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*DISSOCIATION OF RUBIDIUM UPTAKE BY NEUROSPORA CRASSA INTO ENTRY AND BINDING PHASES**

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Communicated by Theodore Shedlovsky, September, 17, 1958

Introduction.—Previous experiments concerned with the inhibitory effect of deoxycorticosterone (DOC) on the growth of *Neurospora crassa* indicated that this steroid interfered with certain permeability processes, inhibiting the uptake of rubidium, amino acids, or sugars.[1] Since DOC has been shown to inhibit the uptake of a physiologically similar ion—potassium— by other cells and tissues,[2, 3, 4] the phenomenon of rubidium uptake by *N. crassa* has been intensively studied, to provide a basis for elucidating the nature of the effect of DOC. Some of the salient features of this work which have a bearing on the general aspects of cell permeability will be described here; a brief report has been made elsewhere.[5]

This paper presents evidence that the uptake of rubidium by *N. crassa* is an active transport process, which can be dissociated into two discrete events. One is concerned with the entry of rubidium into the cell and the other with the subsequent accumulation of rubidium against an *apparent* concentration gradient. The entry appears to be dependent on metabolic energy but lacks specificity, in that competition between potassium and rubidium is not evident in this phase of uptake. The concentrative accumulation of rubidium in the cell appears to involve a rubidium-binding system which is competitively inhibited by potassium and is blocked by DOC. Although these studies deal with the uptake of rubidium rather than potassium, the usual observation of an equivalence of these ions as nutritional requirements and in their permeability characteristics suggests that the following observations and conclusions apply to potassium as well as rubidium.

Methods and Materials.—The methods and the system employed in this study have been described elsewhere,[1] and they will be only briefly summarized here. Rubidium uptake was examined in a system consisting of washed, germinated conidia suspended in buffer (0.02 M NaH_2PO_4 in 0.05 M NaCl, adjusted to pH 5.6–5.8 with NaOH). Rubidium^{-85}, admixed with radioactive rubidium^{-86}, was added as the chloride, and the uptake of rubidium was estimated by either or both of two methods: (*a*) by the decrease of radioactivity in the suspending medium after removal of the cells by centrifugation or filtration and (*b*) from the

radioactivity of hot-water extracts of cells rapidly rinsed with buffer while being harvested in a Büchner funnel (very little loss of intracellular rubidium occurred with this treatment); the results obtained by these methods agreed very well with each other. In some experiments, where rubidium-containing cells were desired at the outset, the potassium of the germination medium was replaced with sodium, and rubidium was added as a growth substitute for potassium. Washing with buffer resulted in only a very small loss of rubidium; i.e., washed cells showed a rubidium content approximately that expected on the basis of radioactivity disappearance from the medium. The dry weight of cells in suspension (7–12 mg/ml) was determined for each sample, and the intracellular content of rubidium is expressed as μg. Rb^+ per mg. cell dry weight; no significant changes in cell dry weight occurred during incubation. Calculations of the intracellular concentration of rubidium

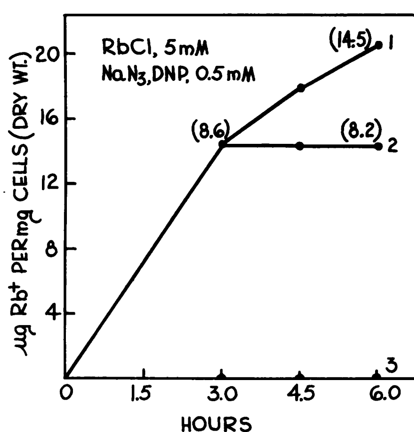

FIG. 1.—Effect of azide and 2,4-dinitrophenol (DNP) on the uptake and retention of rubidium. Curve *1*, control; curve *2*, azide or DNP added at 3 hours' incubation; curve *3*, azide or DNP added 5 minutes prior to the addition of RbCl (time 0). The numbers in parentheses represent the ratios of internal to external rubidium at the associated points.

