# THE EFFECT OF AMMONIA ON THE RATE OF TERMINATION OF DIAPAUSE IN EGGS OF *ACHETA COMMODUS* (WALK.)* (ORTHOPTERA : GRYLLIDAE)

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*Summary*

Certain ammonium compounds applied at a concentration of 0·04M proved highly effective in terminating diapause in eggs of *Acheta*. The only obvious characteristic in common between the effective compounds was that all of them had an organic anion, whereas those with an inorganic acid radical were ineffective.

Gaseous ammonia applied under conditions of high humidity was effective in terminating diapause after a period of exposure of less than 1 hr. No significant difference was found in the effectiveness of the action of the ammonia at 12 and 23°C, but at 27°C it was less effective.

## I. Introduction

When dilute solutions of urea are applied under prescribed conditions to eggs of the common field cricket, *Acheta commodus* (Walk.), urea is absorbed into the eggs. This was demonstrated first with prediapause eggs in which the intake was concurrent with that of water, but was also found to take place in diapausing eggs, after the intake of water had ceased (Hogan 1961).

The entry of urea into diapausing eggs has the effect of accelerating the rate of termination of diapause. This effect, however, does not appear to be due to any direct action by the urea as such, but rather to the supply of ammonia derived from the urea. This conclusion followed from experiments in which [$^{14}$C]urea was applied to the eggs and $^{14}CO_2$ was evolved, thus indicating the breakdown of urea and the probable production of ammonia (Hogan 1962). Ammonium compounds applied to the eggs, in the same way as the urea, also proved to be effective in accelerating the termination of diapause (Hogan 1962).

In this paper the effect of a range of ammonium compounds on the rate of termination of diapause is described; also the effect of gaseous ammonia, which proved to be highly effective.

## II. Methods and Materials

The methods for maintaining a supply of cricket eggs, the standardized procedure for inducing diapause, and the measurement of the rate of termination of diapause have been described in a previous paper (Hogan 1962).

* *Note added in proof.—Acheta commodus* has recently been referred by Chopard (1961) to a new genus, *Teleogryllus*.

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Solutions of various ammonium compounds (see Table 1) at concentrations of 0·04M were applied to diapausing eggs by saturating the disks of blotting paper on which the eggs were placed at the bottom of 2 by 1 in. sealed plastic tubes with the solutions.

Diapausing eggs were exposed to gaseous ammonia by applying 20 $\mu$l of either undiluted ammonia (sp. gr. 0·880) or ammonia which had been diluted 1 : 15 with distilled water to a 1-cm square of filter paper affixed inside the cap of a 2 by 1 in. plastic tube which was kept sealed during the period of treatment. Complete volatilization of the undiluted ammonia solution would give a concentration of ammonia in the tube of approximately 0·3 mg/ml.

TABLE 1

EFFECT OF AMMONIUM COMPOUNDS ON THE RATE OF TERMINATION OF DIAPAUSE IN EGGS OF ACHETA

Ammonium compounds applied at a concentration of 0·04M. Number of viable eggs hatching after incubation for 21 days at 27°C determined

| Organic Ammonium Compounds | Arcsin ($H^{\frac{1}{2}}$)* (degrees) | Retransformed Percentage Hatch | Inorganic Ammonium Compounds | Percentage Hatch |
|---|---|---|---|---|
| Ammonium acetate | 49·8 | 58·3 | Ammonium chloride | 3 |
| Ammonium carbonate | 54·9 | 66·9 | Ammonium nitrate | 3 |
| Ammonium citrate | 54·4 | 66·1 | Ammonium phosphate | 5 |
| Ammonium oxalate | 54·9 | 66·9 | Ammonium sulphate | 5 |
| Ammonium salicylate | 52·4 | 62·8 | Ammonium oxalate | |
| Ammonium tartrate | 72·9 | 91·4 | (control) | 76 |
| Water (control) | 21·2 | 13·1 | Water (control) | 4 |

* $H$ = percentage hatch; difference required for significance at the 5% level = 15·7; at the 1% level = 21·9.

## III. RESULTS

### (a) *Effect of Ammonium Compounds*

The rate of termination of diapause by the ammonium compounds was measured by determining the percentage hatch of viable eggs after incubation for 21 days at 27°C. It is apparent from Table 1 that some of the ammonium compounds are highly effective in terminating diapause whereas the others are ineffective. The effective ammonium compounds have one factor in common, viz. an organic acid radical, whereas the ineffective compounds have acid radicals in which no carbon is present. The different effect of the two series of compounds may be due to differences in the rate of entry of the ammonium radical into the egg rather than to any difference in effectiveness of action within the egg.

Further evidence as to whether the organic acid radicals were directly involved in the diapause-terminating process within the eggs was sought by testing the effect of gaseous ammonia.

### (b) *Effect of Gaseous Ammonia*

(i) *Effect of Dosage Rate.*—In the first experiment diluted ammonia was used as the source of gaseous ammonia in three of the treatments, and undiluted ammonia for a further treatment. Three replicates of 25 diapausing eggs were used for each treatment, and the periods of exposure were either 5, 6, or 7 hr when diluted ammonia was used, but only 35 min for the undiluted ammonia treatment. The period of exposure at each dosage level was based on preliminary tests not described here.

