CHANGES IN pH ASSOCIATED WITH THE APPLICATION OF AMMONIA AND POTASSIUM HYDROXIDE TO DIAPAUSING EGGS OF *TELEOGRYLLUS COMMODUS* (WALK.) (ORTHOPTERA: GRYLLIDAE)

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Summary

Diapausing eggs of *T. commodus* were exposed to ammonia evolved from solutions of ammonium hydroxide in desiccators for specific periods. It was found that the pH of the egg contents increased with increase in concentration of the ammonium hydroxide and the period of exposure.

Toxic symptoms and mortality of eggs were associated with a rise of pH exceeding 0·4 of a unit. This occurred at the higher concentrations, viz. exposure for 3 days to 0·3M or for 2–3 days to 0·1M ammonium hydroxide.

The most favourable effect on the termination of diapause was with exposure for 3 days to 0·01M ammonium hydroxide, which raised the pH to 7·0. This was also the highest non-toxic concentration.

On the basis that the rise in pH might be the causal factor for the termination of diapause, the effect of potassium hydroxide solutions on the pH of the eggs was tested and found to be moderately effective both in this regard and in accelerating the rate of termination of diapause. However, there was some termination of diapause before any significant rise in pH occurred. It appears, therefore, that the action of ammonia and of potassium hydroxide must have a common factor other than raising the pH of the egg contents.

I. Introduction

It has been shown that when diapausing eggs of *Teleogryllus commodus* (Walk.) are exposed to ammonia evolved from solutions of ammonium hydroxide for an adequate period, diapause is terminated in a high proportion of the eggs. There is also evidence of a change in the pH of the eggs (Hogan 1964).

In other organisms an alteration in pH has been quoted by Brachet (1960) as being responsible for embryonic induction. Yamada (1950) found a shift in pH by the use of ammonia effective in one form of embryonic induction, namely, neuralization.

In view of the above, and the known importance of pH in relation to metabolic processes, the possibility that the resumption of development could be induced by a change in the pH of the eggs has been investigated.

II. Materials and Methods

The supply of eggs was from cultures of crickets reared in the laboratory at a temperature of 26(±2)°C. Petri dishes containing moist graded sand were placed in the cages overnight and removed the following morning. They were then held in a sealed container at 23(±0·2)°C for 14 days to induce diapause. The eggs were removed from the sand, when required for the tests, by sieving under water.

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Ammonia was applied by placing the eggs on moist filter paper in plastic trays in a 6-in. desiccator, with 100 ml of ammonium hydroxide solution of specified concentration in the base. For the potassium hydroxide treatments, the eggs were semi-immersed in solutions of specified concentration.

The rate of termination of diapause after the various treatments was measured by hatching counts made after placing the eggs on moist filter paper disks in 2 by 1 in. plastic tubes held in sealed plastic boxes, and incubated at 23°C. The hatching counts were based on eye-spot development and were made at the end of 24 days of incubation.

Prior to the measurement of their pH the eggs were washed, placed in a 100-ml erlenmeyer flask with 50 ml 3% NaOCl, and shaken mechanically for 4 min. This removed the chorion. After rinsing with distilled water the eggs were then given a succession of washings in distilled water. For this purpose the erlenmeyer flasks were held in a shaking machine and the water in them changed at intervals of 2, 5, 10, 10, and 10 min. The eggs in the controls were given the same washing procedure.

The pH measurements were made with an R model Jones pH-meter with a sensitivity of 0·002 pH. The pH-meter was calibrated against a potassium phthalate buffer (pH 4·0) and a phosphate buffer, KH_2PO_4 and $Na_2HPO_4.2H_2O$, with a pH of 6·5. The latter was used to check the setting of the meter before each pH measurement of the eggs. A temperature of 21°C was maintained by circulation of water from a controlled-temperature water-bath through a jacket enclosing the reference electrode.

For the pH measurement a sample of 100 eggs was placed in a microbeaker, 120 ml distilled water added, and the eggs crushed by means of a glass spatula. The supernatant fluid was then drawn off by means of a pasteur pipette.

Samples that were crushed in this way and then centrifuged at 5000 r.p.m. for 20 min gave similar readings to samples prepared without centrifuging but held for a similar period. The simpler and quicker method was therefore adopted.

Further details of method are given in the description of the experiments to which they apply.

III. Results

(a) *Effect of Ammonia on the pH of Diapausing Eggs*

(i) *Evidence of the Influence of Ammonia on the pH of Diapausing Eggs.*—This evidence was presented in a previous paper (Hogan 1964). More detailed evidence of the effects on pH of period of exposure and ammonia concentration is provided by the following experiment. Diapausing eggs were exposed to ammonia evolved from solutions of ammonium hydroxide at concentrations of 0·001–0·1M in desiccators for periods ranging from 1 to 3 days at 23°C. They were then removed, and a sample of 100 eggs from each treatment used for the measurement of pH. Table 1 shows that the pH increased from the mean control reading of 6·73* to a maximum of 9·18 after exposure for 3 days to 0·1M ammonium hydroxide. A consistent increase

* The pH readings of the homogenate give comparative, not the absolute, values. The dilution of the homogenate with distilled water and the NaOCl treatment both tend to raise the pH slightly.

with period of exposure and concentration occurred. As in previous experiments the eggs showed a discoloration by the more severe treatments, ranging from brown to black, and this is associated with failure to hatch in the more severe cases (see Table 3). This was associated with treatments causing a rise of pH exceeding a value of 7·2. Obviously such treatments have no relation to termination effects.