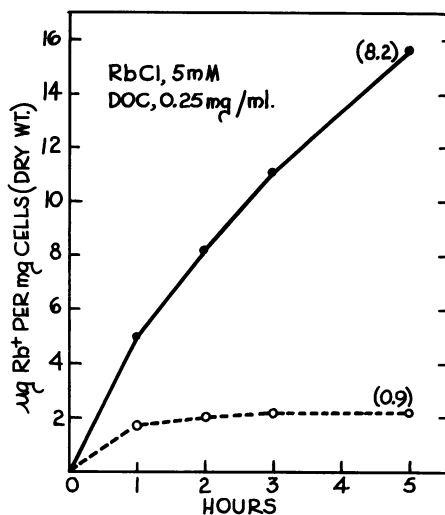

FIG. 2.—Uptake of rubidium in the absence (*solid line*) and presence (*broken line*) of deoxycorticosterone (DOC). The numbers in parentheses represent the ratios of internal to external rubidium at the associated points.

were based on determinations of cell water as 84–85 per cent of the wet weight of filtered cells. Unless otherwise indicated, all the experiments were carried out with 20–30 ml. of suspension in 125-ml. Erlenmeyer flasks, incubated at 30° C. with agitation.

Experimental.—The uptake of rubidium by germinated conidia of *N. crassa* resembles, in certain respects, observations made with potassium (or rubidium) in many other kinds of cells and appears to be a specific, active transport process. Germinated conidia accumulate rubidium to a concentration several times that outside the cell; this accumulation is completely inhibited by 2,4-dinitrophenol (DNP) or azide and is competitively inhibited by potassium. Figure 1 shows the accumulation of rubidium against an apparent concentration gradient and its complete inhibition when DNP or azide are added to a cell suspension almost

simultaneously with rubidium. A unique feature of this system becomes evident when the cells are allowed to take up rubidium for a few hours prior to the addition of DNP or azide. In this case the inhibitors block any further uptake of rubidium, but, more significantly, *an expected efflux of rubidium is not observed.* These observations indicate that the energy requirement for the uptake of rubidium is not the same as that required for the maintenance of intracellular rubidium concentration in excess of that outside the cell.

Another striking characteristic of this system is the unusual stability of the rubidium accumulated by germinated conidia, under conditions where efflux or exchange would be expected. Table 1 indicates the firm retention of rubidium by cells which had been grown in medium where radioactive rubidium replaced the requirement for potassium. These cells were washed and resuspended in buffer

TABLE 1

Stability of Intracellular Rubidium

Additions to Cell Suspension*	Per Cent Loss of Intracellular Rubidium
Experiment I†	2.5-Hr. Incubation
None	<1
Sucrose (0.03)	1.4
RbCl(0.01 *M*)	3.3
KCl(0.01 *M*)	3.7
Experiment II‡	2.0-Hr. Incubation
Sucrose (0.03 *M*)	1.5
KCl (0.05 *M*)	4.1
Sucrose (0.03 *M*) + KCl(0.05 *M*)	4.8

* The cells were suspended in the buffer described in "Methods."

† Initial intracellular rubidium, 45 μg/mg cell dry wt.; initial cell concentration, 8.5 mg/ml suspension.

‡ Initial intracellular rubidium, 38 μg/mg cell dry wt.; initial cell concentration, 9.7 mg/ml suspension.

with various additions. No significant efflux of rubidium was observed upon incubation in buffer, in the absence or presence of sucrose, which is readily metabolized by these cells. Similarly, incubation in the presence of non-radioactive rubidium or potassium resulted in little efflux of rubidium from these cells, nor did the presence of an energy source, sucrose, lead to an exchange with potassium. These results indicate that, once rubidium enters the cell, it can be maintained against a large apparent concentration gradient, and it is not readily available for exchange with rubidium or potassium entering subsequently. Also, it would appear that the observed uptake of rubidium largely represents a unidirectional flow of rubidium into the cell rather than a two-way flux process.

