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NITROGEN METABOLISM IN THE AMERICAN COCKROACH: AN EXAMINATION OF WHOLE BODY AMMONIUM AND OTHER CATIONS EXCRETED IN RELATION TO WATER REQUIREMENTS

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SUMMARY

The effects of dietary nitrogen levels in relation to ammonotelism, cation excretion and water requirements were examined in the American cockroach, *Periplaneta americana* (L.). Very little ammonia is released from the respiratory surfaces; rather it appears to be eliminated in the faeces, presumably as ammonium ions. Microflora present in the hindgut may contribute significantly to the production of excreted ammonia under certain dietary conditions. Injections of buffers containing either NH_4^+, K^+ or Na^+ resulted in normal (NH_4^+ and K^+), or less than normal (Na^+) levels of ammonia excretion. Faeces collected from cockroaches maintained on twelve different diets containing various levels and sources of nitrogen were examined for NH_4^+, K^+, Na^+, Ca^{2+} and Mg^{2+}. Ammonium ions were found to be the major cations contained in the faeces and were excreted in increasing amounts as the dietary nitrogen levels increased. The *ad libitum* water requirements were closely correlated with dietary nitrogen levels, and subsequently with ammonia excretion. Certain aspects concerning the possible factors involved, and the significance of ammonia excretion, are discussed.

INTRODUCTION

Close examination of nitrogen excretion in various ammonotelic organisms has recently led to some interesting and important discoveries. For example, it has been shown that although most of the ammonia produced by terrestrial isopods is dissolved in the urine, a portion of it may be released in gaseous form (Wieser & Schweizer, 1970; Wieser, Schweizer & Hartenstein, 1969; Wieser, 1972). Study of terrestrial snails has revealed that, although little ammonia can be detected in the excreta, appreciable quantities are eliminated in the form of a gas, apparently by diffusion through the shell (Speeg & Campbell, 1968). Ammonia volatilization in dogs has been attributed to its release into alveolar air, being present in amounts of the order of

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magnitude expected if the ammonia in the blood were to equilibrate with alveolar air during its passage through the pulmonary capillaries (Jacquez, Poppell & Jeltsch, 1959). Contrarily, in earthworms (MacDonnell & Tillinghast, 1973), blowflies (Prusch, 1972), and aquatic neuropterans (Staddon, 1955), ammonia release appears to be primarily associated with the gut tissues, and it is excreted with the faeces.

The discovery that the American cockroach does not excrete significant quantities of uric acid externally, and that ammonia is a major nitrogenous product (Mullins & Cochran, 1972, 1973), has stimulated research concerning the site of its release from the intact insect. Also, studies of dietary nitrogen levels and nitrogen and cation excretion as they relate to water balance in *Periplaneta* were conducted in order to evaluate the physiological/biochemical significance of ammonotelism in this species. The results of these experiments are presented here.

MATERIALS AND METHODS

General

The American cockroaches used in this study were obtained from established cultures reared on commercial dog food containing 25% crude protein. The rearing and experimental room was maintained at 28 °C ± 2 °C, 64 ± 3% relative humidity and a 12L/12D photoperiod. All insects used in this study had been maintained on dog food at least one month after their final molt and before being placed on any other dietary regime.

Most of the diets used in this study were formulated after those used by Haydak (1953) and consisted of yeast extract (vitamin source), Hawk Oser Salt mixture No. 3, casein protein or casein hydrolysate, and dextrin. Diets containing uric acid and NH_4Cl as a nitrogen source were mixed with the various other components of the basic diet formulations mentioned above (see Table 4). The diets were diluted with chromatographic grade cellulose (100 g diet up to 120 g). All diets were ground in an Intermediate Wiley Mill (No. 40 mesh) and mixed on a roller mill with glass beads for several hours to insure homogeneity.

The method of anaesthesia used during these studies was that of cold-immobilization at 4 °C for 15 min in ¼ in mesh wire cages. This procedure insured constant conditions of anaesthesia throughout all experimentation and reduced the amount of trauma that generally accompanies such procedures.

Collection of volatile ammonia

An apparatus (Fig. 1) was designed and built to allow for continuous collection of volatile ammonia released from the insects as follows: (1) A battery of air scrubbers to purify the air before it was passed over the insects consisting of three acidic (30 ml 2 N-HCl), one basic (30 ml 0·2 N-NaOH), two water (30 ml) and one condenser column(s) filled with 3 mm glass beads. (2) Insect cages of two types: small (70 ml) glass jars and larger (400 ml) plastic air-tight containers. Both types were fitted with ¼ in mesh screens suspended over solutions (3 or 40 ml) of saturated K_2CO_3 and water (2:1) which was mixed continuously with a magnetic stirrer. Four cages (one control and three experimental cages) could be run simultaneously. (3) Individual air scrubbers, corresponding to each of the cages, consisted of a 25 ml burette containing

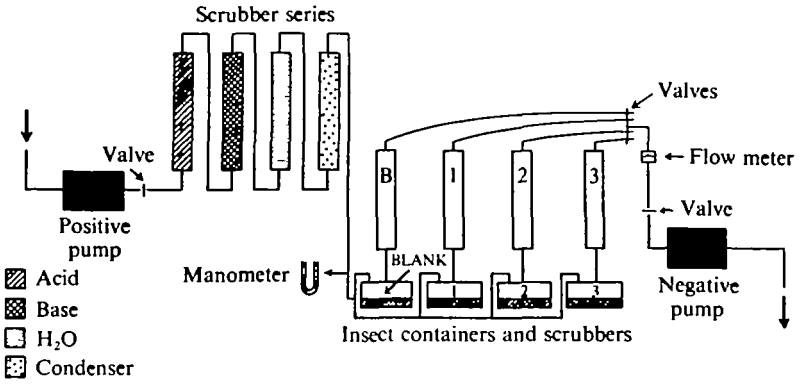

Fig. 1. A diagram of the apparatus designed to collect volatile ammonia produced by encaged cockroaches at ambient atmospheric pressures.