TABLE 1

EFFECT OF PERIOD OF EXPOSURE TO VARIOUS CONCENTRATIONS OF AMMONIA ON THE pH OF THE CONTENTS OF DIAPAUSING EGGS

Eggs were held at 23 °C during the treatment period and for the subsequent incubation period to the hatching stage. Ammonia was evolved from solutions of ammonium hydroxide of differing concentrations

Period of Exposure (days)	pH of Egg Contents for Ammonium Hydroxide Concentrations (M):					
	0 (control)	0·001	0·003	0·01	0·03	0·1
1	6·72	6·72	6·75	6·79	6·88	7·65
2	6·75	6·71	6·80	6·86	7·14	8·68
3	6·71	6·85	6·89	7·02	7·98	9·18

The procedure in this experiment assumes that all the ammonia absorbed on to the shell will be either removed by the washing or remain absorbed during the separation of the egg contents. That none will be removed by further washing has

TABLE 2

EFFECT OF DIPPING DIAPAUSING EGGS IN AMMONIUM HYDROXIDE PRIOR TO TREATMENT WITH NaOCl AND WASHING ON THE pH OF THE EGG CONTENTS

Controls	Eggs Dipped* in:		Eggs Exposed† to Ammonia Evolved from:	
	0·01M NH_4OH	0·03M NH_4OH	0·01M NH_4OH	0·03M NH_4OH
6·75	6·72	6·75	6·88(2)	7·28(2)
6·78	6·79	6·79	6·96(3)	8·03(3)
6·72	6·78	6·78	6·97(4)	8·12(4)

* Period of dipping occupied *c.* 7 sec.

† Period of exposure (days) given in parenthesis.

been found by testing the final washings for the presence of ammonia both by Nessler's reagent and pH effects.

To check whether any remaining ammonia reached the egg contents a test was conducted in which eggs were dipped in ammonium hydroxide solution prior to

their treatment with NaOCl and washing. Their pH was then compared with the normal controls and with eggs treated with ammonia of the same concentration over periods of 2, 3, and 4 days. Table 2 shows that dipping in ammonia prior to the standard washing procedure did not significantly affect the reading of the controls.

(ii) *Effect of Ammonia on the Rate of Termination of Diapause.*—Measurements of this effect have been given in a previous paper (Hogan 1964) but in view of variations in the responses from one batch of eggs to another, presumably associated with intensity of diapause, it was necessary to measure this effect concurrently with the measurement of pH and with eggs from the same culture. The effect of ammonia evolved from solutions of ammonium hydroxide in the range 0·001–0·1M, for periods of 16 hr to 5 days at 23°C, was measured. The solution and the eggs were held in desiccators as in Section III(*a*)(i) and after completion of the treatment the eggs were incubated at 23°C for 24 days and those with eye-spots counted.

TABLE 3

EFFECT OF CONCENTRATION OF, AND PERIOD OF EXPOSURE TO, AMMONIA ON THE RATE OF TERMINATION OF DIAPAUSE IN EGGS HELD IN DESICCATORS AT 23°C

Period of Exposure (days)	Percentage Hatching at Ammonium Hydroxide Concentrations (M):					
	0 (control)	0·001	0·003	0·01	0·03	0·1
7	5	*	*	*	5	32·5†
1	2·5	0	0	2·5	10	22·5†
2	2·5	0	0	2·5	30	2·5†
4	0	0	2·5	40·0	12·5†	*
5	2·5	0	0	57·5	*	*

* Not tested. † Toxicity symptoms.

Table 3 shows that for the periods of exposure used, 0·001M and 0·003M ammonium hydroxide were ineffective and that the remaining concentrations showed effects related to the period of treatment, the termination rate increasing with the period until a toxic level was reached.

(*b*) *Influence of Potassium and Sodium Hydroxides on the Rate of Termination of Diapause*

The correlation between a rise in pH of the eggs as a consequence of treatment with ammonia and an increase in the proportion of eggs emerging from diapause when the treatment was continued for a sufficient period suggested that a similar effect might be obtained by applying other chemicals with a high pH.

On the basis of preliminary tests an experiment was set up in which solutions of potassium and sodium hydroxides at concentrations of 0·12–1·0M were used. The eggs were semi-immersed in the solutions for a period of 4 days. The level of the solution was maintained by the addition of water at the periodic inspections to compensate for any drying out that had occurred.

