Nutating Annular Cage for Measuring Motor Activity

Abstract. *The construction of an activity-measuring device that utilizes a nutating annular animal enclosure is described. The instrument converts motor activity to numerical scores. With mice, the logarithms of these scores assume a normal distribution. A second test occurring 1 day, week, or month after the first fails to exhibit any habituation of the animals.*

The activity cage described in this report is the first known to utilize a mobile annular animal enclosure. This feature, in combination with the nutating disk on which the enclosure rests, provides for an apparatus that is sensitive only to movement in line with the sensing contact on the platform below, and insures uniform sensitivity throughout the entire course of animal movement. The cross-sectional area of the passageway is just large enough to allow the animal to reverse its body orientation.

Construction of the device is relatively simple and can be accomplished with readily available material (*1*). The apparatus as described in Fig. 1 is suitable for mice, but enclosures of larger diameter have been used with the same sensing unit for the measurement of rat activity. This instrument acts as an electrical switch by which a circuit may be repeatedly interrupted in proportion to animal movement. The interrupted circuit can be that controlling any of a number of recording instruments. As used in our laboratories, the activity cage drives a typical low-current vacuum tube relay circuit (*2*), which in turn drives a 28-volt d-c Veeder-Root counter. We find an assembly of 20 such units very convenient for pharmacological studies.

The nature of the activity data recorded by this instrument was determined in the following manner. Each of 500 nonfasted, male, albino mice of the Swiss-Webster strain, weighing 18 to 25 g, were injected intraperitoneally with 0.9-percent sodium chloride solution at a dose of 0.01 ml/g and immediately placed in the activity cage for 1 hour. The frequency distribution of the arithmetic activity counts for these animals was extremely skewed. However, conversion of these raw scores to their logarithms produced a normal frequency distribution. The mean log count was 3.100 ± 0.013 (S.E.), with a standard deviation of 0.280 and a coefficient of variation of 9.0 percent. Chi-square tests of goodness of fit to the normal curve, as well as statistical tests for skewness and kurtosis, indicated that the frequency distribution of log counts does not depart significantly from normality.

Experiments were conducted to determine the effect of the repeated exposure to the apparatus on the motor activity of the animals. On three separate occasions, each of a group of 100 mice was injected intraperitoneally with normal saline and motor activity measured for 1 hour on two consecutive days. In all three cases there was no significant difference between the mean log activity counts of the first and second day as determined by the *t* test. A similar study with 100 mice indicated that a second exposure 1 week after the initial one resulted in a somewhat higher count, possibly owing to continuing maturation of the animals during this time. In another study, a group of 15 male and 15 female rats from the Mead Johnson colony was tested for 1 hour at monthly intervals for a period of 6 months. The male group fell below its initial mean log count only once, and that was by 1.4 percent at the end of the sixth month. The female group remained above, generally showing more activity than the male. Hence, habituation, as demonstrated by reduced activity upon repeated exposure, was not evident with this instrument. As previously suggested (*3*), this lack of habituation may well be due to the positive feedback provided by the movement of the activity cage in response to the animal's own movement. The tilt in the cage floor of approximately 2° is almost imperceptible and does not appear to affect animal locomotion adversely.

The preceding experiments were all performed during normal daytime working hours. The activity cages were housed in a normally lit room in individual cubicles, which were arranged in pigeonhole fashion, visually isolated from each other. The counters were situated at a distance in another room.

In pharmacological studies, we us-

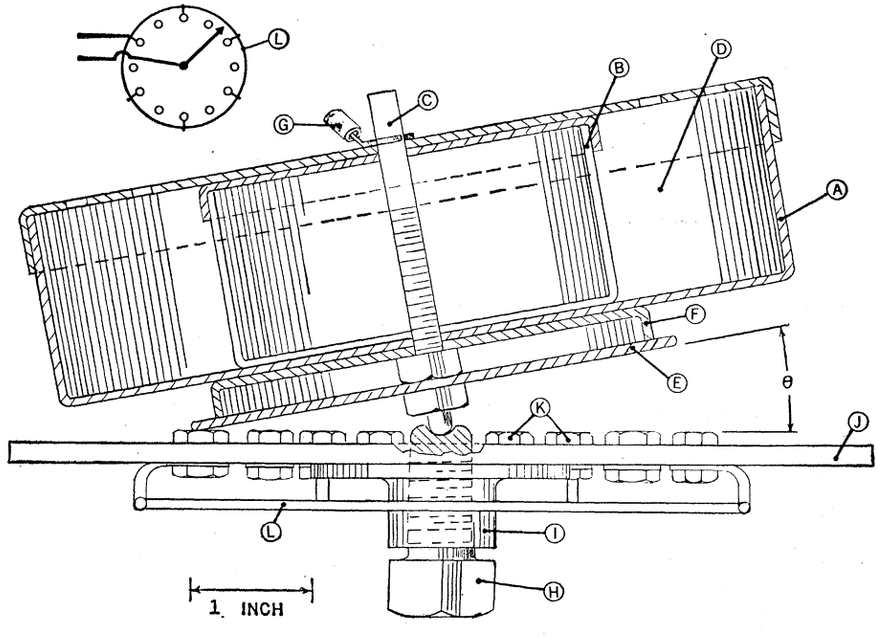

Fig. 1. Construction of the nutating annular cage. Two plastic containers *A* and *B* are concentrically positioned around the central brass shaft *C*, providing an annular passageway *D*. This assembly is supported above a copper disk *E* by a plastic container lid *F*. A solder lug *G* provides a removable closure latch. The central shaft pivots in an indentation in the end of 9/16 inch machine bolt *H*, which is supported by a threaded Castaloy flexaframe foot *I* fastened to a ¼-inch-thick Masonite panel *J*. The bolt *H* provides for setting the angle θ, usually 2.0° to 2.5° (exaggerated in the drawing for clarity). Placed in a circle about the pivot are 24 3/16 inch brass machine bolts *K*, the heads of which support the edge of the disk. Alternating bolts are connected electrically by a wire *L*. The inset illustrates the manner in which the unit acts as a rotary switch.

ually test vehicle-control animals simultaneously with the drug-treated animals. Scores are recorded by groups at the end of an appropriate time interval, converted to their logarithms, and averaged. The difference between these two means represents the response for that particular dose. Linear log dose-response curves have resulted from data recorded in this manner.