10 ml 3 mm glass beads and a collection solution of 5 or 10 ml 0·2 N-HCl through which the effluent air from the cages passed and in which the volatile ammonia was collected.

The use of a positive pressure pump and a control valve (placed before the battery of purifying scrubbers), together with four control valves and a negative pressure pump (placed after the individual air scrubbers), allowed for the maintenance of fairly constant air flow (100 ml/cage/min) and constant air pressure very close to ambient. The effects of stress, due to pressure changes differing from the ambient pressure, were avoided by balancing the positive and negative pump flow rates using a manometer. The ammonia collection apparatus was housed in a room in which the temperature and photoperiod were identical to those to which the insects were accustomed. Due to the humidifying effects of the battery of purifying scrubbers and the dehumidifying effect of the nearly saturated K_2CO_3 solutions present in the cages, the relative humidity was maintained at a somewhat higher level (87%) than that to which the insects had been accustomed.

The ammonia trapped in the scrubber columns was estimated by either of the following methods. (1) The solutions containing ammonia released by individual cockroaches were measured using the phenolhypochlorite reaction with trichloroisocyanuric acid (modified from Wieser & Schweizer, 1970). (2) Solutions containing ammonia collected from groups of insects were measured by routine Nesslerization, the optical density being determined with a spectrophotometer at 490 nm. The modified method of Wieser & Schweizer allowed for a more sensitive detection of ammonia, which was required when individual insects were examined.

Although recovery of ammonia from standard solutions injected into the cages was satisfactory (95%) certain problems were encountered in attempting to measure that contained in fresh faecal pellets. The release of ammonia from a faecal pellet is dependent upon the diffusion of K_2CO_3 into the pellet as well as the subsequent diffusion of ammonia out of the pellet. Furthermore, the decomposition of labile amines in the faeces during such procedures might contribute to errors in measurement of ammonia excretion. Close examination of faecal material obtained from cockroaches on various diets indicates that the presence of such labile nitrogen-containing materials

is not very significant (Mullins & Cochran, 1973). However, a certain amount of error can certainly not be ruled out in the estimates of ammonia released by whole animals.

Total non-ammonia nitrogen excretion

Total non-ammonia nitrogen was determined by collecting lyophilized faecal material from cockroaches contained in Petri dishes for a period of two weeks and maintained on the various diets. The faeces were ground in a Wig-L-Bug Dental Amalgamator and the total nitrogen determined by a micro-Kjeldahl method (Schmidt, 1961) on lyophilized samples weighed on an electrobalance. Measurement of ammonia contained in the dried faecal material was accomplished by a microdiffusion technique (Kirk, 1950). Nitrogen values from the micro-Kjeldahl analysis were corrected for the ammonia content determined by microdiffusion giving the total non-ammonia nitrogen excreted in the faeces. Total nitrogen excretion was calculated by addition of the values obtained for total non-ammonia nitrogen excreted and those for ammonia excretion from insects examined in the volatile ammonia collection apparatus.

Determination of potassium, sodium, calcium and magnesium in the faeces

Faecal material was digested by a modified method of Sanui & Pace (1959). Twenty mg of ground faecal material was placed in 18 × 150 mm Vicor test tubes and 0·75 ml of conc. HNO_3 was added to each tube and placed in a micro-Kjeldahl oven maintained at 190 °C. After drying, 0·75 ml of H_2O: 72% perchloric acid (2:1) was added to each tube after which they were raised to 300 °C. When the samples were again almost dry, they were diluted to a final volume of 7 ml. The samples were diluted to appropriate concentrations and the K^+, Na^+, Ca^{2+} and Mg^{2+} were measured on a Varian Techtron Atomic Absorption Spectrophotometer Model AA-5 (absorption mode).

Determination of the water requirements

The *ad libitum* water requirements were determined by placing groups of four insects in wide-mouth 16 oz jars covered with $\frac{1}{4}$ in wire mesh. The insects were maintained in the culture room and allowed to feed on their particular prescribed diet *ad libitum*.

Water vials used in the determination of water consumption were constructed from 35 × 10 mm plastic Petri dishes (top and bottom sealed) and a plastic mini-vial with a 1 mm hole in the top and bottom through which a small cotton wick was inserted. Water loss from the vials was determined gravimetrically with a four-place analytical balance. Each water vial was calibrated against a series of control vials before and after presentation to the insect groups. The use of these calibration values, calculated on the basis of the rate of water evaporation from the controls, allowed quite reliable correction for evaporation. Each diet series tested consisted of four replicates and were run two or three times for a period of 4 to 5 days.

