IONTOPHORESIS OF CYCLIC AMP

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ABSTRACT The design, calibration, and operation of a source of controlled amounts of cyclic AMP (c-AMP) are described. Typically, 1.5 s pulses containing 10^{10}–10^{12} molecules of c-AMP can be delivered to a region about 10 μm in diameter on an agar plate. The resulting concentration profiles are given as functions of distance and time. The diffusion coefficient of c-AMP in agar was measured to be 0.97×10^{-5} cm$^2 \cdot$ s^{-1} at 21°C.

INTRODUCTION

When the food supply of *Dictyostelium discoideum* amoebae is exhausted, the amoebae enter a period of differentiation called interphase (Bonner, 1963) which lasts for approximately 8 h, and then aggregate (Raper, 1940; Bonner, 1967). This process has been described by many authors (Shaffer, 1962; Gerisch, 1968; Robertson and Cohen, 1972); many of its details are now well understood. It has been shown that cyclic adenosine monophosphate (c-AMP) is most probably used for intercellular communication by the amoebae during aggregation (Konijn et al., 1967, 1968). It has been suggested that individual amoebae release pulses of c-AMP and that their neighbors respond to these pulses by moving towards the signal source and by relaying an identical pulse (Shaffer, 1962; Cohen and Robertson, 1971 *a, b;* Robertson et al., 1972). In order to do this the amoebae must acquire several competences during interphase (Cohen and Robertson, 1972). These include the ability to sense distinct threshold concentrations of c-AMP for chemotaxis and for relaying and for some cells, the ability to produce pulses of c-AMP autonomously.

It was therefore necessary to develop an artificial source of controlled c-AMP signals for two main reasons. Firstly, it was clearly essential to test the hypothesis that a source of pulses of c-AMP could control *D. discoideum* aggregation; secondly, with such a source it should be possible to measure parameters of the system such as thresholds and duration of the natural signal, and to determine the time of emergence for the competences required for aggregation. In this paper we therefore describe the setting up and calibration of a microelectrode system for the delivery of controlled amounts of c-AMP by iontophoresis. The first results obtained with this system have been described earlier (Robertson et al., 1972); more details are given in the accompanying paper (Robertson and Drage, 1975).

MICROELECTRODE SOURCE

A glass micropipet drawn from Corning no. 12-141 melting-point tubing, 2 mm external diameter (Corning Glass Works, Corning, N.Y.), was used as a point source of c-AMP. The tip internal diameter was between 2 and 5 μm, giving impedances between 1 and 5 MΩ. The pipet was filled with phosphate buffer, pH 6.5, and various concentrations of c-AMP. c-AMP dissociates to produce a negative ion at this pH, the dissociation constant K_a being 3.8. The pipet electrolyte was therefore biased positive, via a chlorided silver wire electrode, to retain negative ions. The 10 V positive bias was supplied via a 10 MΩ resistor and was reversed, whenever c-AMP ions were to be expelled from the microelectrode, by a negative pulse to the pipet from a Grass stimulator (model S88AB; Grass Instrument Co., Quincy, Mass.) and isolation unit (model S105A) via a 1 MΩ resistor. An equivalent circuit for the apparatus is shown in Fig. 1. In our calibration runs the pulse current was 10 μA and its duration 1.5 s. These values were obtained by adjusting the output of the Grass stimulator. The pulse interval and bias were obtained from a specially made multivibrator monostable timing circuit. In all experiments to which these calibration runs are relevant current was kept constant at 10 μA, and the number of c-AMP molecules ejected was changed by changing c-AMP concentration. This tends to avoid any nonlinear electrode effects and to ensure that only the amount of c-AMP ejected is changed. 10 μA was chosen because it is in a range in which current is accurately controllable and harmless to cells, and yields enough c-AMP to be effective at convenient ranges of c-AMP concentration in the electrodes.