Having defined certain aspects of rubidium uptake by *N. crassa*, the effect of DOC was examined. The time course of rubidium uptake by germinated conidia in the absence and presence of DOC is illustrated in Figure 2. For a maximal effect the cells were incubated for about 2 hours with DOC before adding rubidium; the control was similarly preincubated without DOC. In the absence of DOC, the rate of rubidium uptake is nearly linear for about 3 hours, and, although the rate diminishes with time, rubidium uptake is still continuing at 9 hours. The presence of DOC reduces the uptake of rubidium, and, characteristically, after about 2 hours no further uptake is observed. In the presence of DOC, the intracellular concentration achieved approximates that of the external rubidium concentration.

This observation suggests that DOC does not prevent the entry of rubidium into the cell but does interfere with the process whereby rubidium is accumulated against an apparent concentration gradient.

This possibility is more clearly indicated in Table 2, which shows the effect of the external rubidium concentration on uptake in the presence and absence of DOC.

TABLE 2

EFFECT OF EXTERNAL RUBIDIUM CONCENTRATION ON UPTAKE IN PRESENCE AND ABSENCE OF DOC

Initial RbCl (mM)	DOC (mg/ml)	Rb^+ Uptake* (μg/mg Cells)	$\frac{[Rb_i]}{[Rb_o]}$
5	...	11.7	6.05
12.5	...	14.6	2.58
25	...	22.8	1.95
5	0.25	2.2	0.93
12.5	0.25	5.1	0.91
25	0.25	11.0	0.90

* 3.0-hr. incubation.

In the absence of DOC the uptake of rubidium increases as the external concentration is increased, but not proportionately. However, in the presence of DOC the uptake of rubidium is directly proportional to the external concentration, and in each case the intracellular concentration approximates that of the concentration of rubidium outside the cell. If, at each concentration of rubidium, the uptake values obtained in the presence of DOC are deducted from those obtained in the absence of DOC, the remainders are found to be quite similar. These results

TABLE 3

EFFECT OF POTASSIUM ON RUBIDIUM UPTAKE

Initial Concentration RbCl (mM)	KCl (mM)	Rb^+ Uptake* (μg/mg Cells)	$\frac{[Rb_i]}{[Rb_o]}$
5	...	14.7	9.1
5	2.5	7.5	3.5
5	5	5.2	2.3
5	10	4.3	1.8
5	20	3.6	1.5

* 3.0-hr. incubation.

TABLE 4

EFFECT OF POTASSIUM ON RUBIDIUM UPTAKE IN PRESENCE AND ABSENCE OF DOC

Initial Concentration RbCl (mM)	KCl (mM)	DOC (mg/ml)	Rb^+ Uptake* (μg/mg Cells)	$\frac{[Rb_i]}{[Rb_o]}$
5	..	...	16.2	9.5
10	..	...	19.6	4.8
5	10	...	5.1	2.2
10	10	...	9.2	2.0
5	..	0.25	2.5	1.03
10	..	0.25	4.7	0.96
5	10	0.25	2.4	0.98
10	10	0.25	5.2	1.06

* 3.0-hr. incubation.

suggest that the capacity of these cells for rubidium in excess of the external concentration is relatively constant. This capacity for rubidium has been found to vary inversely with the potassium content of the cell; however, the sum of intra-

cellular potassium plus the rubidium taken up in excess of the external concentration attains a rather constant maximum value equivalent to about 80 μg. rubidium/mg cell dry weight.[6]

Tables 3 and 4 show the effect of potassium on rubidium uptake in the absence and presence of DOC. The upper half of Table 4 shows that potassium inhibits rubidium uptake in a competitive fashion. In Table 3 it can be seen that the effect of potassium was greater than that expected on the basis of an exact competitive equivalence of potassium and rubidium; for example, with equal concentrations of these ions, rubidium uptake was reduced to about one-third rather than one-half the value obtained in the absence of potassium. It should be noted that as the external concentration of potassium is increased, the uptake or rubidium asymptotically approaches a value corresponding to an intracellular concentration approximating that outside the cell. In other experiments, using still higher potassium concentrations, the uptake of rubidium was about equivalent to that expected on the basis of a passive diffusion of rubidium into the cell.