TABLE 2

EFFECT OF GASEOUS AMMONIA ON THE RATE OF TERMINATION OF DIAPAUSE IN EGGS OF ACHETA

Eggs exposed to gaseous ammonia evolved from undiluted ammonia (sp. gr. 0·880) or from ammonia diluted 1 : 15 with distilled water. Number of viable eggs hatching after an incubation period of 12 days at 27°C determined

| Gaseous Ammonia Obtained from: | Period of Exposure (hr) | Arcsin ($H^{\frac{1}{2}}$)* (degrees) | Retransformed Percentage Hatch | Arcsin ($M^{\frac{1}{2}}$)† (degrees) | Retransformed Percentage Mortality |
|---|---|---|---|---|---|
| Control | 0 | 31·8 | 27·8 | 0‡ | 0 |
| Diluted ammonia | 5 | 62·9 | 79·2 | 15·5 | 7·1 |
| | 6 | 54·3 | 66·0 | 18·2 | 9·8 |
| | 7 | 63·0 | 79·4 | 24·3 | 16·9 |
| Undiluted ammonia | 0·6 | 55·8 | 68·4 | 36·1 | 34·7 |

* $H$ = percentage hatch; difference required for significance at the 5% level; = 13·6; at the 1% level = 19·3.

† $M$ = percentage mortality; difference required for significance at the 5% level = 14·4; at the 1% level = 21·0.

‡ Excluded from analysis.

In Table 2 the percentage of eggs hatching after incubation at 27°C for 12 days is shown for each treatment. All the ammonia treatments showed a highly significant increase in the rate of termination of diapause, but no significant difference was obtained between the ammonia treatments. No mortality occurred in the control treatment but toxic effects showed in all the ammonia treatments and increased with the severity of the treatment.

(ii) *Effect of Temperature.*—In view of the strong influence of temperature on the rate of termination of diapause in eggs of *Acheta*, its influence on the action of ammonia in terminating diapause is of interest.

One difficulty in measuring any such effect is that the rate of intake of ammonia into the egg is likely to be affected by temperature, as has been shown to be the case with urea (Hogan 1961). To ensure that equal quantities of ammonia were

present in the eggs in each treatment, diapausing eggs were exposed to gaseous ammonia evolved from undiluted ammonia at the same dosage rate as for the previous experiment. They were then removed from the ammonia atmosphere and subdivided into groups of 25. Three groups were held for 5 days at each temperature of 12, 23, and 27°C, together with a control group which had not been exposed to ammonia. The assumption in this procedure is that the termination of diapause would not be completed within the 35 min of ammonia treatment but would be during the next 5 days after the entry of the ammonia into the eggs. A 5-day period was chosen because it was considered that this was the longest period that the egg could be incubated without the rate of diapause termination being affected appreciably

TABLE 3

EFFECT OF GASEOUS AMMONIA ON THE RATE OF TERMINATION OF DIAPAUSE IN EGGS OF ACHETA

Eggs at 27°C exposed for 35 min to gaseous ammonia evolved from undiluted ammonia, after which they were transferred to temperatures of 12, 23, and 27°C for a period of 5 days and then incubated at 27°C for 14 days. Number of viable eggs hatching determined

| Temp. (°C) | Arcsin ($H^{\frac{1}{2}}$)* (degrees) | | Retransformed Percentage Hatch | | Arcsin ($M^{\frac{1}{2}}$)† (degrees) | | Retransformed Percentage Mortality |
|---|---|---|---|---|---|---|---|
| | Control | Ammonia | Control | Ammonia | Control | Ammonia | Ammonia |
| 12 | 23·4 | 57·8 | 15·8 | 71·6 | 0 | 15·7‡ | 7·3 |
| 23 | 20·6 | 60·5 | 12·4 | 75·8 | 0 | 27·2 | 20·9 |
| 27 | 19·8 | 42·1 | 11·5 | 45·0 | 0 | 28·3 | 22·5 |

* $H$ = percentage hatch; difference required for significance at the 5% level = 15·2; at the 1% level = 21·3.

† $M$ = percentage mortality; difference required for significance at the 5% level = 18·0; at the 1% level = 27·1.

‡ Including one tube with zero mortality.

due to temperature effects—independently of the ammonia. At the end of the 5 days all the eggs were incubated at 27°C for a further 14 days by which time the greater number of viable eggs had hatched.

Table 3 shows that the presence of ammonia increases the rate of termination of diapause at each temperature. There is no significant difference between the percentage hatching at 12 and 23°C, but there is a significantly lower percentage at 27°C. It would appear from this that the rate of termination of diapause by ammonia is slower at 27°C than at the lower temperatures. Another interpretation is possible, however, viz. that a delay in development, not related to diapause, occurs at 27°C due to sublethal toxicity of ammonia at this temperature, since, after the absorption of ammonia, mortality tends to be higher at the higher temperature. Further investigation will be required to elucidate this point but it is clear that gaseous ammonia is at least as effective at the lower temperatures as at the higher.

## IV. Discussion

The short period of exposure to ammonia, which is effective in terminating diapause, leaves little doubt that the previously reported effectiveness of urea for this purpose is due to the release of ammonia after the breakdown of the urea. This does not eliminate the possibility, however, that urea may be important as a means of accumulating and storing a supply of ammonia during the winter period when the eggs, under natural conditions, are in diapause. Whether urea or ammonia is involved in the previously reported reactions at sub-zero temperatures (Hogan 1960) is being investigated.

The mode of action of ammonia may be the direct activation of a metabolic process or the supply of a deficient compound or compounds. The rapidity of action suggests the former.

It seems unlikely that the superiority found to exist for ammonium compounds with an organic radical, over those with an inorganic radical, is due to any participation of the anions in the diapause-terminating process, particularly in view of the effectiveness of gaseous ammonia. The difference seems more likely to be related to the rate of penetration into the egg. However, it is still to be determined whether the gaseous ammonia enters the egg as $NH_3$ or as $NH_4^+$ after reaction with moisture. Meister's (1957) suggestion that in some vertebrate tissues $NH_3$ may be in equilibrium with $NH_4^+$ is of interest in this regard.

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