Dialysis Studies of the K^+ Binding Capacity of *Physarum polycephalum*

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INTRODUCTION

Anderson (1964) has observed that migrating plasmodia of the slime mold, *Physarum polycephalum*, maintain a higher concentration of K^+ in the region of the advancing front than in the posterior trailing region. He has also found that most of the Na^+ but little of the K^+ in the posterior region can be removed by flushing water over the organism. Previous work by Anderson (1962) had revealed that alcohol precipitates prepared from plasmodial homogenates contain considerable quantities of K^+ which cannot be removed by washing. Based on these discoveries he has suggested that K^+, but not Na^+ is closely associated with some cytoplasmic component.

Regarding the elusive problem of protoplasmic streaming, the occurrence of higher K^+ concentrations in the advancing front of this organism lends support to the concept that this region plays a dominant role in the generation of ameboid motility (Allen, 1961). Kamiya (1965) has demonstrated that the front is a fibril-containing region and a site of contraction in glycerinated models of slime mold plasmodia. Of perhaps greater importance, Anderson's findings and conclusions challenge certain time-honored concepts which are of general physiological significance. It would appear that most investigators presuppose the cell to be a membrane-bound sac containing ions, particularly monovalent ones, in free solution. Since the movement of these ions across living membranes is frequently against a concentration gradient and cannot be accounted for by free diffusion, it has been necessary to postulate the existence of ion pumps and various schemes of active transport. Such metabolically dependent systems have been demonstrated to be thermodynamically impossible and to contradict experimental data (Ling, 1965; Ling, 1965a).

Evidence supporting the view that monovalent cations can be complexed is frequently encountered in the literature (Ling, 1965; Ling, 1965a). The findings of Cope (1967) demonstrate convincingly that large quantities of Na^+ are complexed by muscle and other tissues. His data reveal that a gel-like state is necessary for the type of cation-

binding studied. Cope concludes that such complexing is a consequence of the low activity of water in gels or concentrated suspensions of anions. According to this concept, much of the interstitial water of such materials would be present in hydration shells and the solubility of small ions correspondingly reduced.

It is the purpose of the present study to test severely the K^+ binding capacity of dried plasmodial powders by subjecting them to prolonged dialysis. In accordance with Cope's findings the relationship between K^+ complexing and the concentration of dialyzed material is also explored.

METHODS AND MATERIALS

Preparation of plasmodial suspensions.—The method of Camp (1936) was employed in culturing the plasmodia utilized in all but one of the procedures to be described. One sample was cultured on nutrient media defined by Daniel and Rusch (1961) as modified by Anderson (1964). Dried plasmodia were prepared and stored as powders according to the method of Hatano and Oosawa (1966). Suspensions to be dialyzed were prepared by stirring 650 mg of powder in 10 ml of distilled water, or in 5 ml distilled water for more viscous suspensions, over a period of 60 min. Bovine serum albumin (BSA) was employed as a control and was treated in the same manner as the test material except that it was stirred in a solution of KCl, 10 milliequivalents/liter with respect to K^+. This concentration of K^+ approximates that of the 650 mg/5 ml experimental samples prior to dialysis.

Dialysis procedures.—For samples consisting of 650 mg/10 ml plasmodial powder in water, dialysis was carried out at 9°C for a period of 30 hours against one liter of medium. The medium was changed at eight and twelve hour intervals and a sample of each dialysate was collected for K^+ analysis. Following dialysis the volume of the treated suspension was recorded and the K^+ concentration of the sample analyzed was calculated on the basis of this volume. Separate samples were dialyzed against media containing 0, 5.0, and 10.0 meq K^+ per liter. An additional sample was dialyzed against a solution 10.0 meq/l with respect to Na^+. The more viscous suspensions (650 mg/ml) were dialyzed against one liter of medium for 30 hours with no change in medium.

Powders prepared from the advancing front and from the posterior training regions of plasmodia were tested for their relative K^+ holding capacities by dialyzing samples of each region in separate tubes

but in the same medium. A control specimen of BSA in K^+ was included. Material representing the two regions was obtained from large slime molds which had been allowed to migrate on an "Anderson racetrack" (Anderson, 1962). Media utilized for these dialyses consisted of distilled water, KCl (5.0 meq/l, and a solution of NaCl plus KCl (5.0 meg/l with respect to Na^+ and K^+).

Preliminary tests for particulate and lipid-bound K^+.—Approximately one gram of fresh plasmodium was homogenized in 10 ml cold (9°C), 0.25 M sucrose in a hand homogenizer and centrifuged at 4°C. The centrifugal force was gradually increased to 27,000 x g over a period of 15 minutes. This force was maintained for 30 minutes. The supernatant and a 10 ml suspension of the particulate material obtained were then analyzed for total K^+.

