INDUCED TRANSIENT HYPERAMMONEMIA IN *RANA HEXADACTYLA* WITH REFERENCE TO THE AMMONIA AND UREA EXCRETION

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Summary : Different ammonia toxicity induced by ammonium acetate administration in frogs lead to variable excretion of ammonia and urea into the medium. When 11.45 *mM/kg* body weight of ammonium acetate was administered, ammonia excretion increased while urea excretion decreased. When 4·17 *mM/kg* body weight of ammonium acetate is administered, the urea excretion increased while ammonia excretion decreased. The possible metabolic conversion of ammonia and urea by the animal at high and low ammonium acetate administration has been discussed.

Key words : hyperammonemia, ammonia, urea, frog, excretory patterns, ammonium acetate

INTRODUCTION

In many aquatic and semi-aquatic, animals, ammonia (1) and urea are excreted indicating that body ammonia is excreted either as such and also after conversion into urea. Experimentally induced hyperammonemia was reported to alter the excreted ammonia and urea levels in the fishes (2,3,4) indicating the possible changes in the ornithine cycle system in liver. Amphibians such as *Rana hexadactyla* lead amphibious life leaning towards more of ureotelism than ammonotelism thereby indicating greater potentiality for ornithine cycle system and consequent ureogenesis. In cases of hepatotoxemia as a consequence of ingestion of polluted prey, the ammonotoxemia is imminent with its consequent lethality (5). Since frogs are of utility value as biological insect controls as well as source of food for humans, it will be worthwhile to investigate the patterns of detoxification of ammonia prevalent in the animal under induced ammonotoxemia and test the tolerance levels.

MATERIAL AND METHODS

Medium size frogs, *Rana hexadactyla*, were accilimated to laboratory conditions for a period of one week. Different concentrations of ammonium acetate (in 0.75% saline) was intraperitoneally administered to separate frogs to determine LD_{50} which was found to be at 11.7 *mM/kg* body weight of ammonium acetate in three hour period. Separate batches of frogs received 100, 200, 400 and 500 *µm*/animal representing 2.08, 4.17, 8.33 and 11.45 *mM/kg* body wt of ammonium acetate and were separately placed in the 100 *ml* medium.

Ammonia and urea contents in the medium were estimated using the methods of Ward *et al.* (6) and Natelson (7) respectively and the values were expressed as *µmoles* of excretory product (ammonia or urea)/100 *ml* of the medium and were mean of eight observations.

Ammonia and urea contents were estimated in the medium. Since immediate metabolic responses are envisaged soon after the administration of ammonium acetate, more number of samples in the first hour time sequence (0,5,15,30,60 min) were analysed than the second hour (90,120 min) and third hour (180 min). It is presumed that the system assumes metabolic balance in later periods. The urea-ammonia (U/A) ratios were calculated for three hour samples to determine the optimum dose of ammonium acetate. But to understand the ammonia toxicity, two concentrations alone were studied. Hence, two sublethal concentrations namely 550 (near lethal) and 200 uM (far away from lethal) animal representing 11.45 and 4.17 *mM/kg* body wt of ammonium acetate respectively were selected. The control animals received equal quantity of saline.

Straight line curves for excretory product levels in the medium at different periods were fitted using least squares as the best fit and slopes for the above curves were calculated.

RESULTS AND DISCUSSION

Ammonia and urea content was not detectable in the aquatic medium employed. When saline administered animals were kept in the medium, the ammonia and urea content were detected in traces (0.04 and 1.2 *µmoles* respectively) and the levels can be considered as insignificant. When ammonium acetate administered animals are kept in the medium, there is consistent and persistent increase in the levels of ammonia and urea in the medium containing animals under differential ammonotoxemia, indicating

excretion of both ammonia and urea. However, the rates of excretion seem to vary in these types. In order to find out the critical concentration of ammonium acetate administration that will give U/A ratio comparable to that of the control ratio the following method has been adopted (Table. I). The medium having control animals showed a

TABLE I : Ammonia and urea excretory patterns of the animal after three hours of administration of different concentrations of ammonium acetate. Ammonia and urea contents are expressed as *µmoles/100 ml* of medium and are mean ± S.D. of eight observations.

