Effect of D$_2$O on the Circadian Rhythm of Petal Movement of *Kalanchoe*
Albrecht Maurer and Wolfgang Engelmann
Institut für Biologie Tübingen

(Z. Naturforsch. 29 c, 36–38 [1974]; received October 23, 1973)

Heavy Water, Circadian Rhythm, Petal Movement, *Kalanchoe*

D$_2$O lengthens the free running period of the circadian petal movement of isolated *Kalanchoe* flowers by 1.6% per 10% heavy water. This corresponds to findings reported in the literature for other organisms. 100% D$_2$O administered in the form of 4 hour pulses at various phases of the circadian cycle lead to phase responses zero to maximally 1.5 hour delays. No advancing phase shifts occur. Possible ways in which lengthening of the period occurs are discussed.

The slowing down of circadian rhythms by heavy water has been demonstrated in unicellulars, plants and animals and seems to follow the same quantitative relationships (about 2% lengthening of the period per 10% D$_2$O)\(^1\) (but see also\(^2\)). This feature has been claimed to suggest fundamental similarities of the underlying rhythmic mechanisms. In connection with findings of slowing *rhythms of higher frequencies* with D$_2$O it has been hypothesized that ionic balances across cellular membranes are influenced and are thus parts of the clock mechanism\(^1\). Recent findings of ions being involved in circadian movements of plants\(^3\)\(^,\)\(^4\) support this view.

We have studied the influence of D$_2$O on the *Kalanchoe* petal movement rhythm offered continuously as well as for shorter periods of time. We will discuss different general modes of action of D$_2$O on circadian rhythms and present an alternative interpretation to that of a direct change of the oscillating system by D$_2$O leading to a lengthening of the period.

**Material and Methods**

Isolated flowers of *Kalanchoe blossfeldiana* exhibit a circadian rhythm of opening and closing the petals under conditions of constant temperature and in physiological darkness of weak green light for about one week if mounted in a 0.2 M solution of sucrose. Methods of rearing the plants\(^6\) and details of recording the rhythm have been described elsewhere\(^7\)\(^,\)\(^8\).

Desired concentrations of D$_2$O\(^9\) were prepared and sucrose added to give the standard 0.2 M solution. For experiments in which D$_2$O was offered in the form of 4 hour pulses the flowers were transferred with the acrylic glass plates in which they were mounted from the recording cuvettes to dishes containing the heavy water/sucrose solution. The times of maximum flower opening were determined from the plotted curves and the distances between the maxima used for the calculations of period lengths. Phase shifts due to D$_2$O pulses were determined in respect to undisturbed controls. The temperature was maintained at 22.5 ± 0.5 °C.

**Results and Discussion**

1. Dependence of the period length on the concentration of D$_2$O offered continuously

The period length progressively lengthens in a linear manner with increasing concentrations of D$_2$O (Fig. 1). The regression line has the form

\[
y = 22.97 + 0.038 \times
\]

Fig. 1. Dependence of the period length (ordinate) of the *Kalanchoe* petal rhythm on the concentration of D$_2$O (abscissa). Vertical bars: standard errors.

Requests for reprints should be sent to Dr. W. Engelmann, Institut für Biologie, Universität Tübingen, D-7400 Tübingen, Auf der Morgenstelle 1, Germany.
with a low amplitude was observable. At this concentration the flowers showed severe signs of wilting.

II. Effect of 4 hour pulses of D$_2$O

4 hour pulses of 100% D$_2$O do not damage the flowers. They lead to phase shifts of the petal rhythm the amount of which depends on the affected phase of the circadian cycle. Fig. 2 shows in the upper part the petal movement of a control flower after release into darkness at time 0. From the lower part of Fig. 2 it can be seen, that D$_2$O pulses delay the rhythm maximally (about 1.5 hours) at times when the petals are closed. No phase shift takes place if D$_2$O pulses are administered during the time of fastest opening of the flowers (15 and 38 hours after onset of darkness). No advancing phase shifts are observed. The form of the phase response curve is repeated in later cycles.

For discussions concerning the mechanism of the D$_2$O action on circadian rhythms see$^1$$^2$. We would like to discuss the action of D$_2$O on circadian rhythms in a more formal way to avoid speculations on the mechanism by which D$_2$O acts. Some of the possible ways of D$_2$O in retarding circadian rhythms are schematically shown in Fig. 3 (other alternatives, e.g. simultaneous actions at several of the discussed points, are of course conceivable).

1. D$_2$O could act on a driven oscillation, but the driving oscillation is unaffected by it.

2. D$_2$O could act on the coupling between the driving and the driven oscillator as has been discussed and compared to a slippery belt action$^{10}$.

3. D$_2$O acts only as a perturbation, and a continuous application of D$_2$O acts like a continuous repetition of perturbations. The overall effect is thus the sum of all the effects of single perturbations. The amount of phase shift per cycle can be deduced from the response curve towards 4 hour D$_2$O pulses if the phase shifting action of foregoing D$_2$O pulses is taken into account. Since 4 hour pulses bring forward delays only, the overall effect would be a general delay which shows up as a lengthening of the period.

4. D$_2$O acts on an essential part of the oscillator, thus changing the frequency of the oscillation.

Under the assumption that the driving oscillator (© in Fig. 3) is the light sensitive oscillator, the modes of action of 1. and 2. can be dismissed: We have found that under D$_2$O the phase response curves towards light pulses are changed in such a way as one would expect if the light sensitive oscillator has been slowed by the action of heavy water$^{11}$. Proposition 3 (D$_2$O acts as a perturbation) could be tested in the following way: A 4 hours D$_2$O pulse is given during the phase of maximum responsiveness of the cycle (see Fig. 2) and repeated in several cycles (the phase shifts induced by the foregoing pulses being taken into account). This should add up to a considerable phase shift after several cycles, whereas the same treatment with daily recurring cycles at minima of responsiveness towards D$_2$O pulses should hardly shift the rhythm at all.

This work has been supported by Deutsche Forschungsgemeinschaft under the “Schwerpunktprogramm Biologie der Zeitmessung”. Thanks are due to Dr. E. Bünning, Dr. A. Johnsson and Dr. M. K. Chandrashekaran for critically reading the manuscript.
1 J. T. Enright, Z. vergl. Physiol. 72, 1–16 [1971].
3 R. L. Satter and A. W. Galston, Bioscience, in press.
5 W. Engelmann and V. Vielhaben, Z. Pflanzenphysiol. 55, 54–58 [1965].
9 Roth, Karlsruhe, 99.7 atom%.