In a final exploratory test the acetone utilized in preparing plasmodial powders, and extracts of plasmodia with ethyl ether substituting for acetone were measured for K^+.

K^+ analysis.—Analyses for K^+ were performed by standard flame photometric techniques using the internal standard method. Boiling the sample in water for 5 minutes releases all the K^+ (Anderson, 1962).

RESULTS AND DISCUSSION

Preliminary observations.—The data recorded in Table I reveal that virtually all plasmodial K^+ is located in the soluble fraction. It is therefore apparent that the presence of a K^+-rich organelle cannot account for the regional differences in concentration reported for this ion in the plasmodium.

TABLE I
K^+ Distribution in Particulate and Soluble Plasmodial Fractions

Fraction	meq K^+/l	Total meq $K^+ \times 10^3$
Supernatant	2.20	11.0
Pellet	.....	0.04
Wash	.....	1.32

When plasmodia were extracted with acetone the bulk of the K^+ was also extracted (Table II). This suggests that the ion might be the K^+ removing capacity of the acetone is consequence of its water extracting capacity. After dialyzing suspensions of acetone powders against one liter of distilled water for 30 hours it was found that the

TABLE II
K^+ Analysis of Plasmodia[a]
by Acetone Extraction and Dialysis

Fraction	meq K^+/l	Total meq K^+ × 10^3
Acetone extract	3.33	47.5
Plasmodial supernatant	0.18	2.7
Plasmodial ppt	0.00	0.00
Dialysate	0.02	.....

[a] Camp culture

associated with some lipoid component. The results obtained when the plasmodium was extracted with ether (Table III) suggest that

TABLE III
K^+ Analysis of Plasmodial[a]
Extracts, Suspensions, and Dialysate

Fraction	meq K^+/l	Total meq K^+ × 10^3
Ether extract	0.02	0.39
Acetone extract	7.20	93.6
Plasmodial suspension	6.20	62.0
Plasmodial suspension, dialyzed	1.74	17.4
Dialysate	0.43	42.6

[a] Plasmodia cultured on Daniel and Rusch nutrient medium.

dialyzed material had a K^+ concentration which was about tenfold greater than that of the dialysate. This finding was consistent whether the plasmodium had been cultured on oatmeal or on the, relatively, K^+-rich medium of Daniel and Rusch. The data presented in Table II also reveal that, when dialyzed suspensions are centrifuged and then boiled, all of the K^+ is found to be associated with the water soluble fraction.

Responses of plasmodial powers to media of varying ionic strength.— Regardless of the ionic strength of the dialyzing medium the treated suspension always maintained a higher concentration of K^+ than the dialysate (Table IV). This result was obtained even when dialysis was against a medium 10 meq/l with respect to Na^+, indicating that the affinity of treated material for K^+ is not a simple nonspecific affinity for cations. Plasmodial material dialyzed against 5 meq K^+/l was found to have a higher K^+ concentration than that exhibited by undialyzed suspensions (Table III, plasmodial suspension). The gen-

TABLE IV
K^+ Concentrations of Dialyzed Plasmodial Suspensions[a]

Dialyzing	Final dialysate (meq K^+/liter)	Suspension (meq K^+/liter)
Distilled water	0.0084	0.0589
5.0 meq K^+/l	4.92	8.12
10.0 meq K^+/l	10.00	11.38
10. meq Na^+/l	0.0148	0.0419

[a] Suspension consists of 650 mg of dried plasmodium in 10 ml distilled H_2O

eral pattern of the results of this series of treatments reveal, as the K^+ concentration of the dialyzing medium is increased, that of the dialyzed material also increases, and a concentration gradient is maintained. The differences in *relative* K^+ concentrations decrease, however, indicating that a saturation point is being approached.

Regional responses of concentrated plasmodial suspensions to dialysis treatments.—The final series of dialysis treatments was performed with several objectives in mind. Cope's hypothesis that the cation-binding capacity of materials is a function of anion concentration was tested by doubling the ratio of plasmodial material to water (650 mg/5.0 ml). Powders prepared from the advancing front and from the trailing channelled region of migrating plasmodia were tested for their relative K^+ holding capacity. Finally, the extent to which a Donnan equilibrium could account for the results obtained was tested by dialyzing samples of plasmodial material and a control (BSA in 100 meq K^+/l) against a medium containing both Na^+ and K^+.

