The concentration of ammonia in the excreta of sixth instar larvae of *Lamida moncusalis* Walker (Pyralidae: Lepidoptera) during development

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Summary. The concentration of ammonia in the fresh and dry excreta of *Lamida moncusalis* Walker was determined. It was found that a large quantity of ammonia was lost from the excreta on drying. Ammonia is one of the major excretory products of the larva.

Key words. Lamida moncusalis; excreta; ammonia; development; nitrogenous excretory products.

The classical concept about the nitrogen excretion of terrestrial insects is that they are primarily uricotelic, and it is considered that they do not excrete ammonia as one of the major nitrogenous waste products. If ammonia is present in the excreta of terrestrial insects, it is in small amounts[2–10]. But it has been recently demonstrated that in *Periplaneta americana*, ammonia is a major excretory product[11–13]. A comparative study of nitrogen excretion in 23 cockroach species also showed that ammonia is the major excretory product in most of them[14]. Lazar[15] found that the feeding stages of the sixth instar larvae of *Spodoptera mauritia* were ammonotelic. It is therefore essential to investigate the excretion of different nitrogenous by-products by a variety of terrestrial insects to formulate a general concept of the nature of excretion in this group. In the present study the ammonia concentration in the excreta of sixth instar larvae of *Lamida moncusalis* is estimated.

Materials and methods. Sixth instar larvae of *Lamida moncusalis* Walker reared in the laboratory on fresh mango leaves (*Mangifera indica*) were separated from the colony immediately after molting and were used for experiments. The feeding stages of the larvae were divided into phase I (0–24 h), phase II (24–48 h), phase III (48–72 h), phase IV (72–96 h) and phase V (96–120 h).

Collection of excreta was done according to Lazar and Mohamed[16]. The estimation of ammonia in the excreta and food was done according to Miller and Rice[17]. The ammonia was isolated on a cation exchange resin (Dowex 50W-X16, 20–50 mesh, J.T. Baker Chemical Co., USA). It was then eluted into a known quantity of dilute sodium hydroxide (0.1 N). The ammonia in the aliquots was determined photometrically using the Berthelot reaction. Standard ammonium sulphate processed in the same way was used for calibration. An average dry content of the fresh excreta (14.38%) was used to calculate the weight of dry excreta where fresh material was used for estimation of ammonia.

Results and discussion. The results of the analysis of the fresh excreta for ammonia in the various developmental phases are presented in the table.

The concentration of ammonia in the fresh excreta was at a minimum during phase I. The concentration increased gradually and reached a maximum value during phase IV but declined sharply in phase V.

The table also illustrates the amount of ammonia present in the dry excreta and the percentage of ammonia lost on drying the excreta. The loss of ammonia on drying was very high, i.e., 86.88–89.88%. The loss of ammonia during different phases did not show any significant difference.

It seems from the present study that the sixth instar larvae of *L. moncusalis* employ ammonia as one of the major nitrogenous excretory products as is the case for cockroaches[11–14] and *S. mauritia*[15]. In the larvae of *L. moncusalis* only 10.12–13.12% of the total ammonia was found when the excreta were dried. In the present study all possible measures were taken against the loss of ammonia during the analysis of the excreta. Ammonia is a volatile compound, so it is to be expected that it will be lost on drying or lyophilization. Only non-volatile ammonium salts will be present in the dry excreta. Zielinska[2], while estimating total nitrogen of *Galleria mellonella*, observed that 40% of the material was lost upon drying of the larvae. Aquatic insects[18, 19] and dipteran larvae[20–22] are considered to be ammonotelic on the basis of the quantity of ammonia excreted to the surrounding medium, in which ammonia gets dissolved. In these cases no question of volatilization arose. Pramila and Krishnamoorthy[9] observed that in the larvae of *Bombyx mori* an enormous quantity of ammonia (12.7–174 µmoles/individual/h) was volatilized from the animal. Mullins and Cochran[11, 12, 14], Mullins[13] and Lazar[15] considered the factor of the loss of ammonia during drying of the excreta and took adequate precautions while processing the material.

The analysis of mango leaves showed only traces of ammonia. The feeding of the larvae in phase I is minimal and the excreta also show a minimal amount of ammonia. At phase IV, the feeding activity and growth rate are the highest. Here the excreta show the maximum concentration of ammonia. It is therefore probable that this compound is of metabolic origin. The possibility that it could be produced by the gut microflora is small.

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Ammonia content of excreta

Larval phases	µg/g (fresh excreta analysis)	µg/g (dry excreta*)	% of dry excreta	µg/g (dry excreta analysis)	% of loss of ammonia on drying
I	509.63 ± 22.37	3544.02 ± 155.57	0.35	361.11 ± 34.69	89.88
II	627.14 ± 90.16	4361.28 ± 627.00	0.44	568.89 ± 134.97	86.98
III	654.47 ± 87.03	4551.28 ± 605.23	0.46	601.14 ± 169.73	86.88
IV	733.91 ± 11.85	5033.28 ± 90.31	0.50	647.41 ± 71.57	87.19
V	573.76 ± 132.01	3570.51 ± 627.00	0.36	365.71 ± 6.60	89.78

Five samples each were used in the estimations and the results are the mean of the five determinations with standard deviations. * The mean percentage dry content of excreta (14.38%) was used to convert the values per unit fresh weight to unit dry weight. The difference between the amount of ammonia obtained in the fresh excreta analysis and dry excreta analysis was used to calculate the percentage of ammonia lost on drying.