RESULTS

Examination of the possible release sites of ammonia from intact male cockroaches was done by sealing certain portions of the insects with paraffin and sampling the air which passed over them. Table 1 presents data which show that very little ammoni

Table 1. *Determination of the site of ammonia release from the intact male cockroach*

Experiment*	Individuals†	Replications‡	NH_3-N Releases μg-N/male/day
I. Food and water not provided			
A. Portion of body sealed with paraffin§			Av. ± S.E.M.‖
1. Head and rectum	4	3	0·5 ± 0·1
2. Rectum only	4	3	0·3 ± 0·0
3. Head only¶	4	3	2·2 ± 0·6
B. Body not sealed with paraffin**	1	6	44·4 ± 6·4
II. Food and water provided *ad libitum*** ††	8	9	75·0 ± 4·9

* Insects were maintained on dog food prior to experimentation and were placed in the ammonia collection apparatus for 24 h intervals.

† Number of individuals used per replication.

‡ Number of replications.

§ Portions of cold-immobilized males were dipped briefly into paraffin (60 °C) and immediately into cold water, to diminish the effects of the heat treatment.

‖ Average ± standard error of the mean.

¶ No faecal pellets were eliminated by the insects.

** Faecal pellets were eliminated by the insects.

†† Blank values for ammonia released from the dog food diet (presumably from microbial activities) was 2·7 ± 1·5 μg NH_3-N/day. Appropriate corrections for this source of ammonia were made by subtraction of the blank values from the experimental values.

was released when the rectum and both the head and rectum were sealed. However, when only the head was sealed, a small but measurable amount of ammonia was released even though no faecal material had been produced during the sampling period. The amount of ammonia released from untreated males deprived of food and water was slightly over half of the amount normally released by those individuals allowed access to food and water *ad libitum*.

Fig. 2 presents further evidence that ammonia released from intact cockroaches is associated with the rectum, primarily with the production of a faecal pellet. In these experiments, insects were examined individually and continuously for 8 days under conditions of complete inanition. The body weight, deposition of faecal material and amount of ammonia released were determined for each 24 h period. Examination of the information obtained (Fig. 2), from three insects chosen to represent the series of cockroaches studied in this manner, show that significant quantities of ammonia are released only when a faecal pellet was eliminated from the cockroach (designated by an encircled F in the histogram). However, there were a few intervals where small amounts of ammonia were detected and no faeces had been eliminated.

The information presented in Table 1 and Fig. 2 indicates that ammonia release from the intact cockroach occurs via the rectum, being primarily associated with the faecal pellet. These results are similar to those reported by Staddon (1955). He found that aquatic *Sialis* larva excrete ammonia via the rectum and not across the body surface. The trace amounts of ammonia released from cockroaches whose rectum had been sealed with paraffin (Table 1), or not producing a faecal pellet (Fig. 2), might be attributed to releases across the respiratory surfaces. However, more sophisticated methodology and sensitive techniques may be required to evaluate this possibility.

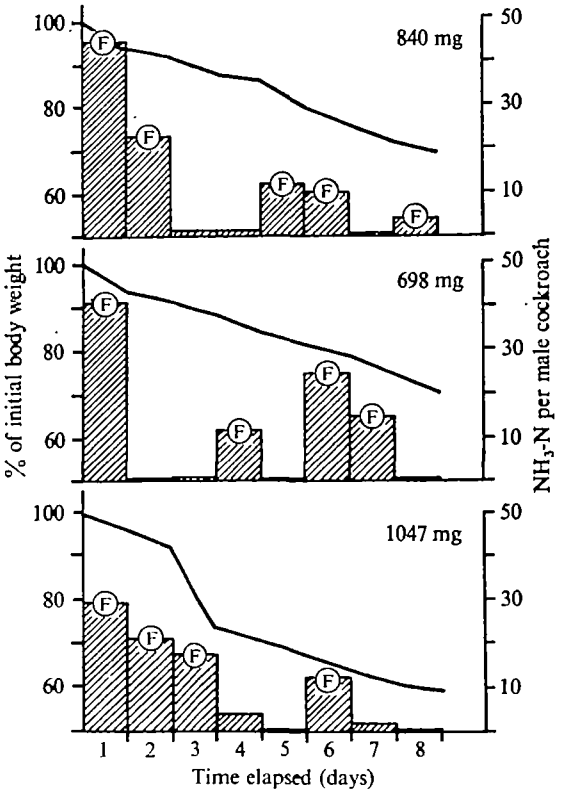

Fig. 2. Elimination of ammonia from three male cockroaches under conditions of complete inanition for 8 days. The histogram portion of the graphs correspond to the ammonia nitrogen released (μg NH_3-N/male/day). The encircled F indicates the deposition of a faecal pellet during a particular 24-h interval. The line portion of each graph represents the percent of initial body weight, and the initial body weight is indicated in the upper right-hand portion of each graph.

Table 2. *Ammonia released from male cockroaches fed on 25% casein protein diets containing antibiotics*

Diet*	Individuals†	Replications‡	NH_3-N Released μg-N/male/day Av. ± S.E.M.§	% of Normal
1% Neomycin	8	6	34±3	35
10% Neomycin	8	6	44±4	45
1% Penicillin	8	6	19±5	19
10% Penicillin	8	6	52±9	53
1% Aureomycin	8	6	30±4	31
10% Aureomycin	8	6	30±2	31
25% Casein protein	8	9	98±2	—

* Insects were maintained on 25% casein protein diets containing antibiotics for two weeks prior to experimentation.

† Number of individuals used per replication.

‡ Number of replications.

§ Average ± standard error of the mean.

