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RELATIONSHIP OF INTRACELLULAR POTASSIUM TO ASEXUAL REPRODUCTION IN *HYDRA*

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SUMMARY

Removal of potassium from *Hydra* culture medium produces a decrease in intracellular potassium and a parallel decrease in asexual growth rate. Rubidium and caesium are ineffective as substitutes for potassium in the maintenance of growth rate. Increases in intracellular potassium parallel increases in growth rate up to a level somewhat below the normal steady-state level of intracellular potassium. The full potassium requirement for maximum effect on budding can be acquired from food or external medium. High levels of external potassium suppress budding but do not alter intracellular potassium levels.

INTRODUCTION

Many researchers have investigated the influence of potassium on the rate of asexual reproduction in hydra (Lenhoff, 1966; Lenhoff & Bovaird, 1960; Loomis, 1954; Muscatine & Lenhoff, 1965; Schulz & Lesh, 1970) as well as in *Cordylophora* (Fulton, 1960, 1962) and on the induction of metamorphosis in *Hydractinia* planulae (Müller & Buchal, 1973). Also established is the direct effect of potassium on various developmental mechanisms such as regulation of gene expression (e.g. Barth & Barth, 1974; Kroeger, Troesch & Muller, 1973) and mitotic control (e.g. Cone, 1971; Pardee, 1971).

In a previous investigation (Koblick & Epp, 1975) we found that a decreased budding rate in *Hydra viridis* grown under conditions of reduced osmotic stress is accompanied by a decrease in intracellular potassium. A hypothesis which we suggest may be drawn from our previous finding is that intracellular levels of potassium may directly determine the budding rate of *Hydra*. If the hypothesis is correct, at least some treatments known to affect budding rate, such as availability of food or the presence or absence of specific ions in the external medium, should do so by increasing or decreasing the level of intracellular potassium. Also, any treatment affecting intracellular potassium levels should also affect budding rate. For example, ouabain, a compound that inhibits active sodium-potassium exchange, thereby decreasing intracellular potassium, would be expected to produce a decreased budding rate if added to the

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culture medium. The focus of this paper is to examine the effects of feeding, environmental potassium and ouabain on budding rate and intracellular potassium in the light of this hypothesis.

MATERIALS AND METHODS

Cultures of *Hydra viridis* obtained from Wards, Rochester, N.Y., were maintained in 'M' solution (Muscatine & Lenhoff, 1965) which contains 10^{-3} M-$CaCl_2$, 10^{-3} M-$NaHCO_3$, 10^{-4} M-$MgCl_2$, 10^{-4} M-KCl, and 10^{-3} M-Tris buffer, pH 7·8, in distilled water. Cultures were routinely fed daily with freshly hatched *Artemia salina* nauplii after the method described by Loomis & Lenhoff (1956). Temperature was 20–22 °C. Illumination was continuous. Reproduction was exclusively asexual under these conditions.

To measure the rate of asexual reproduction, replicate experimental cultures were initiated by placing 5 'uniform' hydra, defined by Lenhoff & Bovaird (1961) as having a single bud in early stages of development and having been starved one day, in plastic petri dishes filled to a depth of about 1 cm with 50 ml of culture medium, observing reproduction over the next 6 days by counting individuals and buds, and calculating first order exponential growth constant for each dish at the end of this time according to the equation $k = 2{\cdot}3/t \log N_t/N_0$, where t = time in days, N_t = number of hydra at time t, N_0 = number of hydra at time 0. Statistical comparisons were made using Fisher's t test (Weast, 1964).

'Uniform' hydra selected for the initial experiment demonstrating the potassium requirement for asexual reproduction came directly from stocks. In all other experiments involving measurements of growth rate, selection of 'uniform' hydra was made from subcultures of stocks conditioned for 6 previous days in the experimental media in which growth rate was to be determined. These subcultures were maintained in rectangular plastic dishes filled to a depth of 1·5 cm with 200 ml of experimental media and fed on alternate days. After establishing experimental cultures feeding intervals of experimental cultures and the subcultures from which they were derived varied together according to experimental design. Culture solutions were changed on non-feeding days. Other culture conditions for subcultures and experimental cultures were as described above for stocks.