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Lack of pressor effect of dopamine in the pentobarbital-anesthetized rat

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Summary. The blood pressure and heart rate responses to intravenous dopamine infusion at 2.5, 5.0 and 10.0 $\mu g \cdot min^{-1} \cdot 100\,g^{-1}$ were studied in conscious and pentobarbital-anesthetized Sprague – Dawley rats. In the conscious rats, dopamine caused a significant dose-related increase in the mean arterial blood pressure which was abolished in the anesthetized rats. The heart rate increased significantly only at the highest dose infused. The responses to equipressor doses of noradrenaline (40 $ng \cdot min^{-1} \cdot 100\,g^{-1}$) and phenylephrine (1.0 $\mu g \cdot min^{-1} \cdot 100\,g^{-1}$) were also suppressed in the anesthetized rats. The results suggest that pentobarbital anesthesia depresses the blood pressure response to dopamine infusion in the rat through a depression of activation of alpha-adrenoceptors.
Key words. Dopamine; blood pressure; heart rate; anesthesia.

Previous reports on the blood pressure effect of dopamine in the rat are scanty. A dose-related pressor effect has been described in the conscious rat[1]. This pressor effect of dopamine is, however, affected by various factors. Thus, on a low sodium diet, the blood pressure response in the conscious rat is attenuated[2]. Also, there is general agreement that the administration of dopamine causes a reduction of the blood pressure in animals pre-treated with alpha-adrenoceptor blocking angents[3,4]. This hypotensive response is thought to be due to vasodilation of the renal, mesenteric and coeliac beds[5], and/or partly due to the release of a dilator prostaglandin.[6]

In recent years, increased attention has been given to the importance of alteration in cardiovascular dynamics produced by general anesthesia[7,8]. In general, the cardiovascular system of the animal is depressed and the response to exogenous agents can be markedly affected by anesthesia[6]. The effect of anesthesia on the cardiovascular response to dopamine administration is unknown.

The purpose of this study, therefore, was to compare the blood pressure and heart rate responses to i.v. dopamine in the conscious and in the pentobarbital-anesthetized rat. To determine the role of alpha adrenoceptor stimulation in the response, equipressor doses of noradrenaline and phenylephrine were also used in the study.

Materials and methods. Eighty-two male Sprague-Dawley rats weighing between 120 and 240 g were used. The animals were bred and housed in the department under natural light and environmental conditions. They were allowed free access to a commercial feed (Pfizer Products, Lagos, Nigeria) and tap water to drink. They were subsequently divided into:

a) *Conscious rats:* Under sodium pentobarbital anesthesia (60 mg/kg, i.p.) two polyethylene catheters were inserted and secured: one into the right carotid artery, and the other into the left jugular vein. The free ends of the catheters were passed s.c. behind the ear on the right side of the neck and exteriorized. Catheters were filled with about 0.10 ml of heparinized saline (1000 units/ml), and plugged. The cut skin was sutured and the animal allowed to recover. 24 h later, when the animal was fully awake, the heparinized saline was removed from the catheters and discarded. A fresh 0.20 ml of heparinized saline was then injected to keep the carotid catheter patent, while through the jugular vein, 5% dextrose solution (154 mmol/l) was infused (Ealing Universal Infusion Pump, Walford, England) at a constant rate of 1.0 ml/h for 60 min (equilibration period); the last 20 min was taken as the pre-drug infusion period (Pre-drug). Thereafter, dopamine (3,4-dihydroxyphenylethylamine hydrochloride; Sigma Chemical Co., St. Louis, MO, USA) was infused at 2.5, 5.0 and 10 $\mu g \cdot min^{-1} \cdot 100\ g^{-1}$ for another 20 min (Experimental period). Dopamine was dissolved in 5% dextrose and infused at a constant rate of 1.0 ml/h. In the subsequent 20 min (Recovery period), 5% dextrose was again infused. In a control group of rats, 5% dextrose only was infused continuously for 60 min after the equilibration period. The pulsatile arterial blood pressure was measured via the carotid artery using Statham P23ID pressure transducer and recorded on a Gilson Model 5/6H-Polygraph at 0.1 mm/s chart speed. The mean arterial blood pressure was taken as the diastolic blood pressure plus one-third of the pulse pressure. To obtain the heart rate, the chart was speeded to 5.0 mm/s and the pulse peaks counted. During the infusion, all rats were unrestrained and put on a stainless steel pan to which they were accustomed prior to the experiment.

b) *Anesthetized rats:* Anesthesia was induced with sodium pentobarbital (60 mg/kg i.p.). The rats were placed on a thermostatically heated table that maintained the body temperature constant at 37.0 ± 0.5 °C. They were thereafter prepared for dopamine infusion as in the conscious group, except that in addition, the trachea was also intubated. After surgery, the animals were allowed at least 45 min of equilibration during which 5% dextrose was infused at 1.0 ml/h and the blood pressure had stabilized. At the end of the equilibration period, the pre-drug infusion data were recorded. The subsequent protocol was similar to that in the conscious rats. In another group of control rats, 5% dextrose was infused continuously for 60 min after the equilibration period.

c) Equipressor ($\simeq$ 40 mmHg increase in mean arterial blood pressure in the conscious rat) doses of noradrenaline (40 $ng \cdot min^{-1} \cdot 100\ g^{-1}$) and phenylephrine (1.0 $\mu g \cdot min^{-1} \cdot 100\ g^{-1}$) were also similarly infused in further groups of conscious and anesthetized rats. The conscious and anesthetized rats for noradrenaline and phenylephrine infusions were prepared as above.

Statistical analysis: All values are given as the mean ± SEM. The data were analyzed using Student's t-test with Dunnett's correction to the t-value. A p value less than 0.05 was considered significant.