In some experiments, fluorescein was also included in the pipet electrolyte. It forms a negative ion of mobility similar to that of c-AMP. In UV illumination its fluores-

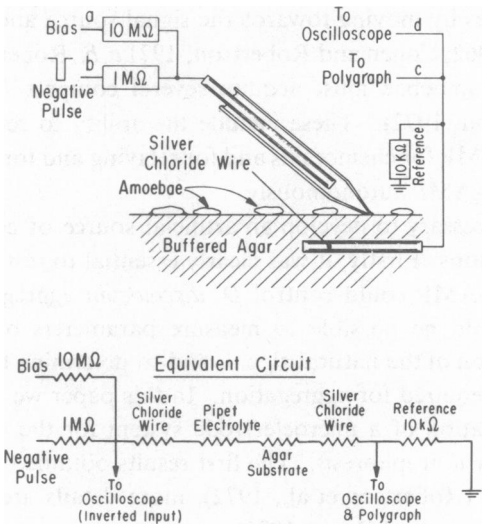

FIGURE 1 *Top:* Pipet detail. *Bottom:* Equivalent circuit showing impedances.

cence therefore roughly indicates the c-AMP spatial distribution. Magnitudes of positive biases which prevent both leakage out of the pipet and drift of negatively charged ions up the tube can then be selected.

The pulse duration, interval and current were continually monitored, with a polygraph, from the ground electrode across a 10 kΩ reference resistor. The system was calibrated using a differential input oscilloscope, Tektronix 565 (Tektronix, Inc., Beaverton, Ore.), with plug-in amplifier no. 3A3. With the negative input switched off the voltage across the reference resistor could be measured, while with it switched on the voltage across the pipet-agar combination could be measured (see Figs. 1 and 2).

The only component of the system with variable impedance is the pipet electrolyte (see Fig. 1 *b*); this varies with the size of the pipet opening and the cross section of the tapered portion of the tip; the agar is given a nominal impedance of 20 kΩ.

EXPERIMENTAL ARRANGEMENT

In experiments involving amoebae the system was set up as shown in Fig. 2 for video tape recording; it was used also with conventional time-lapse cinemicrography. 2% agar (Difco Bacto-agar; Difco Laboratories, Detroit, Mich.) was made up in phosphate buffer, as used in the micropipet. The agar was poured into a small transparent plastic box ($1\frac{1}{4} \times \frac{7}{8} \times \frac{3}{4}$ in) with hinged lid and allowed to cool, forming a layer about $\frac{1}{4}$-in thick in the bottom half of the box. On this substrate cells were spread out at the required density. The lid of the box had a 5 mm diam hole drilled through it to allow introduction of the pipet, which was held in place by modeling clay. The inside surface of the lid was coated with detergent (Triton X) to stop fogging by condensation of water vapor. Before the pipet tip was positioned on the agar surface, similar cholorided silver wire electrodes were inserted both into it and into the agar substrate

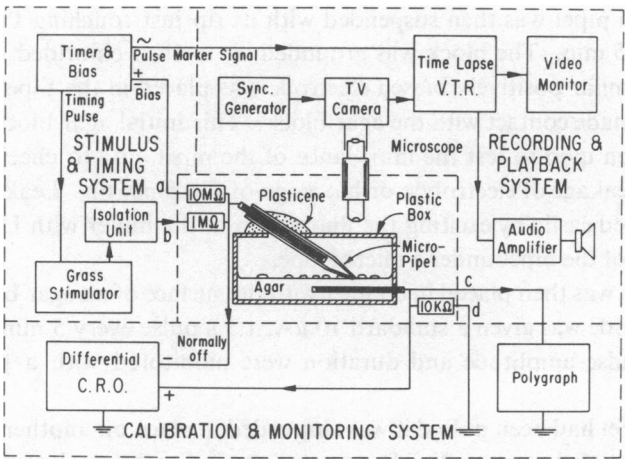

FIGURE 2 Logic diagram of complete pulsing system.

(through a small hole in the side of the box). The latter grounded the agar via the reference resistance.

During video tape experiments one monostable in the timer sent a 15 s duration 60 Hz signal to the audio channel of a video tape recorder to mark the occurrence of pulses, giving a 4.8 KHz pip on playback. During film experiments light flashes were superimposed on a corner of the appropriate frame.