Flame photometry was used for measurement of intracellular potassium content of hydra. The several hundred hydra necessary for this technique were obtained by pooling the experimental cultures with the subcultures from which they had been derived. These hydra were starved for 24 h, rinsed several times in a 50 mM sucrose/distilled water solution and placed on a pre-weighed Gelman Type GM-13 filter. Each of these filters was supported on silicate paper to absorb excess wash water. The loaded filters were then dried in a vacuum oven at 50 °C overnight, brought to room temperature, weighed, and sample weights calculated by difference. Each filter was then transferred to a polyethylene centrifuge tube and 0·5 ml of concentrated HNO_3 added. The tubes were then heated to 50 °C for 1 h. After cooling, 10 ml of distilled water was added to each tube. The addition of water produced a fine white precipitate both in tubes containing loaded and unloaded filters; digests were, therefore, centrifuged and the clean supernatants used for photometry. Supernatants from digests of unloaded filters did not contain measurable potassium.

Table 1. *Effects of variation in potassium concentration on asexual growth rate and the specificity of the potassium requirement*

(Cations were added to potassium-free culture medium (as chlorides) to the concentrations shown. Means of growth constants ± standard deviation are presented ($n = 3$). O_s = survival for 6 days without reproduction. O_d = death of all individuals within a 6 day period. All cultures fed twice during 6 days.)

Cation concentration (as chlorides)	Growth constants				
	0 M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
K^+	0·20 ± 0·02	0·24 ± 0·02	0·27 ± 0·01	0·27 ± 0·01	0·23 ± 0·02
Rb^+	—	0·17 ± 0·01	0·17 ± 0·02	O_s	O_s
Cs^+	—	0·13 ± 0·02	O_s	O_d	O_d

Table 2. *Time course of intracellular potassium changes after removal of potassium from culture medium*

Time (h)	Tissue potassium content (m-equiv./g dry wt)	
	K^+-free medium	Complete medium
0	0·22	0·21
24	0·24	0·25
48	0·18	0·23
96	0·16	0·21

RESULTS

Demonstration of the potassium requirement for asexual reproduction.

Hydra placed in potassium-free culture medium and fed twice during 6 days (days 2 and 5) show a growth constant of 0·20 (Table 1). This compares with a value of 0·27 in complete culture medium (10^{-4} M-K^+) under the same feeding conditions (Table 1). Reduction of the potassium concentration to 10^{-5} M has no effect but reduction to 10^{-6} M results in a growth rate intermediate between that in 10^{-4} M-K^+ and potassium-free medium. A tenfold increase in potassium concentration above that in culture medium produces a decrease in growth rate. Substitution of rubidium or caesium for potassium depresses growth at low concentrations and stops reproduction at higher concentrations. Caesium is toxic at 10^{-4} M.

Determination of tissue potassium level in potassium-free medium

To determine whether growth depression produced by removal of potassium from the culture medium is accompanied by a decrease in intracellular potassium two stock culture vessels were used, one containing regular culture medium and one with regular culture medium replaced by potassium-free medium. Both cultures were fed after 48, 72 and 96 h. Samples of several hundred hydra were taken at intervals over a period of 4 days for determination of intracellular potassium (Table 2). The 24 h values are slightly higher than the zero time levels. This is probably not physiologically significant as the increase occurs in the controls as well. After 2 days the level of potassium in the experimental culture is lower than that in the control and remains so at 96 h.

Table 3. *Effects of external K^+ and ouabain on asexual growth rate and intracellular potassium of* H. viridis

(Means ± standard deviations of growth constants calculated after 6 days ($n = 5$). ( ) = m-equiv. K^+/g dry wt obtained after pooling all hydra from a given culture medium.