Table 3. *Ammonia released after injection of ammonia, sodium and potassium into the abdomen of adult males maintained on a 25% casein protein diet*

	Micrograms NH_3-N released/male/day† ‡				
Injection*	Day 1 Av. ± S.E.M.	Day 2 Av. ± S.E.M.	Day 3 Av. ± S.E.M.	Total NH_3-N	Comparison of means to control§
Control	97 ± 5	74 ± 21	82 ± 23	253	—
NH_4^+	92 ± 15	71 ± 14	86 ± 18	249	N.S.
Na^+	63 ± 16	40 ± 7	41 ± 8	144	S.
K^+	86 ± 12	83 ± 19	69 ± 25	238	N.S.

* Fifty μl. of the following solutions were injected into the abdomens of cold-immobilized males on the first day: Control = 0·4 M glucose; NH_4^+ = 0·1 M-NH_4Cl + 0·1 M-NH_4HCO_3 (pH. 7·7); Na^+ = 0·1 M-NaCl + 0·1 M-$NaHCO_3$ (pH 8·0); K^+ = 0·1 M-KCl + 0·1 M-$KHCO_3$ (pH 8·3). The solutions were made up with distilled water (4 °C) immediately before injection.

† These cockroaches were maintained on a 25% casein protein diet for 2 weeks prior to and after the injections *ad libitum*. Values of NH_3-N released are expressed as micrograms per day (average ± standard error of the mean).

‡ Uninjected controls release 98 ± 2 μg NH_3-N/male/day when maintained on a 25% casein protein diet.

§ Comparison of the means of the NH_4^+, Na^+ and K^+ injected insects compared to the injected controls over the 3 day period following the injections. The standard student's *t*-test was used: N.S. = not significant and S. = significant at the $P < 0.05$ level (Downie & Heath, 1970).

Antibiotic feeding

To study the involvement of gut microbes in ammonia excretion, male cockroaches were fed 25% casein protein diets containing antibiotics. The results obtained from feeding diets containing 1 and 10% levels of neomycin, penicillin and aureomycin are presented in Table 2. Ammonia excretion was reduced in all cases, ranging from 19 to 53% of the levels normally released by males maintained on a 25% protein diet. Considerable variability in the amount of ammonia released is shown in this table. These results serve to indicate that the gut microflora may be involved to some degree in whole body ammonia releases. However, the effects of such treatments were not evaluated with regard to their influence on the normal cockroach metabolic activities, or mycetocyte metabolism which may be important.

The physiological functions and activities of the hindgut are undoubtedly complex and not very well understood (Maddrell, 1971). The length and comparable time required for the contents to pass along the hindgut (Snipes & Tauber, 1937), the distribution of nematode populations along the hindgut lumen with respect to diet (Hominick & Davey, 1972) and their hypothesized neuroendocrine control (Gordon, 1968), reflect the significance of the hindgut in this insect. Work reported by Todd (1944) showed that the presence of ammonia increased the ability of infective eggs of a nematode species to mature *in vitro*. This evidence, along with the detection of ammonia in the hindgut and the isolation and incubation of three hindgut bacterial strains which produced ammonia in culture, led Todd to suggest that ammonia levels might have an influence on the nematode population. The use of antibiotics may not provide an adequate evaluation of the role of the gut microflora in ammonia excretion,' since Greenberg, Kowalski & Karpus (1970) found that treatments of antibiotics fed at fairly high levels did not completely repress the gut microflora. Closer examination of the metabolism of isolated whole hindgut and *in vitro* incubations were beyond the scope of this study, but might be quite useful.

Table 3 presents data obtained from injections of equimolar (approximately equi-osmolar) solutions of solutes into the abdominal haemocoel of male cockroaches. These solutions were formulated to produce an equimolar presence of solutes or ions, iso-osmotic to the haemolymph (about 400 mOsm, Wall, 1970) and having a pH within a reasonable range of physiological acceptability. The amount of ammonia-nitrogen (140 μg NH_3-N) was about 1·5 times the amount normally excreted by uninjected males (97 μg NH_3-N/male/day) maintained on a 25% casein protein diet. It can be seen from this table, that the total amount of ammonia nitrogen excreted, 3 days after injections of the ammonium and potassium buffers, was similar in the injected groups and the uninjected controls. However, injection of the sodium buffer resulted in a significantly lower amount of NH_3-N excretion.

Injections of salt solutions into the haemocoel of *Periplaneta* by various workers has indicated that it has a very high tolerance for rapid increases in haemolymph ions (McEnroe, 1956; van Asperen & van Esch, 1956; Munson & Yeager, 1949). Theoretically, the injection of ammonia into the haemocoel should result in one or more of the following responses: (1) rapid removal of ammonia by excretion or release across respiratory surfaces, (2) rapid incorporation of ammonia into amino acids and other small nitrogen-containing compounds, and/or (3) rapid synthesis and storage of uric acid/urates. The results of the experiments presented here indicate that rapid removal of ammonia does not occur by excretion or elimination across respiratory surfaces, since injection of ions in concentrations 1·5 times the amount normally excreted daily did not increase the level of ammonia nitrogen excretion.

Although McEnroe (1956) did not explore the possibility that injected ammonia might be eliminated rapidly by excretory or respiratory processes, he did nevertheless find that it disappeared from the haemolymph. Normal haemolymph ammonia concentrations of about 1 mg/% were measured six hours after injections of 33–180 μg of dibasic ammonium citrate (corresponding to an estimated elevation of haemolymph ammonia levels from 17 to 90 mg/%, respectively). Examination of the fat body after successive injections of ammonium citrate showed that uric acid/urate concentrations increased, and *in vitro* incubations showed *Periplaneta* fat body capable of fixing 9 μM-NH_4/100 mg tissue in three hours (McEnroe, 1956).