CALIBRATION OF ELECTRODES

The calibration of microelectrodes used for iontophoresis is normally carried out by making a rough estimate of the partial conductance of the ionic species of interest (Bures et al., 1967). Knowledge of the total electrolyte conductance and the duration and current of the applied pulse then leads directly to an estimate of the number of ions of interest expressed per pulse. However, as microelectrode operation is not thoroughly understood and involves complicated nonlinearities and as such partial conductances can only be estimated very roughly, this procedure may not be adequate for our purposes. It is essential to measure the amount of c-AMP released from an electrode in our experimental conditions and to compare it with an estimate based on partial conductivities.

We therefore used tritiated c-AMP (purchased from Schwartz/Mann, Orangeburg, N.Y.; 12.8 Ci/mol in a 10^{-4} M aqueous solution) diluted as appropriate with "cold" c-AMP and made up in solution with the usual phosphate buffer and 10^{-3} M fluorescein. Thus in each determination, at total c-AMP concentration of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} M the respective proportions of tritiated c-AMP were 1%, 10%, 100%, 100%. We started with five similar pipets with impedances between 2 and 5 MΩ. Because of their fragility, one or two of the pipets normally broke during the numerous transfers required. The procedure was as follows. Pipets were loaded with test solution and carefully washed with buffer to remove traces of c-AMP on their external surfaces. Each pipet was then suspended with its tip just touching the surface of an agar block for 5 min. The block was grounded via a silver chlorided wire as in Fig. 1 (top), and a similar positively biased electrode was placed in the pipet electrolyte before the pipet made contact with the agar block. This initial agar block was discarded after it had been used to test the impedance of the pipet, and to check whether there was excessive leakage of electrolyte or blockage of the pipet tip. Leakage or blockage could be checked easily by exciting the fluorescein in the buffer with UV light and observing the tip of the pipet under a microscope.

The pipet tip was then placed in contact with the surface of an agar block weighed to 1 g. The electrode was given a standard 10 μA, 1.5 s pulse every 5 min for a period of 1–3 h. The pulse amplitude and duration were monitored with a polygraph chart recorder.

After the pipet had been pulsed it was suspended in place on another 1 g agar block. The bias current alone was applied for the same period as the total duration of pulsing. During the leak period a small amount of c-AMP escaped from the pipet. This indi-

cated the amount of leakage occurring between pulses in normal use and was subtracted from the total amount of c-AMP released during the pulsing period. Two or three of the remaining pipets were allowed to leak first and were pulsed second in order to assess the range of the background leakage, and to determine whether the pipets were being exhausted. No systematic differences were observed. In the worst case in the calibration runs (10^{-6} M) 0.3% of the total c-AMP in the pipet would have been used, using our smallest measured electrolyte volume of 30 μl. For 10^{-3}, 10^{-4}, and 10^{-5} M, 0.14, 0.10 and 0.07%, respectively, of the c-AMP was used. Thus, even in a real experiment involving a 10-fold increase in time only a few percent at most of the c-AMP would be used, so that there would be no significant change in electrolyte concentration, or the amount of signal released in a pulse.

The agar blocks from these trials were then placed in test tubes and melted, using a double-boiler method. 0.5 ml of the liquid agar was drawn off, deposited directly into a polypropylene centrifuge tube containing 5 ml of Triton X + Permafluor scintillation fluid and mixed vigorously. The mixture was centrifuged at 15,000 *g* for 1 min. The supernate was placed into a scintillation vial and the radioactivity recorded. The residual agar was found to contain only 5% of the initial radioactivity which was ignored as it was well within the total experimental error. Disintegrations from dilutions of the original tritiated c-AMP were also counted in the same fashion to give overall counting efficiency.

The results for all electrodes are shown in Table I: number of molecules of c-AMP per pulse expressed by 10 μA for 1.5 s together with its mean and variance for each concentration, and the number of molecules leaked per second between pulses. There was no significant correlation observed between the number of molecules per pulse or the number leaked and the electrode impedance.