No. days fed ...	2/6	3/6	6/6	2/6	3/6	6/6
K^+ ...	0	0	0	10^{-4} M	10^{-4} M	10^{-4} M
Ouabain						
0	0·22 ± 0·01 (0·15)	0·24 ± 0·01 (0·18)	0·28 ± 0·02 (0·19)	0·26 ± 0·01 (0·24)	0·32 ± 0·01 (0·23)	0·34 ± 0·02 (0·24)
2×10^{-5} M	0·20 ± 0·02 (0·17)	0·26 ± 0·01 (0·18)	0·27 ± 0·03 (0·18)	0·26 ± 0·01 (0·22)	0·31 ± 0·02 (0·20)	0·32 ± 0·03 (0·22)
10^{-4} M	0·22 ± 0·01 (0·15)	0·23 ± 0·02 (0·17)	0·28 ± 0·03 (0·19)	0·28 ± 0·01** (0·20)	0·33 ± 0·02 (0·21)	0·33 ± 0·01 (0·22)
5×10^{-4} M	0·22 ± 0·01 (0·16)	0·24 ± 0·03 (0·19)	0·30 ± 0·03 (0·20)	0·27 ± 0·01* (0·20)	0·32 ± 0·02 (0·20)	0·32 ± 0·03 (0·22)

* = $P < 0·05$. ** = $< 0·01$.

Effect of feeding, external potassium and ouabain on growth rate and intracellular potassium

In potassium-free medium and with minimal feeding of 2 out of 6 days, both growth rate (0·22) and intracellular potassium levels (0·15 m-equiv/g dry wt) are at an observed minimum (Table 3). Both values increase as feeding increases, to an observed maximum when animals are fed daily of 0·28 for growth rate and 0·19 m-equiv/g dry wt for intracellular potassium. Addition of ouabain in various concentrations to potassium-free medium does not produce significant change in any of these observed values.

With potassium available in the culture medium, higher growth rates are observed, again varying directly with feeding, from 0·26 with minimal feeding to 0·34 with daily feeding. However, although intracellular potassium level is also higher than observed in potassium-free medium, it does not change with changes in feeding, remaining at about 0·24 m-equiv/g dry wt at all feeding regimes. Under each feeding regime, the addition of ouabain at all concentrations tested to culture medium containing potassium consistently produces a small decrease (about 10%) in intracellular potassium level when compared to controls. However, the expected corresponding decrease in growth rate is not observed; growth rates are largely unaffected. Unexpectedly in two instances, at 10^{-4} M and 5×10^{-4} M ouabain and when fed on 2 out of 6 days, there were significant increases in growth rate when compared to the pooled mean of similarly fed hydra.

Since a decrease in growth rate was noted when potassium concentration was increased above that in normal culture medium (Table 1), we measured intracellular potassium and the effect of ouabain under these conditions. Table 4 shows that increasing potassium in the culture medium from 10^{-4} to 10^{-3} M reduces the growth rate of *Hydra* fed on alternative days from 0·33 to 0·31 ($P < 0·01$). Intracellular potassium levels were, however, essentially unchanged. Addition of ouabain to culture medium containing 10^{-3} M potassium had no clear effect on growth rate nor level of intracellular potassium.

Table 4. *Effect of high levels of external potassium and ouabain on growth rate and intracellular potassium of* H. viridis

(Means ± S.D. (n = 5). Hydra fed 3 of 6 days.)

K^+ conc. (ouabain conc.)	Growth rate (intracellular K^+)†
10^{-4} M-K^+ (0)	0·33 ± 0·01* (0·23)†
10^{-3} M-K^+ (0)	0·31 ± 0·01 (0·24)
10^{-3} M-K^+ (10^{-4} M Ou.)	0·31 ± 0·01 (0·23)
10^{-3} M-K^+ (5×10^{-4} M Ou.)	0·31 ± 0·02 (0·22)

* $P < 0{\cdot}01$.

† m-equiv K^+/g dry wt obtained after pooling all hydra from a given medium.

DISCUSSION

Earlier observations (Koblick & Epp, 1975) that a decrease in osmotic load, produced by addition of sucrose to the culture medium, resulted in a decrease in intracellular potassium and a parallel decrease in asexual growth rate of *Hydra viridis* suggested to us that budding in hydra is controlled by intracellular potassium.