Since ammonia can be quite toxic in elevated concentrations it must be removed fairly rapidly. Schutt & Holzer (1972) have described a closely mediated feed-back system in *E. coli* which is capable of rapid incorporation of ammonia into glutamine, and have suggested that it protects the cells from the potential toxic effects of ammonia. Concentrations of haemolymph ammonia exceeding 7 mg/% were found to be toxic to *Sialis* larvae (Staddon, 1955). Staddon suggested that the mechanisms utilized by *Sialis* in resisting the toxic effects of elevated levels of ammonia present in its environment might include the incorporation of ammonia into glutamine/glutamate, and subsequent transamination reactions related to the amino nitrogen pools. Even in the absence of specific information concerning the toxicity of haemolymph ammonia to *Periplaneta* it is probably reasonable to assume that it must be dealt with fairly rapidly. Although McEnroe (1956) has shown that injections of ammonia into the haemocoel of *Periplaneta* ultimately results in increased uric acid/urate deposition in the fat body, initial incorporation of the injected ammonia nitrogen into various intermediates must occur prior to uric acid synthesis. The enzymic capability for such

Table 4. *Total non-ammonia, total ammonia, and total nitrogen excreted by males maintained on twelve diets*

Diet Number and composition	Nitrogen content %	Equivalent protein level %	Micrograms nitrogen excreted/male/day: Non-NH_3-N* Av. ± S.E.M.‡	NH_3-N† Av. ± S.E.M.	Total N	NH_3-N/Total N (%)
1. Dog food (25 % crude protein)	4	25	128 ± 10	75 ± 5	203	37
2. 25 % Casein protein	4	25	66 ± 9	98 ± 2	164	60
3. 25 % Casein hydrolysate	4	25	66 ± 8	83 ± 6	149	56
4. 42 % Casein protein	7	42	100 ± 6	191 ± 33	291	66
5. 76 % Casein protein	12	76	186 ± 5	464 ± 85	650	71
6. 25 % Casein protein + 1 % UA-N§	5	32	80 ± 13	180 ± 14	260	69
7. Dextrin	0	0	45 ± 5	25 ± 2	70	36
8. Dextrin + 1 % UA-N	1	6	47 ± 6	26 ± 3	73	36
9. Dextrin + 1 % NH_3-N‖	1	6	79 ± 3	46 ± 6	125	37
10. Dextrin + 2 % NH_3-N	2	13	118 ± 5	44 ± 6	162	27
11. Dextrin + 4 % NH_3-N	4	26	195 ± 6	41 ± 8	236	17
12. Dextrin + 6 % NH_3-N	6	39	170 ± 12	45 ± 12	215	21

* Insects were maintained on the diets for 6 weeks prior to a 2-week collection period for faecal analysis.

† Insects were maintained on the diets for at least 4 weeks prior to the ammonia collection and analysis.

‡ Average ± standard error of the mean.

§ UA-N = uric acid nitrogen incorporated into the diet as free uric acid.

‖ NH_3-N incorporated into the diet as NH_4Cl.

reactions are apparently present in *Periplaneta*, since McAllan & Chefurka (1961) have shown the fat body to have a very active glutamic dehydrogenase whose equilibrium favours the amination of alpha-ketoglutarate producing glutamate. They also demonstrated a glutamate-aspartate transaminase whose equilibrium favours the disappearance of glutamate and production of aspartate.

The total non-ammonia nitrogen, total ammonia nitrogen, and total nitrogen excreted by males maintained on twelve diets for 8 weeks is given in Table 4. The nitrogen content and equivalent protein rating have been included as an index of percentage nitrogen input. It can be seen that generally as the dietary nitrogen level increases, there is a corresponding increase in the total amount of nitrogen elimination. Ammonia excretion increases in response to increased dietary protein consumption and correspondingly, contributes a larger portion of the total nitrogen excreted. Those insects consuming diets containing ammonium chloride (9 through 12) in concentrations ranging from 1 to 6 % total nitrogen excreted about the same amount of ammonia nitrogen. However, non-ammonia nitrogen excretion tends to increase as the NH_4Cl content of the diet increases, except for the 6 % NH_4Cl diet. Insects on this diet generally consumed less and suffered from a higher mortality rate. Also males consuming dextrin (no nitrogen) excreted some nitrogenous materials of which 35 % was found to be ammonia-nitrogen. Those insects feeding on dextrin + 1 % uric acid nitrogen showed a similar pattern, indicative of uric acid-nitrogen absorption from the diet (Mullins & Cochran, 1972).

