Reports

Relation of Ammonia Excretion to Urine pH in the Guinea Pig

In 1856 Claude Bernard (*1*) observed that the urinary *p*H of carnivorous animals is markedly lower that that of herbivorous animals. Some years later Walter (*2*) described the excretion of ammonia in various animals that had been rendered acidotic and stressed the protective and base-saving features of this mechanism. Since then, numerous studies on the patterns of ammonia excretion in man, dog, and rat, especially during acidosis, have been published. On the other hand, very few studies on these patterns in the rabbit have been made and, as far as we are aware, none in the guinea pig. The current theories of ammonia excretion are, therefore, largely based on studies in species that normally excrete an acid urine.

In spite of much work in this field, three major questions await conclusive answers. What is the physiological stimulus for ammonia excretion? What is the relative importance of enzymatic and physicochemical factors in the mechanism of ammonia excretion? What is its physiological significance? During studies on ammonia excretion in the guinea pig, we have made observations that are surprising when they are considered in the light of present concepts of ammonia formation and excretion.

The ammonia output of guinea pigs (1 to 4 microequivalent/30 min kg) is low compared with that of rats or dogs and varies with age, sex, and dietary habits. In acute experiments (*3*) guinea pigs were rendered acidotic or alkalotic; and urine, collected under oil, was analyzed for *p*H (*4*) and ammonia content (*5, 6*). The urinary ammonia concentration was expressed as microequivalents per milliliter and the output as microequivalents per 30 minutes, per kilogram of body weight. Data from seven acute experiments are plotted on Fig. 1, using the urinary *p*H as abscissa and ammonia concentration or output as ordinate (*7*). The graph shows that both the concentration and the relative output of ammonia in the urine rise, not only during acidosis as in man, dog, and rat, but also during alkalosis. The same is true if the relative ammonia output for each animal per 30 minutes is plotted against the *p*H. The correlation between deviations of *p*H from the normal range and increased ammonia output is not strict but is statistically significant. A similar pattern has been observed in a few experiments with rabbits.

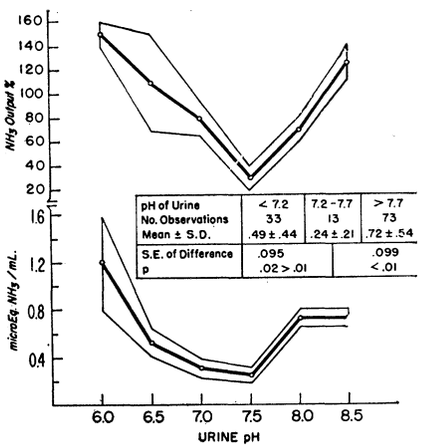

Fig. 1. The relation of ammonia excretion to urine *p*H in the guinea pig.

The increased ammonia concentration in alkaline urines cannot be explained as a function of urine flow, as could an increased output. It must, therefore, be assumed that there is increased ammonia production or transfer into alkaline, as well as acid, urine.

These observations suggest reconsideration of current hypotheses regarding the stimulus. Decreased plasma bicarbonate or *p*H or increased titratable acidity or decreased *p*H of the urine have been proposed as the proximate stimulus for the increase in ammonia output during acidosis. None of these, however, could cause the marked increase in ammonia output observed during alkalosis. Information now available does not indicate whether the same or different stimuli act during acidosis and alkalosis.

Ammonia excretion is currently considered to be the resultant of two independent mechanisms, one being enzymatic, the other being physicochemical in nature. Investigations during the past few years indicate that glutaminase activity in the kidneys is one of these factors (*8*). Transport of ammonia from tubular cells into urine, be it by passive diffusion (*9*) or active exchange for sodium (*10*), is held to be the second limiting factor for ammonia excretion. Our experiments demonstrate that ammonia excretion in the guinea pig is nearly as efficient in the alkaline as in the acid range. It seems improbable, therefore, that physicochemical transport is the limiting factor in ammonia excretion in the guinea pig. If it were, different mechanisms in the acid and alkaline ranges would have to be postulated.

At urine *p*H's of 8, 8.5, and 9 the fractions of ammonia present in an un-ionized form as free ammonia are respectively 4, 11, and 29 percent (*11*). Back diffusion of the ammonia during the flow of the urine from the distal tubules to the bladder should, therefore, also be taken into account in studies on ammonia excretion in the alkaline range. This aspect has not yet been explored.

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References and Notes

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2. F. Walter, *Arch. exptl. Pathol. Pharmacol.* 7, 148 (1877).
3. The animals were anesthetized with pentobarbital and ether, and a stomach tube and bladder catheter were inserted. They were given $NaHCO_3$ or HCl by stomach tube. The experiments lasted 2 to 6 hours, and 6 to 15 urine samples were collected during each experiment.
4. Determinations of *p*H were done with a Cambridge Research *p*H meter using the syringe-type glass electrode.
5. A modification of the microdiffusion method of D. Seligson and H. Seligson [*J. Lab. Clin. Med.* 38, 324 (1951)] was used. The working range of this method in our hands was between 0.5 and 100 microequivalents of ammonia nitrogen.
6. This investigation was supported by a research grant, RG-3795, from the National Institutes of Health, U.S. Public Health Service.
7. Owing to individual variations in the level of ammonia excretion, the average ammonia excretion in each experiment was assigned a value of 100, and the individual values were expressed as percentages of this figure.
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11. The *pK* for the reaction $NH_4^+ \rightleftharpoons H^+ + NH_3$ was taken as 9.4 [E. J. Conway, *Microdiffusion Analysis and Volumetric Error*, C. Lockwood (London, ed. 2, 1947)], p. 88.