Table 4 shows that in the presence of DOC, potassium has no inhibitory effect and that the uptake is approximately that expected for a passive diffusion of rubidium. The inability of high concentrations of external potassium to prevent entry of rubidium into the cells and the lack of any competitive effect of potassium on rubidium uptake in the presence of DOC indicate that the entry of rubidium is a relatively non-specific step. On the other hand, the accumulation of rubidium against an apparent concentration gradient appears to be a specific event, as evidenced by the competition between rubidium and potassium.

The data presented so far could suggest that rubidium enters the *Neurospora* cell by a passive process and is then concentrated by some other process. However, it has already been observed (Fig. 1) that DNP or azide practically completely blocks the uptake of rubidium. Since this effect was obtained under conditions where concentration against an apparent gradient could occur and a small uptake might be relatively obscure, the effect of these inhibitors was examined in the presence of DOC. Table 5 shows that DNP or azide almost completely abolishes rubidium uptake under these conditions. These results indicate that the entry of rubidium is not "passive" but is an "active," energy-requiring event under conditions where rubidium does not accumulate against an apparent concentration gradient.

TABLE 5

EFFECT OF AZIDE OR DNP ON RUBIDIUM UPTAKE IN PRESENCE AND ABSENCE OF DOC

Initial Concentration RbCl (mM)	Initial Concentration NaN_3 or DNP (mM)	DOC (mg/ml)	Rb^+ Uptake* (μg/mg Cells)	$\frac{[Rb_i]}{[Rb_0]}$
5	...	...	12.0	6.0
5	0.5	...	0.25	0.1
5	...	0.25	2.5	1.02
10	...	0.25	4.8	0.98
5	0.5	0.25	0.25	0.1
10	0.5	0.25	0.50	0.1

* 3.0-hr. incubation.

The separateness of entry and intracellular accumulation against an apparent gradient can also be demonstrated by the differential effect of temperature on

these phases of rubidium uptake. With an initial external concentration of rubidium of 5 mM, the uptake values in the absence of DOC represent mainly the specific accumulation process, while in the presence of DOC the uptake values represent mainly the entry process. The data given in Figure 3 indicate that the accumulation process is more sensitive to temperature changes than the entry process. Thus, lowering the temperature from 32° to 22° causes a marked decrease in rubidium uptake, in the absence of DOC, whereas a similar temperature change has little effect on the rate of entry, in the presence of DOC. With a further decrease of 10°, the rate of uptake is decreased in both cases, again suggesting that the entry process is also dependent on cellular activities.

Discussion.—The data presented are schematically summarized in Figure 4, where the uptake of rubidium is visualized as a diphasic phenomenon consisting of

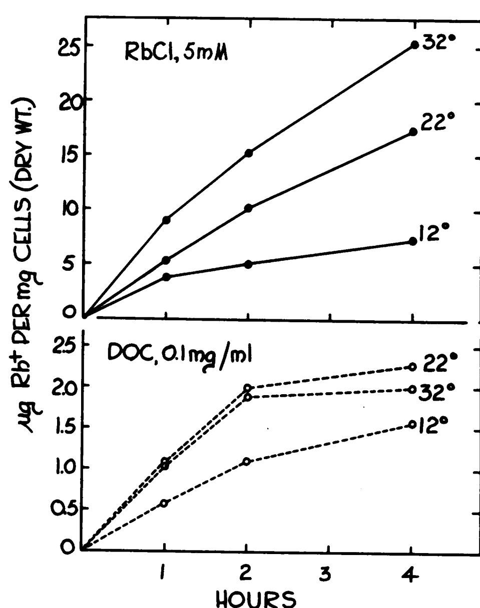

FIG. 3.—Effect of temperature on the uptake of rubidium in the absence (*solid lines*) and presence (*broken lines*) of deoxycorticosterone (DOC). The cell suspensions were incubated at 30° C. for 2 hours with or without DOC prior to the addition of RbCl (time 0) and subsequent incubation at the temperatures indicated. Note the difference in the ordinate scales of the upper and lower portions of this figure.