Preliminary studies of nitrogen excretion in *Periplaneta* have already shown that

Table 5. *Potassium, sodium, calcium and magnesium concentrations in the faeces obtained from males maintained on twelve diets for eight weeks**

Diet Number and composition	Micromoles per gram of faecal material				Total metallic cations (μM/mg)
	K^+ Av. ± S.E.M.†	Na^+ Av. ± S.E.M.	Ca^{2+} Av. ± S.E.M.	Mg^{2+} Av.—S.E.M.	
1. Dog food	647 ± 4	735 ± 39	446 ± 6	265 ± 6	2·09
2. 25% Casein protein	941 ± 54	578 ± 34	167 ± 4	143 ± 2	1·83
3. 25% Casein hydrolysate	933 ± 14	1359 ± 39	247 ± 20	187 ± 5	2·73
4. 42% Casein protein	504 ± 32	425 ± 29	130 ± 10	136 ± 7	1·20
5. 76% Casein protein	399 ± 60	291 ± 50	110 ± 13	86 ± 19	0·89
6. 25% Casein protein +1% UA-N	714 ± 63	561 ± 21	187 ± 17	162 ± 3	1·62
7. Dextrin	708 ± 17	277 ± 19	287 ± 9	137 ± 5	1·41
8. Dextrin + 1% UA-N	624 ± 2	304 ± 37	269 ± 9	116 ± 6	1·31
9. Dextrin + 1% NH_3-N	494 ± 28	225 ± 17	245 ± 26	89 ± 3	1·05
10. Dextrin + 2% NH_3-N	419 ± 17	229 ± 15	226 ± 21	86 ± 3	0·96
11. Dextrin + 4% NH_3-N	356 ± 17	223 ± 31	162 ± 18	79 ± 4	0·82
12. Dextrin + 6% NH_3-N	350 ± 27	188 ± 12	162 ± 17	93 ± 5	0·79

* Faecal samples were collected for 2 weeks after the insects had been maintained on their respective diets for 6 weeks.

† Average ± standard error of the mean.

as the dietary level of nitrogen is elevated, ammonia excretion is increased (Mullins & Cochran, 1972, 1973). The values for ammonia excretion reported here are generally higher than those previously reported which is most likely due to the incorporation of cellulose into the diet formulations adding absorption area to the food bolus/faecal pellet, and to the use of a more reliable apparatus for the collection of ammonia. Studies conducted on other organisms such as the earthworm *Lumbricus terrestris* (Tillinghast & Janson, 1971) and pigeons, *Columba livia* (McNabb, McNabb & Ward, 1972) have shown that ammonia excretion increases as the dietary nitrogen level is increased. Diets containing different nitrogen levels as well as different nitrogen sources (uric acid and ammonium chloride) were used to examine more precisely the ammonotelic response to these parameters. Labelled ^{14}C uric acid has been detected in fat body tissues isolated from cockroaches fed on diets containing labelled uric acid (Mullins & Cochran, 1972), and Sedee (1958) was able to show the bluebottle fly *Calliphora erythrocephala* capable of utilizing dietary ammonia for amino acid synthesis. It should be pointed out that as the dietary nitrogen level increases, there is also a corresponding increase in internal nitrogen storage in the form of urates (Mullins & Cochran, 1974*a*, *b*, *c*; Haydak, 1953; McEnroe, 1956).

Potassium, sodium, calcium and magnesium concentrations contained in the excreta produced by male cockroaches maintained on the twelve diets are presented in Table 5. The total content of these 4 metals is tabulated in the last column. It can be seen from this table that the total cationic content of these four metals ranges from 0·79 to 2·73 μM/mg faeces. The faecal material collected from insects maintained on the casein hydrolysate diet (3) contained the highest concentration of these metals and sodium was the major contributor. This high sodium content found in the faeces was due to a comparatively high sodium content in the diet (0·28 μM/mg contained in the casein hydrolysate diet compared to 0·12 μM/mg for the 25% casein protein diet

Table 6. *Comparison of the concentrations of potassium, sodium, calcium, magnesium and ammonium ions in the faecal material obtained from males maintained on twelve diets with the* ad libitum *water requirements*

Diets Number and composition	Metallic* cations (μM/mg)	Ammonium† cations (μM/mg)	Total cations (μM/mg)	NH_4^+ Cations‡ Total cations (%)	H_2O Consumed (μl/male/day) Av. ± S.E.M.
1. Dog food	2·09	1·30	3·39	38	104 ± 4
2. 25 % casein protein	1·83	2·62	4·45	59	93 ± 6
3. 25 % casein hydrolysate	2·73	2·71	5·44	50	131 ± 7
4. 42 % casein protein	1·20	3·87	5·07	76	137 ± 6
5. 76 % casein protein	0·89	6·50	7·39	88	179 ± 8
6. 25 % casein protein + 1 % UA-N	1·62	4·23	5·85	72	117 ± 10
7. Dextrin	1·41	0·67	2·08	32	59 ± 3
8. Dextrin + 1 % UA-N	1·31	0·57	1·88	30	66 ± 4
9. Dextrin + 1 % NH_3-N	1·05	0·77	1·82	42	87 ± 12
10. Dextrin + 2 % NH_3-N	0·96	0·92	1·88	49	86 ± 5
11. Dextrin + 4 % NH_3-N	0·82	0·78	1·60	49	100 ± 11
12. Dextrin + 6 % NH_3-N	0·79	1·06	1·85	57	109 ± 6

* See Table 5 for the concentrations of K^+, Na^+, Ca^{2+} and Mg^{2+} found in the faecal material.

† The values presented for NH_4^+ are estimates based on the data in Table 4 and the average mg of faecal material excreted by the insects maintained on the different diets.

‡ Percentage of the total cations excreted that may be attributed to ammonia.

Except for the casein hydrolysate diet, the metal cation concentrations detected in the faeces follow the general trend $K^+ > Na^+ > Ca^{2+} > Mg^{2+}$. A comparison of the dietary concentrations with the faecal concentrations is presented in the companion paper.