Macklin (1967) observed that the inner surface of the ectodermal layer in hydra is the site of a sodium-potassium exchange pump. This pump extrudes sodium from the cells into intracellular spaces which communicate with the gastrovascular cavity and accumulates potassium in the opposite direction. The extrusion of sodium is accompanied by water, either because the processes are coupled or because accumulation of solute in the gastrovascular cavity draws water out of the cell osmotically at a rate equal to that at which it is taken in at the outward facing membrane under osmotic forces. Presumably this sodium–potassium exchange process is under some kind of control which maintains cell water content constant. When osmotic water uptake is reduced, as it would be in hydra grown in sucrose solutions, the pump slows down and the steady-state intracellular potassium concentration is thereby lowered.

The present results offer additional evidence supporting the hypothesis that intracellular potassium regulates asexual growth rate. Under similar feeding regimes, removal of potassium from the culture medium causes a marked decrease in asexual growth rate (Tables 1, 3). This effect is specific for potassium – neither rubidium nor caesium support asexual reproduction when substituted for potassium (Table 1). Removal of potassium also causes a corresponding decrease in intracellular potassium (Tables 2, 3). Intracellular potassium and growth rate both vary directly with feeding when potassium is not available in the external medium (Table 3).

With potassium available in the external medium, intracellular potassium is constant at all feeding regimes and at a higher level than observed in potassium-free medium (Table 3). This indicates that a normal steady-state level of potassium (about 0·24 m-equiv K^+/g dry wt) is acquired from the external medium. Continued increases in growth rate in proportion to feeding under this condition suggest a maximum level of intracellular potassium which can affect the metabolic process associated with budding. Beyond this level other controlling factors related to

nutrition, e.g. food reserves, or independent mechanisms such as temperature (Schulz & Lesh, 1970), become more important. This 'maximum effect' level must be below the normal steady-state level since daily-fed hydra grown in potassium-free medium have a lower intracellular potassium level (0·19 m-equiv/g dry wt, Table 3) than do minimally fed hydra grown in complete medium (0·24 m-equiv/g dry wt–steady state, Table 3), yet have similar growth rates (0·28 and 0·26 respectively, Table 3). Moreover, the fact that potassium obtained exclusively from food can provide the maximum amount of intracellular potassium utilized for budding clarifies reports by other investigators (e.g. Muscatine & Lenhoff, 1965; Lenhoff, 1966) that potassium in the medium is not an absolute requirement for normal growth of well-fed hydra.

The hypothesis that intracellular potassium levels control budding was also tested by growing hydra in media containing ouabain (Table 3), a compound known to inhibit active sodium–potassium exchange in many systems (Skou, 1965). Its application should produce a lowering of the steady-state intracellular potassium level and thereby a reduction of growth rate. Reduction in intracellular potassium and growth rate by ouabain was not seen in hydra grown in potassium-free medium. That potassium was being entirely acquired by the hydra from food rather than by transport from the external medium explains this result. In culture medium containing potassium, ouabain did produce a slight reduction in steady-state intracellular potassium levels. However, it did not produce a decrease in growth rate because intracellular potassium did not fall below the level at which maximum effect of potassium on budding still occurs (about 0·19 m-equiv K^+g dry wt). The two instances in which ouabain stimulated budding are unexplained.

High levels of external potassium inhibited budding (cf. Muscatine & Lenhoff, 1965; Lenhoff, 1966). Although this inhibition occurred without corresponding changes in intracellular potassium (Table 4), numerous investigators (Cone, 1971; Pardee, 1971; McDonald *et al.* 1972; Cone & Toniger, 1973) have shown that high levels of external potassium act as an effective mitotic inhibitor and repressor of nucleic acid synthesis through alteration of cell membrane potential. In this environment we found that ouabain had little effect on growth rate or intracellular potassium. Since ouabain lowered intracellular potassium levels only slightly in normal culture medium, any effect when external potassium was increased tenfold would have been concealed.

The present results also suggest interesting possibilities for further investigation. Control of the cell cycle by potassium through chromosomal alteration (Stambrook, Sachs & Ebert, 1975) or alteration of cell membrane potential (see above) may have bearing on the findings of Campbell & David (1974) that differences in cell cycle parameters are associated with differences in interstitial cell differentiation. In addition the work of Barth & Barth (1974) suggests that the classical developmental concepts of induction and gradients, studied extensively in *Hydra* (reviewed by Lesh-Laurie, 1973), may be explained in part by the ionic composition of the tissues.

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