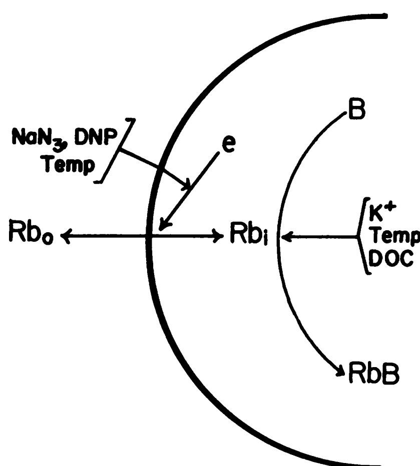

FIG. 4.—A schematic summary of rubidium uptake

entry and an accumulation process involving selective binding. *Neurospora crassa* is considered here as presenting a barrier, perhaps the cell membrane, through which rubidium cannot pass without an expenditure of energy (e). Thus, when the energy supply is reduced, either by a large decrease in temperature or especially by DNP or azide, the entry or rubidium is completely inhibited. Although energy-dependent, the entry of rubidium resembles a passive diffusion process, since rubidium appears to be equally distributed between cell water and the external medium when accumulation is blocked by DOC or high external potassium con-

centration. Moreover, the entry event appears to lack specificity, since potassium does not exhibit any competitive effect on the entry of rubidium, in the presence of DOC. Similarly, when rubidium uptake has been so reduced by high potassium concentrations that the intracellular rubidium concentration approximates that outside the cell, further increases in potassium concentration no longer affect the entry of rubidium. These observations indicate that the competitive interaction between rubidium and potassium observed during uptake probably occurs subsequent to entry, in the accumulation process. The lack of such specificity, in the absence of accumulation, indicates that a transport hypothesis involving the presence of rubidium-potassium specific carrier in the cell membrane is not applicable to the entry phase of rubidium uptake by *N. crassa.*

At present, the mechanism of the entry event is obscure, but any proposals on its nature should take into account its energy-dependence and lack of specificity. These characteristics would obtain for a mechanism such as pinocytosis,[7] which would require energy for membrane movement, resulting in a non-specific transfer of rubidium into the cell; similar features could be envisioned for a contractile-lattice membrane[8] or a contractile process involving an interaction of intracellular and surface proteins.[9] As sole determinants of permeability processes, such proposals have encountered conceptual difficulties in providing for specificity and concentrative features.[10] These difficulties are obviated in the work presented here, since the entry of rubidium lacks specificity, and it can be dissociated from the concentrative event. Thus a mechanical action of the cell surface merits serious consideration as the initial phase in rubidium uptake by *N. crassa* and perphaps as a component of permeability phenomena in other organisms as well.

The process by which rubidium is accumulated in excess of the external concentration is characterized by (*a*) the relatively constant capacity of *N. crassa* for rubidium; (*b*) the maintenance of the internal rubidium concentration in cells incubated in the absence of external rubidium or in the presence of DNP or azide; (*c*) the relatively insignificant exchange between intracellular rubidium and extracellular rubidium or potassium; and (*d*) a competition between potassium and rubidium. These characteristics are not consistent with a hypothesis which assumes flux across the cell membrane in both directions and no differences between the states of intra- and extracellular rubidium or potassium. The operation of a specific pump mechanism, dependent upon metabolic energy for the maintenance of intracellular rubidium against a concentration gradient, is negated by the lack of effect of DNP or azide on the level of accumulated rubidium, although these inhibitors prevent the entry of rubidium.

