

# Excretory electrolytes and habituation in the turtle<sup>1</sup>

IRWIN M. SPIGEL AND KENNETH R. ELLIS

UNIVERSITY OF TORONTO

The relation of excretory  $\text{Na}^+$  and  $\text{K}^+$  to stress was examined during the course of habituation of fresh-water turtles to a new surround and/or a change in feeding schedule. Only when both conditions were imposed simultaneously were higher cation levels evident during the initial stages of the experimental treatments, and a gradual decline in the excreted electrolytes observed over a 20-day period.

Evidence of increased urinary sodium and potassium in humans under stress has been provided by Hoagland (1961), presumably reflecting hypersecretion of adrenal steroids. Although this urinary cation increment has, as yet, not been observed in stressed rats (Pare, 1964), Spigel and Ellis, in an unpublished series of experiments, have shown that both excretory  $\text{Na}^+$  and  $\text{K}^+$  are augmented in fresh-water turtles severely stressed by sustained electric shock administration. The latter study also demonstrated the feasibility of analyzing the water in which these reptiles are maintained for  $\text{Na}^+$  and  $\text{K}^+$  content, thus providing a continuing index of stress in otherwise undisturbed Ss.

The current investigation was designed to examine the sensitivity of excretory electrolyte analysis to a more subtle mode of stress, specifically, the course of habituation to a new surround and/or a change in feeding schedule. Not only is there intrinsic comparative interest in the physiological correlates of habituation, but a sensitive chemical index—in addition to the neural and behavioral measures now employed—would provide for a more complete profile of organismic adaptation to stress.

## Subjects

Twenty-five infant male *Chrysemys picta marginata* were employed. Ages at the start of the experiment ranged from 15 to 18 weeks, as estimated from information furnished by the supplier.

## Apparatus

For seven weeks prior to the start of the experiment, Ss were mass-housed in a glass tank, with a 1/2 in. water level. Flat, dry surfaces were available for basking. Ss were allowed ad lib feeding on frozen halibut for 1 h each day. For the experiment proper, plastic dishes, 2-1/2 in. by 3-1/2 in., housed Ss individually, and contained 6 ml of water. Dishes were tilted so that half the floor area of each remained dry. Illumination during the day averaged 10.5 ft-c at floor level, 3.5 ft-c between 7:45 PM and 9:00 PM, and remained below 1.0 ft-c throughout the night.

## Procedure

Groups of 8, 6, 6, and 5 Ss, respectively, were randomly constituted. Ss of the first group were introduced into the individual plastic dishes and allowed to feed for 1 h every third day. Ss of the second group were fed daily for 1 h. After each 23-h period, the residual water, which was replaced daily, was analyzed for  $\text{Na}^+$  and  $\text{K}^+$  content with a Coleman flame photometer. The remaining Ss were identically housed and fed daily for 21 days, after which they were returned to the original mass tank and continued on daily feeding. Ten days later they were replaced in the individual dishes where the third group was fed every third day and the fourth group fed daily— $\text{Na}^+$  and  $\text{K}^+$  analysis was carried out on the residual habitat water in the manner used with Ss of the first two groups. Ss of all groups remained in the experimental dishes for 20 days of analysis.

## Results

Since the response of the galvanometer used with the Coleman photometer and the sodium filter is nonlinear below a value of 20 mEq/l, direct scale readings were used, and are labeled gEq in Figs. 1 and 2.

Figure 1 contains the excretory electrolyte records for the nonhabituated Ss of Groups 1 and 2; i.e., those fed daily and every third day, respectively. Figure 2 shows these records for the Ss of Groups 3 and 4; i.e., those pre-exposed to the individual dishes for a 21-day period 10 days prior to the experimental period.

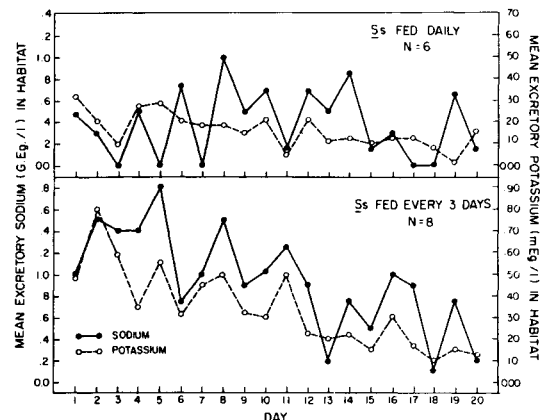


Fig. 1. Twenty-day record of excretory  $\text{Na}^+$  and  $\text{K}^+$  for Ss not previously exposed to the test dishes.