	Control	*2.08 mM*	*4.17 mM*	*8.33 mM*	*11.45 mM*
Ammonia	0.250	0.260	10.017	20.80	13.90
	±0.050	±0.01	±2.36	±3.5	±2.00
Urea	2.430	2.320	48.622	23.491	18.830
	±0.167	±0.10	±4.03	±3.67	±3.960
U/A ratio	9.72	9.0	4.85	1.12	1.33

U/A ratio of 9.7. In the medium containing experimental frogs, the U/A ratios showed a decrement upto 8.33 mM of ammonium acetate administration. While at 11.45 *mM/kg* body wt of ammonium acetate administration, the ratio showed a slight increase. These findings indicate that the urea production is affected under high doses of ammonium acetate administration. The ratio which alters the U/A ratio least as compared to the control can be considered as optimum dose which is 2.08 *mM/kg* body wt of ammonium acetate administration. This concentration is taken as critical optimal dose since increase or decrease in the ammonium acetate concentration will reverse the U/A ratios. With the increase in ammonium acetate concentration the urea excretion increases initially and decreases at later concentrations. Hence it is felt desirable to select two concentrations of ammonium acetate which will fall either at the lower or higher concentrations levels from that of the critical optimal dose for detailed investigations.

Ammonia content is usually maintained at fairly constant level in the body (8) and increase in ammonia content would invariably result in its excretion. An inbuilt regulatory mechanism for the conversion of ammonia into a safer product namely urea (9,10) also operates in the animal so as to reduce the hyper-ammonemic toxemia. However, under extremely high ammonia levels in the body, this inbuilt metabolic machinery may be totally incapable of detoxification of excess ammonia. Under these conditions only part of ammonia will be converted into urea and the rest excreted as such.

The ammonia and urea content in the medium containing animals under mild and intense ammonotoxemia, showed a continuous increase in the course of three hour investigation period suggesting that both these agents are continuously excreted by them. However the rates of excretion of these two components seem to vary. In animals under mild ammonotoxemia is less and the rate of excretion of urea is high when compared to the animals under intense ammonotoxemia.

This difference in the rates can be clearly envisaged in terms of the slope values obtained from the metabolite excretion in the temporal sequence of the animals staying in the medium. Under mild ammonotoxemia, the slope is 2.9 while under intense ammonotoxemia it is 3.9 indicating that intense ammonotoxemia results in increased excretion of ammonia into the medium (Fig. 1).

The slope values for urea excretion in temporal sequence show a reverse trend though the excretory rates are generally showing an increase with time (Fig. 2). At mild ammonotoxemia, the slope value is 19.3 while in higher ammonotoxemia it is 6.2.