Table 6 compares the cation content of the excreta produced by males maintained on the twelve diets for 8 weeks with their corresponding *ad libitum* water requirements. The data show that the estimated ammonium ion excretion may constitute from 30 to 88 % of the total cations measured in the faeces. The increases in the total cation content (NH_4^+, K^+, Na^+, Ca^{2+} and Mg^{2+}) are largely due to the increases in NH_4^+ excretion. The *ad libitum* water consumption is shown to increase with the level of dietary nitrogen and subsequent ammonia excretion.

The relationships between dietary nitrogen level, *ad libitum* water consumption and nitrogen excretion are illustrated in Fig. 3. The regression coefficients for dietary nitrogen level-water consumption and dietary nitrogen level-total nitrogen excretion were $r = 0{\cdot}974$ ($p < 0{\cdot}001$) and $0{\cdot}975$ ($p < 0{\cdot}001$), respectively. Comparison between water consumption and total nitrogen excretion resulted in a regression coefficient of $r = 0{\cdot}886$ ($p < 0{\cdot}001$). The test for the significance of Pearson's r used here is described in Downie & Heath (1970).

The data presented in Tables 5 and 6 clearly show that the cationic contribution due to ammonium ions in excreta is considerable. The increased presence of excreted cations, particularly ammonium, appears to increase the water requirements of the cockroach. The physiological effects of a high ionic, and consequently a high osmotic, content in the faeces may elicit one of at least two responses: (1) reabsorbtion of water in the rectum against a high osmotic gradient, resulting in an expenditure of energy to produce a dry faecal pellet, or, (2) increased water consumption allowing for the elimination of a moist faecal pellet. Wall, Oschman & Schmidt-Nielson (1970), Wall &

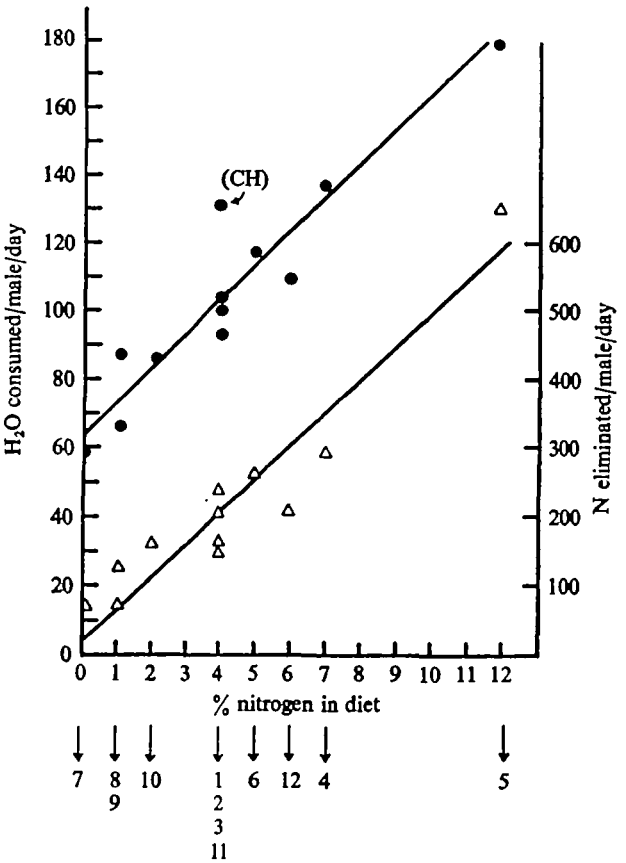

Fig. 3. Comparison of water consumption and total nitrogen excretion with dietary nitrogen levels. Water consumption is expressed as μl. water consumed/male/day (●) and total nitrogen excretion is reported as μg. N eliminated/male/day (△). The lower legend on the abscissa designates the individual diets fed and their corresponding nitrogen levels. For a description of the composition of a particular diet, see Table 4. CH indicates the casein hydrolysate diet.

Oschman (1970) and Wall (1971), have shown that the rectum of *Periplaneta* is able to absorb water from the lumen of the rectum. They hypothesized that the rectal pads are able to concentrate the rectal lumen fluids by net absorption of water in excess of solutes by a process of solute recycling in the rectal pads (Wall & Oschman, 1970). From their proposed model, the absorptive capabilities must be governed to some extent by the osmotic concentrations in the rectal lumen, since increasing the osmotic concentration of solutions injected into the rectal lumen resulted in much greater osmotic concentration increases in the intracellular spaces (Wall *et al.* 1970). Although no quantitative measurements of the water content of the faeces produced by cockroaches maintained on the different diets is available, visual inspection of their faecal deposits reveals quite clearly that those on higher nitrogen diets produce quite amorphous, moist faecal pellets. The results presented here are similar to those reported by McNabb *et al.* (1972), who demonstrated that pigeons on a high protein diet showed increases in water drinking rates, increases in minimum water requirements, together with increases in ammonia excretion and urine flow rates.

Heit, Sauer & Mills (1973) have shown recently that the *ad libitum* water consumption in *Periplaneta* increases in response to increasing concentrations of sodium chloride in the drinking medium. Although the water requirement values they reported are lower than those reported for starved males by other workers (Gunn, 1935 and Wharton & Wharton, 1961), they demonstrated a close relationship between these two parameters. The data obtained from the casein hydrolysate diet did not show a close correlation between the dietary nitrogen level and water consumption (Fig. 3). The relatively higher water consumption rates observed might be explained by the comparatively higher sodium content in the diet and faeces, which may have presented the insect with a situation quite similar to drinking hyperosmotic solutions of sodium chloride (Heit *et al.* 1973).