TABLE I

MEAN PULSE SIZES (η) FOR c-AMP CONCENTRATIONS OF 10^{-3}, 10^{-4}, 10^{-5}, AND 10^{-6} M IN THE MICROELECTRODE

c-AMP concentration	Pulse size η	Leak between pulses	$\bar{\eta}$	$\Delta\eta$
M	*molecules*	*molecules/s*		
10^{-3}	1.18×10^{12}	6.3×10^{9}	1.14×10^{12}	3.16×10^{11}
	1.5×10^{12}	2.5×10^{6}		
	7.3×10^{11}	3.0×10^{9}		
10^{-4}	2.6×10^{10}	5.9×10^{7}	6.08×10^{10}	2.33×10^{10}
	7.6×10^{10}	3.0×10^{8}		
	5.4×10^{10}	2.9×10^{7}		
	8.7×10^{10}	1.2×10^{8}		
10^{-5}	4.4×10^{9}	5.4×10^{6}	7.93×10^{9}	4.25×10^{9}
	1.38×10^{10}	5.7×10^{6}		
	3.4×10^{9}	6.1×10^{6}		
	1.01×10^{10}	9.6×10^{5}		
10^{-6}	2.04×10^{9}	1.8×10^{6}	2.04×10^{9}	

The pulse size η appears to be approximately linear in the c-AMP concentration C within the variances $\Delta\eta$. Accordingly we formed the ratio $y = \eta/C$. The variance Δy is $\Delta\eta/C$ because the error in C is small. A least squares fit to the values of y for 10^{-3}, 10^{-4}, and 10^{-5} M c-AMP solutions, weighted by $(\Delta y)^{-2}$, gave

$$\eta = (7.9 \pm 2.3) \times 10^{14} C$$

with C in moles per liter (molar). The value of χ^2 was 1.8 which, for 2 degrees of freedom, gives $Q = 0.4$ for a just acceptable confidence level.

A linear dependence of η on C is reasonable for the small values of C used here and is consistent with the observed independence of η on impedance. To check whether a value of 8×10^{14} for η/C is reasonable, we estimated it using partial conductances. We assumed a linear superposition of partial conductivities σ_j to give the total electrolyte conductivity, ignoring any nonlinear electrode effects or strong electrolyte effects, the latter being at most 20–30% in our concentration range. The calculation is straightforward; the concentrations C; equivalent conductances, Λ_j, and partial conductivities of the ionic species involved are shown in Table II. Using

$$\eta = \sigma_o \Big/ \sum_j \sigma_j \; (it/1.6 \times 10^{-19}),$$

where i is in amperes and t in seconds and σ_o ($j = o$) is the partial conductivity of the c-AMP ions, which we neglect in the denominator we found $\eta = 10.2 \times 10^{14} C$ with C in moles · liter^{-1}. The partial conductance estimate and the direct calibration agree to 28%, which is well within their combined errors. The agreement of the two estimates lends substantial mutual support.

TABLE II

PARTIAL CONDUCTIVITIES FOR c-AMP, FLUORESCEIN, AND IONIC SPECIES USED IN THE BUFFER

j	Species	C_j	Λ_j	$\sigma_j = 10^{-3} C_j \Lambda_j$
		M	$\Omega^{-1} \cdot cm^{-1}$	$10^{-3}\,\Omega^{-1} \cdot cm^{-1}$
0	c-AMP$^-$	10^{-3}	36.4	0.0364
1	Fluorescein$^-$	10^{-3}	37.5	0.0375
2	K^+	2.42×10^{-2}	71.7	1.74
3	Mg^{++}	2.03×10^{-3}	106	0.215
4	$HPO_4^=$	3.48×10^{-3}	99.6	0.347
5	$H_2PO_4^-$	1.69×10^{-2}	35.2	0.595
6	$SO_4^=$	2.03×10^{-3}	152	0.309

$\sum_{j=1}^{6} C_j \Lambda_j = 3.24\,\Omega^{-1} \cdot cm^{-1}$.