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Taming and Susceptibility to Audiogenic Convulsions

Several recent papers have reported that taming influences both growth and behavior in the albino rat. Tamed animals are described as larger, heavier, and more resistant to the effects of physical and emotional stress than untamed ones. They have also been characterized as

Table 1. Frequency of *N*, *R*, *C* responses, mean frequency and intensity scores, median latencies of first running, and epileptoid attacks in tamed and nontamed rats.

Group	No.	*N*	*R*	*C*	*F*	*I*	Latencies trial 1st running attack	Latencies trial epileptoid phase
Nonhandled	16	48	59	53	0.70	0.65	26.5	84.5
Handled	8	36	41	3	0.55	0.55	40	Indeterminate
Handled prior to test	7	39	22	9	0.44	0.48	62	Indeterminate

more active and less fearful, and they are poorer hoarders but better learners (*1–6*).

The purpose of the present experiment was to reevaluate the relationship between taming and audiogeic seizures. Previously reported differences are possibly confounded by differences in strain, and endocrine or nutritional status or both (*7*). In addition, currently available data are in conflict. One study (*8*) associates taming with low susceptibility, while a second claims tamed animals to be more sensitive to convulsion (*9*).

The experimental subjects were 31 albino rats of a strain inbred by the department of physiology, Emory University Schools of Medicine and Dentistry, for about 25 years, 47 days of age at the time of the first test. Sixteen, the untamed group, were whelped in a lightproof, extremely quiet room and separated into individual cages at weaning. They were touched only when they were transferred once a week to clean cages and when they were placed in and removed from the test chamber. This involved briskly picking up each animal by the tail. They were exposed to light and noise only during the test period. In contrast, the 15 tamed animals (originally there were 16 but one died) were reared in the regular colony room. At weaning, they were segregated three to a cage and subjected to taming. This consisted of 5 minutes of handling each day, the animal being petted, fondled, and allowed to crawl about in the experimenter's lap. Taming was continued throughout the experiment. In addition, seven were given 2 more minutes of petting just prior to being tested to determine whether a drastic change in treatment enhanced sensitivity. All were allowed free access to Purina Lab Chow and water from the time of weaning.

The animals were tested by being placed in a metal container inside a sound-resistant box and exposed to 2 minutes of noise (average level, 101 db; range, 90 to 104 db) generated by a 4-inch doorbell. Each animal received ten tests on alternate days. During each test the latencies of the first running attack and of the epileptoid seizure were recorded when these responses occurred. After each test the subject's test behavior was rated on a three-point scale from *N* (nonreaction) through *R* (running attack) to *C* (epileptoid convulsion). Frequency (*F*) and intensity (*I*) scores were derived from the data of the ten tests according to the following equations:

$$F = \frac{R + C}{N}\,;\; I = \frac{R(0.50) + C(1.00)}{R + C}.$$

The rationale of these scores and the criteria defining response categories is presented in an earlier paper (*10*). Table 1 presents a summary of these data.

Since the frequencies of *N*, *R*, and *C* ratings were derived from ten tests on 31 animals, the statistical criterion of independence is violated, making a chi-square evaluation impossible. The mean *F* and *I* scores were compared by means of Student's *t* test for small independent samples. Because there were animals in both tamed and untamed groups that did not react on any test, certain of the latencies were indeterminate. Median latencies are therefore reported, and statistical evaluation was accomplished by the Mann-Whitney *U* test for independent groups of unequal *N*.

Inspection of Table 1 suggests that taming has a protective effect directly proportional to its magnitude or immediacy or both. This conclusion, however, is not borne out by the statistical evaluation of the data. There is no reliable difference between the tamed group and the tamed group that was given further handling just before tests (*F* scores: $t = 0.65$, $P > 0.50$; *I* scores: $t = 0.44$, $P > 0.60$). When the scores of these two groups are pooled and compared with those for the unhandled group, differences in all but one case again lack significance. (*F* scores, $t = 1.58$, $P > 0.10$; *I* scores, $t = 1.39$, $P > 0.10$; latency per trial per running attack, $U = 161$, $P < 0.10$; latency per trial per epileptoid phase, $U = 168.5$, $P < 0.04$). Thus taming can be said to have a protective effect only in the sense that the tamed animals require a longer time to have a full-blown epileptic fit.

The basis of these negative results is a matter for speculation. They are probably not due to insufficient handling, since the amount given the present animals compares favorably with that reported in previous studies (*2, 8*). One possibility lies in the strain used. Our rats, derived from Wistar stock, are extremely docile, gentle, and easily handled. It is possible that the petting given the tamed groups could add only an insignificant increment to already maximally tame subjects. Inspection of the wide variability in responsiveness in both handled and nonhandled groups meanwhile suggests that much larger samples would be required to demonstrate the suggested beneficial effects of handling on seizures. Should this be the case, its importance to an understanding of the seizure mechanism is probably limited.

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Protoplasmic Streaming in Plants Sensitive and Insensitive to Chilling Temperatures

Many plant species of tropical and subtropical origin are subject to chilling injury when exposed to temperatures below about 10°C but above their freezing points. Severity of injury generally increases as chilling temperature decreases and as duration of exposure increases. Symptoms may develop during or after exposure.

In a study of physiological responses associated with chilling (*1*), I made some observations on protoplasmic streaming. Effects of temperature on streaming were observed by Sachs (*2*), who noted that streaming ceased at about 11°C in *Cucurbita pepo* and *Lycopersicon esculentum*, both of which are chilling-sensitive plants. In contrast, Sachs cited observations by previous workers that in several lower plants streaming ceased at or near 0°C. This suggests differential responses by chilling-sensitive and chilling-insensitive plants.