In view of the above characteristics, the accumulation of rubidium in excess of the external concentration seems best explained by postulating the existence of a specific cytoplasmic system for binding rubidium. This system is assumed to involve a limited number of binding sites (B) which can combine with either rubidium or potassium in a form (RbB or KB) which is not readily dissociated. Since potassium does not readily exchange with rubidium already present in the cell, presumably as RbB, the site of potassium competition would be a specific mechanism responsible for the formation of RbB. The inhibitory effect of DOC on rubidium uptake also appears to involve the binding event, since DOC does not prevent the entry of rubidium. The action of DOC should be directed toward the dissociation

of RbB, its formation, or an alteration of B, but the data do not permit a decision between these possibilities. Similarly, it also remains to be determined whether temperature changes directly affect a specific concentrating system or other related processess. While various cytoplasmic mechanisms have been proposed to account for K accumulation in cells,[11-15] the present data are insufficient to decide between these or to formulate other mechanisms. The resolution of this problem in detail awaits the demonstration and characterization of a specific rubidium-binding system in vitro.

Certain features of rubidium uptake in *N. crassa* have more or less close counterparts in other micro-organisms. Thus in *Escherichia coli* there appears to be a limited capacity for potassium accumulation, efflux is not easily effected, nor does intracellular potassium readily exchange with external potassium unless certain carbon sources are present.[11,16] With *Alcaligenes fecalis*, on the other hand, exposure to a potassium-free medium results in an efflux of most of the intracellular potassium; also complete exchange of intracellular potassium readily occurs.[15] The variations in the stability of bound potassium (or rubidium) among these cells raise the possibility that a spectrum of cytoplasmic binding agents (B) exists which have different affinities for potassium. In a given cell type, intracellular potassium might be associated with one or more kinds of B. The postulate that different types of B can coexist in the same cell could explain the heterogeneity of intracellular potassium in kidney,[17] muscle,[18, 19, 20] and other tissues,[21] with respect to exchange or efflux.

Superficially, the uptake of rubidium by *N. crassa* resembles the active transport of potassium in other cells, in that rubidium *seems* to be taken up against a concentration gradient and that this uptake has an energy requirement. However, the evidence presented indicates that two dissociable processes are operative, each exhibiting some, but not all, of the features of the over-all phenomenon. It does not necessarily follow that this description of active transport in *N. crassa* can be applied to other cells. However, these observations may have a general significance by suggesting that attempts to explain permeability phenomena should not be limited to considerations of a single primary mechanism.

* This work was supported by a grant from the Commonwealth Fund.

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*THE THERMAL CONDUCTIVITY OF CARBON DIOXIDE IN THE REGION OF THE CRITICAL POINT**

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Communicated by Frederick G. Keyes, September 15, 1958

Introduction.—Thermal conductivity phenomena very close to the critical point of CO_2 have not been determined. Reported attempts either have completely skipped the region of most interest or are obviously in error. A new effort based on extensive analysis of the problem was undertaken with equipment exceptionally well suited to the rigorous treatment of the data from which the thermal conductivity can be obtained.[1]

Equipment.—The cell was of the massive concentric cylinder type with a uniform conductivity gap, d, of 0.06955 cm. along the vertical axis and at the bottom. Heat was supplied in a center well of the inner cylinder, and temperature differences were measured by Chromel-P Alumel thermocouples placed at the top and center of the cylinders. A heat guard at the top was brought to the same temperature as the inner cylinder, and thus heat loss along the thermocouple and heater wires was avoided. The thermocouples were calibrated in place against a platinum resistance thermometer contained in a thermostat bath regulated within ±0.001° C. All voltage measurements were made on an Eppley thermocouple potentiometer, which was carefully calibrated. Pressures were measured on a dead-weight gauge calibrated for the vapor pressure of CO_2 at 0° C., and they were controlled by a special regulator system to about 1 part in 100,000.

Theory.—The energy conducted across length, l, of the annulus, d, between two infinite concentric cylinders is

$$Q = \frac{2\pi K l \Delta T}{\ln r_2/r_1}.$$

The constant for the cell is obtained from this term and a similar term for the bottom,

$$Q = \frac{\pi r_1^2 K}{d} \Delta T,$$

together with two correction terms, one for the corner at the bottom, and another