MEASUREMENT OF c-AMP DIFFUSION CONSTANT

Given a calibrated source of c-AMP it is necessary, in order to calculate c-AMP concentration profiles as a function of distance from the source and time after release of a pulse, to know the diffusion constant (D) of c-AMP in 2% agar. To measure D it is also necessary to show that diffusion is isotropic, that is that there are no preferred directions for diffusion. Originally we had assumed that there was a thin aqueous film on the agar surface and that D in this film was approximately 4×10^{-6} cm^2/s and negligible in the agar (Cohen and Robertson, 1971*a*). We therefore performed the following experiments to test these assumptions.

(*a*) A small drop of buffer containing washed carbon particles from Indian ink was deposited on a plain buffered agar surface. The drop was absorbed with very little spreading of the carbon particles. There is thus no evidence for a permanent aqueous film on the agar surface. The absorption of water in the drop was accompanied by local swelling of the agar.

(*b*) A micropipet with 20 μm tip opening and containing 10^{-3} M fluorescein solution in buffer was butted against a plane agar block. The block was illuminated with UV light from a mercury lamp and the apparatus photographed every 10 s. Contours of constant brightness, and therefore constant fluorescein concentration, were semicircular, centered on the microelectrode tip. Diffusion of fluorescein, which is ionized in solution with the same charge as c-AMP and a similar ionic weight, is therefore isotropic. A micropipet, as used in our experiments, may therefore be considered to be a point source from which c-AMP diffuses into a semi-infinite medium, i.e. with a hemispherical concentration front.

(*c*) We measured D at 21°C by butting together two plugs of 2% agar, one doped with 5% tritiated c-AMP. The plugs were formed in the cylindrical portions of 1 cm^3 tuberculin plastic syringes with their tapered ends cut off. A plane normal surface on the plug was produced by positioning the open end of the syringe against a cover slip. The syringe was held in a Plexiglas vee-block. When the agar had set, two syringes, one doped and one containing only buffered agar, were butted together and held in position in another Plexiglas vee-block for fixed times.

When the syringes were separated, the plugs, which had volumes of 0.2 ml, were dissolved in 5 ml of distilled water. The resulting solutions were too dilute to gel. 0.5 ml of each sample was mixed with scintillation fluid and its radioactivity counted, to obtain the relative amounts of tritiated c-AMP in each plug.

The solution of the diffusion equation in the geometry of the experiment is straightforward and results in

$$\frac{1}{2}\frac{N_1 - N_2}{N_1 + N_2} = \sum_{n=0}^{\infty} \frac{\exp\left[-\left(n + \frac{1}{2}\right)^2 \pi^2 Dt/L^2\right]}{\left[\left(n + \frac{1}{2}\right)\pi\right]^2}, \tag{1}$$

where N_1 is the number of counts from the initially doped plug and N_2 that from the other, L is the length of the plug (1.10 cm), and t is the time during which diffusion was allowed to occur. Provided t is 5 h or longer, the contributions of all terms with $n > o$ to the rhs of Eq. 1 will be less than 1% of the $n = o$ term. Consequently Eq. 1 can be rewritten

$$q \equiv -\log \tfrac{1}{2}[(N_1 - N_2)/(N_1 + N_2)] = 0.9032 + 7341\, Dt, \tag{2}$$

with t in hours and D in square centimeters · second^{-1}.

Diffusion was allowed for 5, 10, 12.5, 15, 20, 20.25, 21, 25, 30, 41, and 42.5 h. For those values of t where several determinations of q were made, 4 at 5 h, 5 at 15 h, 4 in the range 20–21 h, and 2 at 25 h, the scatter in the values of q was too large to be explained on the basis of counting error or errors in the length L and time t determinations. The principal source of error was probably nondiffusive mixing that occurred during the initial butting of the agar plugs. Thus the initial concentration in the diffusion experiment departs from the step function assumed in obtaining Eq. 1. Consequently each term in Eq. 1 is multiplied by a factor $(1 + b_n)$, where b_n gives the correction arising from the unknown and presumably variable initial mixing. After 5 h, the modified form of Eq. 1 reduces to a modified expression for q:

$$q = a + 7341\, Dt, \tag{3}$$

with $a = 0.9032\ (1 + b_o)$, and with best values of b_o and D to be determined from the data.