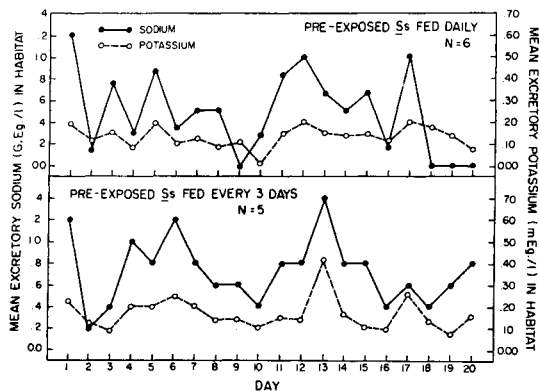


Fig. 2. Twenty-day record of excretory  $\text{Na}^+$  and  $\text{K}^+$  for Ss previously habituated to the test dishes.

Wilcoxon tests were employed for both  $\text{Na}^+$  and  $\text{K}^+$  levels between the first 10 and last 10 days of the experiment on Ss of all groups. Only in the case of nonhabituated Ss fed every third day were both sodium and potassium values significantly different ( $t=0$ ). The descent over the 20-day period is clearly evident in the lower panel of Fig. 1. The potassium levels alone were significantly different between Days 1-10 and Days 11-20 for the nonhabituated daily-fed Ss ( $t=0$ ). For Ss of all other groups, excretory electrolyte levels did not differ significantly between the first and last 10 days.

Mann-Whitney U tests failed to yield a significant difference in  $\text{Na}^+$  and  $\text{K}^+$  levels between habituated Ss which were fed daily and those fed every third day. However, both  $\text{Na}^+$  and  $\text{K}^+$  values differed significantly between nonhabituated Ss on the two feeding schedules ( $U=59$  and  $89$ , respectively). When all daily-fed Ss were compared with Ss fed every third day, U tests showed significant differences for  $\text{Na}^+$  and  $\text{K}^+$  for Days 1-10, and for  $\text{Na}^+$  alone for Days 11-20.

Although the day to day variations do not allow for any precise statement with respect to consistent rhythmicity, the previously habituated Ss appeared to produce peaking of  $\text{Na}^+$  levels less frequently than did nonhabituated Ss. A final observation was the highly consistent covariation, evident from the curves of both figures, of  $\text{Na}^+$  and  $\text{K}^+$  over the 20 days.

#### Discussion

It was clear from both the graphic record and the statistical analysis of excretory cation levels that only in the case of previously nonexposed Ss fed every

third day was there a general decline over time. In short, only in these Ss were initial  $\text{Na}^+$  and  $\text{K}^+$  values higher, and a gradual reduction observed during the 20-day test period in the individual dishes. It appeared that neither the new surround alone, nor the abrupt shift to a more restricted feeding schedule was sufficient to potentiate the excretory electrolyte content, but that the simultaneous imposition of both treatment alterations did indeed produce urinary cation increments which gradually subsided to a more stable level. It would appear that if excretory  $\text{Na}^+$  and  $\text{K}^+$  are indexing an adaptational response, the sensitivity is limited to some minimal level or severity of the stress employed. Such being the case, the cation records in the current experiment do seem to reflect habituation to the imposed conditions.

The covariation of excretory  $\text{Na}^+$  and  $\text{K}^+$  levels over days as seen in Figs. 1 and 2 may be attributable either to identity in the mechanisms underlying electrolyte metabolism or simply to a gross cyclic urinary pattern. The latter, however, is rendered less likely by the results of a recently completed study. It was shown that with shock-induced stress over a 6-day period, excretory  $\text{Na}^+$  in the habitat water remained higher than for nonstressed controls, while  $\text{K}^+$  levels differed only for the initial experimental days. These findings remain in accord with the current observations, however, in that when the residual contents of the bladders of Ss of the earlier experiments were analyzed, both  $\text{Na}^+$  and  $\text{K}^+$  levels were significantly higher in the stressed group.

Efforts to derive some systematic pattern or rhythm in the observed daily variation were unsuccessful. The cyclicity that did appear seemed neither synchronous with, nor dependent upon, feeding schedule, although superficially there appeared to be greater periodicity in nonhabituated Ss. More precise analysis of excretory electrolyte rhythms over time will have to await further research.

#### References

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#### Note

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