It is immediately apparent that the K^+ holding capacity of plasmodial material is not enhanced by doubling the concentration of the suspensions to be dialyzed (Table V). Cope's findings apparently

TABLE V
K^+ and Na^+ Concentrations of Dialyzed Plasmodial Samples[a]

Sample	Dialyzing Medium			
	Distilled Water	5 meq/l K^+	5 meq/l K^+ and Na^+	
			(K^+)	(Na^+)
Advancing front	1.221	9.72	10.75	9.33
Posterior channeled region	0.656	9.16	7.66	6.80
BSA-K^+	0.342	6.66	6.68	6.77
Dialysate	0.171	5.00	5.00	5.00

[a] 650 mg plasmodial powder in 5 ml. of suspending medium

provide no explanation for the results obtained in this study. This conclusion is supported by the observation that the complexing of Na^+ as reported by Cope is a rather nonspecific phenomenon, since Na^+ can be replaced by other cations (Cope, 1967). Also, Cope observed that Na^+ can be removed from muscle homogenate pellets by a single washing. It is clear that the type of binding observed in this study depends on a more stable association between ion and complexing agent. The only accepted mechanism which might account for this close association would appear to be a chelating of the ion, as is known to occur in the case of certain heavy divalent metals (Fruton and Simmonds, 1961).

By comparing the response of the BSA-K^+ control to dialysis against both Na^+ and K^+ with the results that would be expected from a Donnan equilibrium:

$$\frac{(Na^+)_i}{(Na^+)_o} = \frac{(K^+)_i}{(K^+)_o}$$

it is clear that the results obtained for the control can be accounted for by this relation. It appears that the response of the plasmodial material cannot be accounted for so simply. It would seem reasonable to suggest that something other than Donnan equilibrium is involved in the K^+ holding capacity of the plasmodium. This conclusion is also supported by the results of dialysis against higher concentrations of Na^+ (Table IV).

Prior to dialysis, suspensions of advancing front material were found to have higher K^+ concentrations than suspensions of posterior channelled material (11.98 meq/l and 10.08 meq/l respectively). This agrees well with Anderson's findings. The advancing front also *holds* K^+ better than the channelled regions against dialysis (Table V). In further accord with Anderson's results, the affinity of the trailing region for Na^+ does not exceed that of the BSA-K^+ control. However, the advancing front exhibits a notable affinity for Na^+. Since the material which Anderson found to be so easily leached free of Na^+ was obtained from the posterior channelled region, it is not clear whether this latter observation is in conflict with his findings. However, it should be pointed out that, since equal masses of the two regions were dialyzed, the difference in K^+ affinity must be a consequence of qualitative differences in composition and not simply a reflection of higher protein (etc.) concentrations. This too is in keeping with Anderson's measurements of relative protein content in the two regions.

CONCLUSIONS

Membrane-free material of *P. polycephalum* exhibits an affinity for K^+ which cannot be accounted for by anion concentration or Donnan equilibrium. This affinity is specific from the standpoint that it is not eliminated by the presence of relatively large quantities of Na^+. It is not clearly specific from the standpoint that the advancing front also shows an affinity for Na^+ which is superimposed upon its affinity for K^+. The preference of the advancing front for K^+ is a small, subtle one. The channelled regions hold K^+ against dialysis, but not Na^+. The difference in K^+ affinity between the front and channelled region is a result of qualitative differences.

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REFERENCES CITED

Allen, R. D., 1961, Ameboid Movement, pp. 135-186 *in* J. Brachet and A. E. Mirsky (ed.), The Cell, vol. 2: New York, Academic Press, Inc.

Anderson, J. D., 1962, Potassium loss during galvanotaxis of slime mold; J. Gen. Physiol., 44, pp. 567-574.

Anderson, J. D., 1964, Regional Differences in Ion Concentration in Migrating Plasmodia, pp. 125-134 *in* R. D. Allen and N. Kamiya (ed.), Primitive Motile Systems in Cell Biology: New York, Academic Press, Inc.

Camp, W. G., 1936, A method of cultivating myxomycete plasmodia: Torrey Bot. Club, Bull., 63, pp. 205-210.

Cope, F. W., 1967, NMR evidence for complexing of Na^+ in muscle, kidney, and brain, and by actomyosin. The relation of cellular complexing of Na^+ to water structure and to transport kinetics: J. Gen. Physiol., 50, p. 1353.

Daniel, J. W. and H. P. Rusch, 1961, The pure culture of *Physarum polycephalum* on a partially defined medium: J. Gen. Microbiol., 25, pp. 47-59.

Fruton, J. S., and Sofia Simmonds, 1961, General Biochemistry: New York, John Wiley and Sons, Inc.

Hatano, S., and F. Oosawa, 1966, Isolation and characterization of plasmodium actin: Biochem. Biophys. Acta, 127, pp. 488-498.

Kamiya, N., and K. Kuroda, 1965, Movement of the myxomycete plasmodium. I. A study of glycerinated models: Proc. Japan. Acad. 41, pp. 837-847.

Ling, G. N., 1965, Physiology and anatomy of the cell membrane: the physical state of water in the living cell: Federation Proc., 24, pp. 5103-5112.

_____, 1965a, The membrane theory and other views for solute permeability, distribution, and transport in living cells: Perspectives Biol., Med., 9, p. 87.