We performed a least squares analysis grouping data taken at the same, or similar times. The results are given in Table III. The deviation of a from 0.9032 indicates a value of b_o of 0.185, which is nine times the relative probable error of a, $\Delta a/a$, and therefore significant. Random errors such as counting errors and errors in L and t are already included in the analysis. Systematic errors of course are not. It is extremely

TABLE III

DIFFUSION CONSTANT (D) FOR c-AMP IN AGAR

$a = 1.070$, $\bar{D} = 9.71 \times 10^{-6}$ cm^2·s^{-1}, $\Delta D = 0.35 \times 10^{-6}$ cm^2·s^{-1}, $\Delta D/D = 3.6\%$, $\chi^2 = 0.457$, for a confidence level of 98% with 4 degrees of freedom.

i	D_i	ΔD_i	t
	$10^{-6}\ cm^2 \cdot s^{-1}$	$10^{-6}\ cm^2 \cdot s^{-1}$	h
1	9.47	1.49	5(4)*
2	10.08	1.74	10, 12.5
3	10.98	3.63	15(5)
4	10.51	1.77	20, 20.25(2), 21
5	9.67	1.85	25(2), 30
6	9.55	0.666	41, 42.5

*Numbers in parentheses indicate the number of runs of that duration if more than one.

unlikely, however, that these are as large as the value of b_o. Accordingly, we estimate that total random plus systematic error in D to be no more than 10%.

The final value of D, 9.7 $\times$ 10^{-6} $cm^2 \cdot s^{-1}$ at 21°C. is 2.4 times larger than the value 4 $\times$ 10^{-6} $cm^2 \cdot s^{-1}$ estimated via the Stokes-Einstein equation and a rough radius of 5 A° for c-AMP (Cohen and Robertson, 1971 *a*). For molecules in water with diffusion coefficients in the range 5.7 to 35.9 $\times$ 10^{-6} $cm^2 \cdot s^{-1}$, the ratio of experimental to calculated diffusion constant ranges from 1.7 to 4.5 and averages 2.7 for those molecules with values of D within 20% of that of c-AMP (Jost, 1952). The value of D measured by us for c-AMP in agar can therefore be regarded as consistent with present understanding of molecular diffusion in H_2O. There should be little difference between the diffusion coefficient of c-AMP in H_2O and in 2% agar. The effective medium theory (Cohen and Jortner, 1973) of diffusion in an inhomogeneous medium would give a 3% reduction in the agar.

CONCENTRATION PROFILES, APPLICATIONS

In placing the electrode in contact with the agar, care is taken not to pierce the surface of the agar. Upon releasing the electrode, the surface tension of the agar presses the electrode upwards. The plasticene yields and permits a reduction of the surface depression. We estimate the maximal surface depression to be less than about 5 μm. There is, in addition, a meniscus of water pulled out of the agar which has appreciable thickness out to about 10 μm.

It is very difficult to quantify the effects of the meniscus and the surface depression upon the concentration profiles. The simplest approximation, and one which becomes exact at distances large compared to the displacement, h, of the tip center below the unperturbed surface and to the size of the meniscus, is to replace the microelectrode by a point source at the tip center. Of course, the microelectrode is not a point source, and the upper limit to the c-AMP concentration in the immediate vicinity of the tip is the concentration of c-AMP in the electrolyte inside the micropipet, C_e. Under the conditions of operation of the microelectrode, the distance over which ions are transported from within the pipet is much larger than the distance within the agar over which the c-AMP ions can diffuse during a pulse. Thus the concentration just outside the pipet closely approaches C_e. This "point source" releases a total of η_p c-AMP molecules into the agar at a constant rate η_p/t_p over a time t_p during each pulse. The background leakage between pulses is neglected.