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References and Notes

1. Plastic containers suitable for animal enclosure construction are available as stock merchandise from Tri-State Plastic Molding Co., Henderson, Ky.
2. I thank J. G. Jackson for assistance in selecting this recording system. The relay unit was designed and constructed by G. F. Stoltz, South Central Broadcasting Corp., Evansville, Ind.
3. S. Irwin, *Rev. Can. Biol.* **20**, 239 (1961).

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Ureotelism of Echidna and Platypus

Abstract. *Analyses of plasma and urine and the demonstration of arginase in the liver indicate that both the adult echidna* (Tachyglossus aculeatus) *and platypus* (Ornithorhynchus) *are ureotelic.*

Data reviewed by Needham (*1*) show that the echidna excretes 80 to 90 percent of its nitrogen in the form of urea, and this animal is therefore classified as ureotelic. There is no published information on the platypus. The generalization has been made that animals with a closed egg are uricotelic and this raises interesting questions concerning the monotremes which are egg layers. The echidna lays a soft noncalcareous egg from which the young emerge in the pouch at an early stage and proceed to feed on the milk which exudes into the pouch. For these reasons the pouch has been regarded as "a uterus located in an unusual position," and the echidna may therefore be classified as viviparous. Thus, this exception to the generalization has been tentatively argued away. As Needham writes, "It would be very interesting to investigate the nitrogen partition in the urine of the platypus which allows its eggs to develop outside the body." Our results indicate that both the adult echidna and platypus are ureotelic. Since we do not possess any information concerning the nitrogen metabolism and water relations of these animals in the egg stage, it cannot be determined how completely the generalization concerning the relationship between the closed egg and uricotelism will have to be modified. Smith (*2*) raised doubts on this proposition when reporting that turtles excrete most of their nitrogen as urea.

Table 1 shows the analyses of blood plasma and urine. The urea was determined by the method described by Hawk (*3*), allantoin by the method described by Young and Conway (*4*), and uric acid by the Folin method (*5*).

The results show that there is considerable excretion of nitrogen in the form of urea in both the platypus and the echidna. In the echidna, urea is present in the plasma at a concentration similar to that found with mammalian species. Unfortunately we do not have any determinations on the plasma of the platypus. Table 1 also shows that uric acid and allantoin excretion occurs in the monotremes, but in comparison with sheep, a known excretor of allantoin, and the chick, a known excretor of uric acid, the amounts in the specimens we collected are small.

To confirm these results, the presence of arginase was sought in the livers. A simple homogenate of fresh liver was prepared by dispersing 1 g of tissue in 25 ml of 0.1*M* Na_2HPO_4 with the aid of a Potter-Elvehjem homogenizer. The homogenate (1 ml) was mixed with a solution of arginine (40 m*M*) which had previously been adjusted to the same *p*H, and the mixture was incubated at 38°C. The disappearance of arginine and the appearance of ornithine were followed chromatographically by taking serial samples over a period of 1 hour. The solvent system was butanol, acetic acid, and water (80:10:10, by volume), and the chromatograms were run for 2 days. The chromatograms clearly indicated the presence of arginase in considerable amounts: the hydrolysis of 40 μmoles was complete in less than 40 minutes. Under the same conditions liver tissue from the rat completely hydrolyzed the arginine in 20 minutes, but liver tissue from the chick showed no trace of activity. Determination of stoichiometric amounts of urea formed confirmed that the enzymatic activity measured was that of arginase. We therefore conclude that both the echidna and the platypus use urea as the main pathway of nitrogen excretion.

Table 1. Urea, uric acid, and allantoin per 100 ml of blood plasma (P) and urine (U) collected from two echidna (*Tachyglossus aculeatus*), a platypus (*Ornithorhynchus*), a sheep, and a chicken.

Urea		Uric acid		Allantoin
U (g)	P (mg)	U (mg)	P (mg)	U (mg)
		Echidna		
	65	*	0.1	
3.43	49	24	0.9	13
		Platypus		
3.4		18		8
		Sheep		
		*	0.3	60
		Chicken		
	2		5.6	

*Positive.

Because of the difficulty of obtaining further animals we thought that these results were sufficiently conclusive at this stage to warrant publication (*6*).

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References and Notes

1. J. Needham, *Chemical Embryology* (Cambridge Univ. Press, 1931), vol. 2, pp. 1141, 1145.
2. H. W. Smith, *From Fish to Philosopher*, (Little, Brown, Boston, 1953), p. 245.
3. P. B. Hawk, B. L. Oser, W. H. Summerson, *Practical Physiological Chemistry* (Blakiston, New York, ed. 13, 1957).
4. E. G. Young and C. F. Conway, *J. Biol. Chem.* **142**, 835 (1942).
5. O. Folin, *ibid.* **101**, 111 (1933).
6. We thank the director of the Sir Colin MacKenzie Sanctuary, Healsville, for his assistance with the collection of specimens.

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