DISCUSSION

Although the role or mode of ammonia excretion is not well understood, it is clear that ammonia is a major cation in the excreted faecal pellet, and its presence does influence the water balance (requirements) of this insect. Under normal conditions ammonia is released even though a significant amount of nitrogen may be stored internally as uric acid. It is also released in smaller amounts when no dietary nitrogen is available, but under conditions where the stored reserves of uric acid are being mobilized (Mullins & Cochran, 1974*a*, *b*).

The mode of ammonia release is associated with the gut, not from respiratory surfaces. Precise determination of the origin of ammonia present in the gut is undoubtedly complex, since the microflora appear to be involved to some extent as indicated by feeding antibiotics and by the information presented by Todd (1944). However, it is quite probable that ammonia is also released by the gut tissues. Auclair (1959) and Boadle & Blaschko (1968) have shown a very active amino acid oxidase activity in the malpighian tubules of *Periplaneta*. Corrigan, Wellner & Meister (1963) has shown an unusually high D-amino acid oxidase activity in the malpighian tubules of several insects including *Periplaneta*. The hindgut may also be involved in ammonia excretion, since it has been shown to be the major site of ammonia secretion in blowflies (Prusch, 1972). Tillinghast & MacDonnell (1973) have examined the distribution of two ammonia generating enzymes (L-serine dehydrase and 5′-AMP deaminase) in the gut of *Lumbricus*, and have suggested two potential centres for ammonia production.

Ammonia excretion in *Periplaneta* is most likely the result of several activities which may include the gut microflora, nitrogen status and ion balance of the insect. A summary of the possible interactions, roles and physiological and biochemical reasons for ammonia releases from *Periplaneta* is presented here in an attempt to point out some of the complexities surrounding the releases of ammonia observed in these experiments.

(1) Ammonia may serve as a *bona fide* excretory product which is excreted only when the dietary nitrogen is in excess of its requirements or as a byproduct of general metabolism. However, that this is the only function of excreted ammonia is doubtful, since this insect is quite capable of synthesis and storage of uric acid in response to high dietary nitrogen levels, and ammonia is a significant nitrogenous excretory product even in *Periplaneta* when maintained on a nitrogen-free diet.

(2) The presence of ammonia in the gut may serve to buffer the contents of the gut for enzymatic and microbial activities. Greenberg *et al.* (1970) have shown the pH of the gut to be slightly acidic, but quite stable, with a slight but consistent, decreasing gradient of acidity from the crop to the rectum.

(3) Ammonia releases may be the result of deamination of D-amino acids which presumably might be produced by mycetocyte bacteriods and by gut microflora. The apparent absence of D-amino acids from animal tissues has led to the suggestion that the function of D-amino oxidase is to destroy D-amino acids produced by bacteria (Corrigan *et al.* 1963; Corrigan, 1969). Since *Periplaneta* contains both mycetocyte bacteroids and gut microflora, it is possible that their metabolic activities might produce D-amino acids in concentrations requiring removal from the insect by the D-amino acid oxidases. Support for this point might be obtained from the fact that insects fed on dextrin for 8 weeks and, which may be assisted in achieving mobilization of their stored urates by the mycetocyte bacteroids (Malke & Schwartz, 1966; Pierre, 1964; Mullins & Cochran, 1974*a*, *b*), continue to excrete ammonia.

(4) It is possible that ammonia is released into the gut so as to provide the gut microflora with a nitrogen flux with which they might produce materials that if absorbed could prove to be useful to the host on a low nitrogen diet. The possible nitrogen metabolic inter-relationships of gut micro-organisms with the host has been examined recently (Salter, 1973), and poses some interesting questions. A system may be available in the gut tissues for absorption of small nitrogen-containing materials produced by the gut microflora. Balshin & Phillips (1971) have proposed that there is an active transport mechanism for glycine and possibly other amino acids in the rectal wall of locusts. Although Wall & Oschman (1970) did not distinguish between ammonia and amino nitrogen present in the gut, they showed that in dehydrated *Periplaneta*, total amino nitrogen concentrations decreased as gut lumen contents approached and were held in the rectum. Their results suggest that some absorption of amino nitrogen under certain conditions may occur prior to excretion of a faecal pellet.

(5) Finally, ammonia excretion in this insect might facilitate water and ion conservation, particularly that of sodium. Injections of sodium into these insects resulted in a reduction in the amount of ammonia excreted when compared to that produced by equimolar ammonia and potassium injections. Wall & Oschman (1970) have shown that under normal, hydrated and dehydrated conditions, the sodium concentration decreases as the gut lumen contents approach and are held in the rectum. They have suggested that the rectum absorbs sodium in proportion to water in dehydrated cockroaches. Wall (1971) cited some unpublished data which indicates that sodium and potassium and their accompanying anions account for only about 50% of the total osmolality of the rectal pad tissue and intracellular fluid. Amino nitrogen could make major contributions to the maintenance of this osmolality, since in hydrated animals about 20% of the rectal pad sinus fluid was found to be composed of free amino nitrogen (Wall, 1971). Prusch (1972) has suggested that although ammonia excretion by the blowfly functions primarily to eliminate waste nitrogen, it may also be involved in conservation of sodium and potassium.

The excretion of ammonia and cations appears to be related to the insect's general metabolism, particularly that of the fat body. An examination of cation storage and

release in relation to nitrogen balance has been found to be significant and is the subject of the following paper.

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