of the total nitrogen, is the major nitrogenous component of the excreta of larvae of *Sialis* during starvation. Since the insects are primarily a terrestrial group, and the majority of the terrestrial forms which have been examined uricotelic, it would seem probable that the larva of *Sialis* is secondarily ammonotelic. It cannot be concluded, however, on the evidence presented in this communication, that reversal is complete. Nevertheless, evidence (as yet unpublished) has been obtained which indicates that uricogenesis either does not occur or is at most trivial in normal circumstances.

The pupa and adult of *Sialis* are terrestrial. Evidence (unpublished) has been obtained that these stages in the life history are uricotelic. Thus excellent support is given to Delaunay’s (1931) concept correlating the major nitrogenous component of the excreta with the nature of the environment, aquatic or terrestrial, in which an animal lives. Analyses which have been performed on the excreta of other aquatic insects give further support to this concept. Thus results have been obtained which show that ammonia, varying in amount from 70 to 90% of the total N, predominates in the excreta of nymphs of *Aeschna cyanea* (Odonata), larvae of *Phryganea striata* (Trichoptera), adults of *Acilius sulcatus* (Coleoptera) and *Dytiscus marginalis* (Coleoptera) and adults of *Notonecta glauca* (Hemiptera).

There is little reason to doubt that ammonia is being excreted by the Malpighian tubules. Further progress on the elimination side of the ammonia excretory mechanism awaits the development, however, of a procedure capable of submitting to quantitative analysis the small amount of fluid found in the tubules.

Now although 90% of the nitrogen excreted by larvae of *Sialis* is in the form of ammonia there is no accumulation in the body when excretion is prevented. It is presumed that deamination is still proceeding in such circumstances but that the resulting ammonia is being ‘stored’ in some way. Support was obtained for this view when it was shown that ammonia disappeared in the tissues when present in high concentration. The obvious possibility that ammonia may be undergoing synthesis into uric acid has not been discussed in this communication, but results (unpublished) which have been so far obtained indicate that this is not in fact the case. The further distinct possibility that ammonia may be undergoing storage in the form of glutamine has not yet been examined. Glutamine has in fact been found in the haemolymph of a number of insects (Pratt, 1950).
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The question arises whether this mechanism plays any part in the metabolism of the normal larva. The similarity of the concentration of ammonia in the haemolymph of normal larvae and larvae prevented from excreting for a period of 5 days suggests that this may well be the case. Thus the operation of the ‘storage’ mechanism does not seem to require a high threshold of ammonia in the haemolymph.

It will therefore be of interest to see whether the ‘storage’ mechanism is reversible. If so, it may be that the ‘storage’ mechanism is in fact a mechanism for transporting ammonia from the tissues to the Malpighian tubules.

There is the further possibility that the real significance of the ‘storage’ mechanism lies in preventing accumulation of ammonia when the larva leaves the water prior to pupation and enters the marginal soil. It will therefore be of great interest to see at what precise stage during metamorphosis uricogenesis commences.

SUMMARY

1. A study has been made of the excretion and storage of ammonia by the aquatic larva of Sialis lutaria.
2. About 90% of the nitrogen excreted by the larva of Sialis during starvation was in the form of ammonia. The daily ammonia output averaged 10 \( \mu g \) N/100 mg. wet weight.
3. Ammonia was found to be excreted into the hindgut, presumably via the Malpighian tubules. The concentration of ammonia in the hindgut fluid averaged 136 mg. N/100 ml.
4. Evidence was obtained that the tissue fluids are not maintained completely ammonia-free. Thus the total ammonia content of the body averaged 1.0 \( \mu g \) N/100 mg. wet weight of tissue. The concentration of ammonia in the haemolymph averaged 0.5 mg. N/100 ml.
5. Evidence was obtained that the larval tissues are capable of ‘storing’ appreciable quantities of ammonia. Thus ammonia did not accumulate in the tissue fluids of larvae prevented from excreting for a period of 5 days. Furthermore, it was found experimentally possible to raise the concentration of ammonia in the tissue fluids, the ammonia subsequently disappearing. The possible significance of this ‘storage’ mechanism was discussed.
6. The method used for raising the concentration of ammonia in the tissue fluids, by immersing the larva for some time in a solution of dilute ammonia, was considered in some detail, particularly with respect to toxic effects. When the concentration of ammonia in the haemolymph had reached a level in the region of 7.0 mg. N/100 ml. toxic symptoms started to appear.

This formed part of the work carried out while in receipt of a D.S.I.R. Maintenance Allowance. I wish to thank both my supervisor Dr E. T. Burtt and Mr J. Shaw (who introduced me to microtechnique) for their willingness to offer constructive criticism and advice. Finally, I must thank Professor A. D. Hobson for affording me the facilities to work in his department.
REFERENCES