After a pulse of η_p molecules, we obtain for the c-AMP concentration at the surface of the agar at time t

$$C(r,t) = \frac{\eta_p}{t_p} \int_0^{\inf(t,t_p)} dt' \frac{2e^{-[(r^2 + h^2)/4D(t - t') + (t - t')/\tau_F]}}{[4\pi D(t - t')]^{3/2}}. \quad (4)$$

In Eq. 4, r is the distance of the point on the surface from the projection of the pulser tip onto the surface and must be large enough for the point source approximation to

work. τ_F is the relaxation time for c-AMP associated with conversion into 5'-AMP by extracellular phosphodiesterase. When PDE activity can be neglected, the formula to use for the concentration is just

$$C(r,t) = (2\eta_p/\pi 4Dt_p\rho)(\mathrm{erf}[t_d/(t - t_p)]^{1/2} - \mathrm{erf}[t_d/t]^{1/2}), \quad (5)$$

where $\rho = (r^2 + h^2)^{1/2}$ and $t_d = \rho^2/4D$.

The microelectrode can also be used as a steady leak instead of in the pulsing mode. Currents of about 1 μA are then convenient. The rate of leakage of c-AMP molecules is $\dot{\eta}_p$ which, if c-AMP flux were linear in the current, would be

$$\dot{\eta}_p = 5.3 \times 10^{13} C_e \text{molec. c-AMP} \cdot \text{s}^{-1}$$

for a 1 μA current. The resulting concentration profiles are

$$C(r,t) = (2\dot{\eta}_p/4\pi D\rho)\,\mathrm{erfc}(t_d/t)^{1/2} \quad (6)$$

in the absence of phosphodiesterase (PDE). If the source has been turned on for much longer than t_d, but not long enough for c-AMP to have reached the bottom surface of the agar, Eq. 6 simplifies to

$$C(r,\infty) = 2\dot{\eta}_p/4\pi D\rho, \quad (7)$$

a time-independent concentration. Such a concentration profile is very convenient to work with. However, the profiles are scarcely more complex in the presence of PDE. Eq. 4 holds for $C(r,t)$, with t as the upper limit. For $t \gg (t_d\tau_F)^{1/2}$, $C(r,t)$ becomes time independent and takes the form

$$C(r,\infty) = (2\dot{\eta}_p/4\pi D\rho)\exp(-\rho/\sqrt{D\tau_F}). \quad (8)$$

DISCUSSION

We have described the design, calibration, and operation of a source of controlled amounts of cyclic AMP. The source consists of a micropipet containing an electrolyte solution comprised of buffer, c-AMP, and sometimes fluorescein. Electrodes in the micropipet and in the agar permit transfer of c-AMP to the agar by pulsatile or continuous iontophoresis. With proper choice of electrode and pulse parameters, quantities of c-AMP comparable to those released naturally by amoebae of *D. discoideum* can be released on the natural time and distance scale. Such an artificial source can be used to mimic natural c-AMP signals, to expose cells to c-AMP signals under controlled circumstances, and to elicit quantitatively the parameters of a natural c-AMP signaling system. It can of course be used for molecules other than c-AMP.

We established that c-AMP ejected from the electrode tip diffuses three dimensionally into agar and that the diffusion coefficient is 0.97×10^{-5} cm$^2 \cdot$ s^{-1} at 21°C. We also calibrated the electrodes and showed that the number of molecules released in

a 1.5 s, 10 μA pulse was $(7.9 \pm 2.3) \times 10^{14}$ C_e for the buffer and electrodes used. With this information available, concentration profiles can be calculated from Eq. 4.

The accompanying paper (Robertson and Drage, 1975) describes specific quantitative and qualitative applications to the study of differentiation for aggregation and of aggregation in *D. discoideum*. The method, however, should be useful in other preparations.

A. Robertson is an Alfred P. Sloan Foundation Fellow, 1973–75.
This work was supported by National Science Foundation grant GB-30784 to the University of Chicago.

Received for publication 6 August 1974 and in revised form 21 April 1975.

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