Table 4 shows that some effect on the rate of termination of diapause was obtained in all treatments, the effect increasing with both concentration and period

TABLE 4

PERCENTAGE OF EGGS HATCHING WITHIN THE DIAPAUSE-FREE PERIOD AFTER TREATMENT FOR 4 DAYS WITH POTASSIUM AND SODIUM HYDROXIDES AT THE CONCENTRATIONS INDICATED AND AT A TEMPERATURE OF 27 °C

Potassium Hydroxide Concn. (M)	Arcsin ($H^{\frac{1}{2}}$)*	Retransformed Percentage Hatch	Sodium Hydroxide Concn. (M)	Arcsin ($H^{\frac{1}{2}}$)*	Retransformed Percentage Hatch
0 (control)	10·6	3·4	0 (control)	10·6	3·4
0·12	21·1	13·0	0·12	29·2	23·8
0·25	36·7	35·7	0·25	35·2	33·2
0·50	42·3	45·3	0·50	39·1	39·8
1·00	42·3	45·3	1·00	24·5	17·2

* H = percentage hatch; difference for significance at 5% level = 9·6, at 1% level = 13·2.

of treatment until a toxic level was reached. The results paralleled those obtained with ammonia but much higher concentrations were required.

TABLE 5

PERCENTAGE OF EGGS HATCHING WITHIN THE DIAPAUSE-FREE PERIOD AT 23 °C AFTER TREATMENT WITH VARIOUS CONCENTRATIONS OF POTASSIUM HYDROXIDE FOR 1, 4, AND 7 DAYS

Potassium Hydroxide Concn. (M)	Period of Exposure (days)	Arcsin ($H^{\frac{1}{2}}$)*	Retransformed Percentage Hatch
0 (control)	1	15·8	7·4
0·1	1	16·1	7·7
0·3	1	20·4	12·2
0·5	1	16·1	7·7
0 (control)	4	9·9	2·9
0·1	4	20·1	11·8
0·3	4	22·1	14·2
0·5	4	33·7	30·8
0 (control)	7	16·5	8·1
0·1	7	23·9	16·4
0·3	7	32·1	28·3
0·5	7	39·1	39·8

* H = percentage hatch; difference for significance at 5% level = 9·4, at 1% level = 12·8.

In a further trial (Table 5) potassium hydroxide concentrations of 0·1–0·5M and exposure periods of 1–7 days were used. The 1-day period proved to be ineffective but otherwise the rate of termination increased with concentration and with period

of exposure. However, in this test the eggs did not show toxicity symptoms in any of the treatments.

(c) *Effect of Potassium Hydroxide on the pH of Diapausing Eggs*

Diapausing eggs were semi-immersed in solutions of 0·5M potassium hydroxide for 4 and 7 days at a temperature of 23°C. After treatment the eggs were removed, rinsed in distilled water, then treated with 3% NaOCl, and washed with water as in Section III(*a*)(i). The controls were dipped in 0·5M potassium hydroxide, rinsed in distilled water, and then put through the NaOCl and water-washing procedure. After 4 days there was no significant rise in the pH of the egg contents (pH 6·80, control 6·72), but after 7 days a significant increase (to pH 7·00) occurred.* This level roughly corresponding to that reached by eggs exposed to the weakest of the effective ammonia treatments (see Tables 1 and 3).

In these experiments the rate of termination of diapause was increased after 4 days but at this stage the rise in pH was not statistically significant.

IV. Discussion

In the experiments described in this paper, information on the effect of ammonia on the acceleration of the rate of termination of diapause, and the effect in raising the pH of the egg contents, has been extended and confirmed.

The effects of potassium and sodium hydroxides were tested and it was found that the rate of termination of diapause was accelerated by these compounds, but at considerably higher concentrations than with ammonia. However, eggs treated with potassium hydroxide in this way showed no significant rise in pH until after some acceleration in the rate of termination of diapause had occurred.

The rise in pH following the application of ammonia or potassium hydroxide is then presumably incidental to this treatment rather than being a causal factor in the termination of diapause.

What then is the common factor in the action of ammonia and potassium hydroxide? One possibility is that the supply of ammonia may still be the critical factor and in some way, perhaps by a localized rise in pH not perceptible in the bulk egg measurement, ammonia is released by the application of potassium hydroxide.

In favor of this are the results of Beck and Alexander (1964) obtained by injection of ammonium acetate into larvae of *Ostrinia nubilalis*. There is also an analogy between the resumption of development after the termination of diapause and the "unchannelling of metabolic processes in the egg" after fertilization (Runnstrom *et al.* 1939). Brachet (1960) cites work by Orstrom (1941) demonstrating the transient production of ammonia after the fertilization of sea-urchin eggs, and also by Pasquinelli (1954) who found that the rate of ammonia absorption is at a maximum in freshly fertilized sea-urchin eggs.

Action on the cuticle would be in alignment with the view of Slifer (1958) that the permeability of the eggshell of *Melanoplus* to water, via the hydropyle, is the factor involved. McFarlane (1962) has shown that the permeability of non-diapausing

* Difference required for significance at 5% level = 0·09, at 1% level = 0·13.

eggs of the cricket *Acheta assimilis* is affected by immersion in buffer solutions of pH 6 and 7. Yet permeability of the eggshell to water can hardly affect the development of *Teleogryllus* eggs in which water uptake is completed simultaneously with the onset of diapause. Nevertheless, an effect on the permeability of membranes within the egg could well be the critical factor.

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VI. References

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