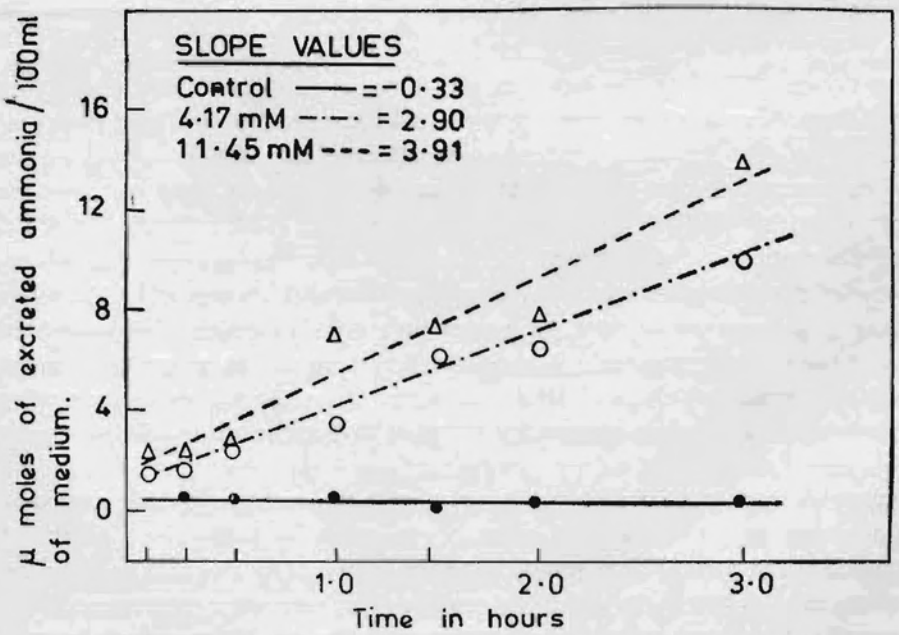

Fig. 1 : Slope representation of ammonia and urea levels in the medium in which control and experimental frogs receiving 4.17 and 11.45 *mM/kg* body wt of ammonium acetate are kept separately.

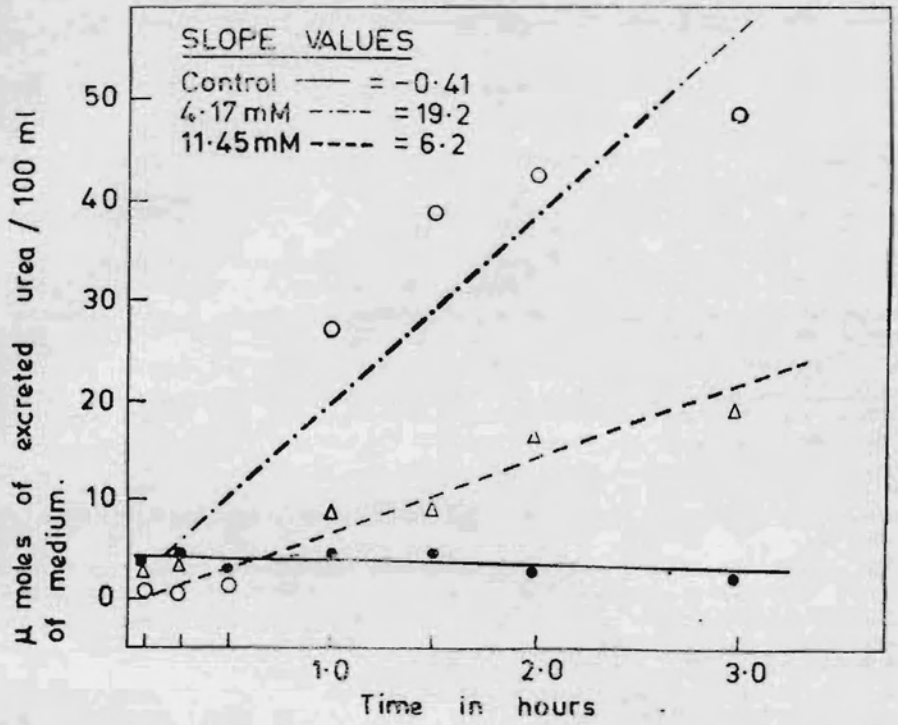

Fig. 2 : Slope representation of ammonia and urea levels in the medium in which control and experimental frogs receiving 4.17 and 11.45 *mM/kg* body wt of ammonium acetate are kept separately.

These observations indicate an elevated excretion of urea at mild ammonotoxemia which is extensively retarded at higher ammonotoxemia. Since the rate of excretion of urea is an index of ammonia conversion into urea in the body and later excretion, it is reasonable to presume that under milder ammonotoxemia there is an efficient and stepped up ureogenesis in the animal. At intense ammonotoxemia, decreased urea excretion is observed. As the tissues have shown high urea levels (11) some derangement in urea transport excretion is suspected since permeability properties and many transport processes across the membranes are reported to be affected in ammonotoxemia (12). Hence it is reasonable to expect excretion of ammonia to increase as conversion of ammonia to urea might not have been occurring due to extensive urea deposits in the tissues and impairment in urea excretion. Interestingly the ammonia excretion does not show a corresponding

increase under high ammonotoxemia when compared to low stress indicating a possibility of triggering new metabolic events in the animal at higher ammonotoxemia.

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