THE ELECTROCHEMICAL STATE OF CELLS OF BROAD BEAN ROOTS

II.* POTASSIUM KINETICS IN EXCISED ROOT TISSUE

By C. K. Pallaghy†‡ and B. I. H. Scott†

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Summary

The electrochemical state of potassium in excised broad bean root tissue is described. Mature cortical cells predominated in the tissue segments used.

No net flux of Na^+ or K^+ was observed during the period 1–5 days after excision for tissue bathed in the standard growth medium, which contained Na^+, K^+, and Ca^{2+} (each 1 mM) and 0·25 mM Mg^{2+}. Nevertheless, oxygen consumption fell by about 60% during this period and there were corresponding falls in bulk fluxes of potassium between the tissue compartments and the external medium. A compartmental analysis using ^{42}K as tracer indicated that this fall in flux could be accounted for on a cellular basis if individual cells were ceasing to exchange K^+ with the external medium, the proportion of such cells increasing with aging of the tissue. The analysis indicated that the tonoplast flux was considerably greater than the plasmalemma flux, particularly in recently excised tissue, when the ratio was about 12.

The vacuolar potential changed from $-138\pm1\cdot4$ mV 8 hr after excision to a steady value of $-112\pm1\cdot4$ mV after 24 hr. The results were not inconsistent with the hypothesis that potassium is moving passively (according to the Ussing–Teorell criterion) within the cells under the conditions of the experiment.

The Appendix gives an exact solution of the flux equations for a three-compartment system if there is no net flux, and considers the modifications necessary if there is net flux or if the fluxes change with time.

I. Introduction

In an earlier paper, Scott, Gulline, and Pallaghy (1968) attempted to apply electrochemical theory to describe the movement of ions between the cells of intact roots of growing broad bean seedlings and the external medium.

It was obvious that the simple Nernst criterion for passive ion movement (see Dainty 1962) was not applicable to the cells under investigation (in the first 2 cm of the root tip) because they were far from being in a state of flux equilibrium. The rapid increase in cell volume was accompanied by large inwardly directed net fluxes of water and solutes. For these cells it may be more valid to use the Ussing–Teorell criterion for passive ion movement, and this requires a knowledge of the separate influxes and effluxes of the ion across cellular membranes.

Isotope tracer methods are widely used for measurement of ion fluxes in cells and tissues. Because of the multi-compartment nature of plant cells and slow exchange between vacuoles and exterior, complete isotope analyses usually occupy periods of

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† Department of Physics, University of Tasmania, Hobart, Tas. 7000.

‡ Present address: Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra, A.C.T. 2601.

15–30 hr. These methods are therefore not suitable for tissues, such as those in the young root, in which the cells are changing rapidly in size and ionic properties. In the intact seedling, further complications may arise because of the non-homogeneous cell population, and ion fluxes along the root axis from the cotyledon. Although Scott, Gulline, and Pallaghy (1968) argue that valid estimates of fluxes in growing root cells can be obtained in short-term (1–2 hr) isotope experiments in some circumstances (particularly for sodium), it is obvious that these tentative conclusions need to be examined further, in more complete kinetic analyses which necessarily must be made on non-growing root tissue.

In the investigations described below, the experimental material was excised from mature parts of broad bean roots. In an endeavour to obtain reasonably homogeneous samples of cells, the stele was removed so that the remaining tissue was predominantly from the cortex. Investigations of potassium exchange between the tissue and bathing medium were made using ^{42}K as tracer and at a temperature of 23°C. This temperature was chosen so that the tissue would be near its normal state of development and to permit comparisons with results obtained on intact roots (Scott, Gulline, and Pallaghy 1968). In a recently described investigation on barley roots by Pitman and Saddler (1967), a temperature of 5°C was chosen to slow down ion exchange across the plasmalemma so that ion movement into the cytoplasm could be distinguished from movement into the free spaces of the root. Lüttge and Laties (1967) used a temperature of 0·5°C in their studies of maize root tissue. The lower temperature did not appear to be necessary to separate potassium compartments in bean root tissue, the rate constant at the outer membranes of these cells being about one-third of that for barley root tissue.

II. Materials and Experimental Methods

Seedlings of a long-pod variety of *Vicia faba* were grown as described previously (Scott, Gulline, and Pallaghy 1968). For these experiments, the growth medium was running tap water to which concentrated salt solutions were added automatically to make total concentrations of ions as shown in the following tabulation:

	Concn. (mM)		Concn. (mM)
K^+	1·00	Cl^-	3·00
Na^+	1·00	NO_3^-	0·50
Ca^{2+}	1·00	$H_2PO_4^-$	0·32
Mg^{2+}	0·25	SO_4^{2-}	0·14
		HPO_4^{2-}	0·20

This medium was also used for bathing tissue segments. The temperature was maintained at $23 \pm 0{\cdot}5$°C.

The following procedure was adopted to provide approximately homogeneous and apparently undamaged samples of root cortical tissue. A longitudinal incision was made in the root of a 2-day-old seedling with a sharp razor-blade just deep enough to reveal the stele. After excising the first 8 mm of the root, the stele of the remaining more mature portion of the root was then carefully gouged out with a sharp, thin-walled glass capillary of suitable diameter, leaving the cortex intact. The segment of cortical tissue lying between 10 and 20 mm from the original position of the root tip was excised and washed for 10 min to remove the initial exudate. Segments were then placed in open flasks containing the growth medium, were shaken at 50 strokes per minute,

and maintained at 23°C. The solution to tissue volume ratio in the flasks was 100 : 1 (henceforth denoted by STVR = 100), the solution being initially replaced four times at 2-hourly intervals. Thereafter, tissues could be maintained in an apparently healthy state for up to 9 days by replacing the solution about every 5 hr, although slight browning of the tissue, probably due to phenoxidase activity, appeared 24 hr after excision. Tissue samples were selected after various aging periods (measured from the time of excision) for the experiments described in Section III.

Analyses of cation content of the tissue were made by flame-photometry (Scott, Gulline, and Pallaghy 1968), which was also used to determine changes in ion concentration in the bathing medium and hence net fluxes of ions between tissue and medium. In these latter experiments, STVR was 11 to allow concentration changes to be detected in a medium that was already 10^{-3} molar in both sodium and potassium. Samples were taken initially at half-hourly intervals, increasing gradually to 4-hourly intervals, the solution being completely replaced at each sampling. Ion concentrations in the bathing media never departed from the nominal values by more than a small percentage.

Influx of potassium was followed using ^{42}K as tracer. Five tissue segments were mounted in a small stainless steel wire cage, which held them in position but permitted free circulation of the labelled solution (STVR = 150). The activity of the tissue was measured periodically by removing the cage from the solution, washing in running inactive solution for about 10 sec, quickly blotting the cage, and placing it under an EKCO N664A scintillation counter which was set to register gamma-rays only. The tissue was then replaced in the solution, the interruption to normal uptake being no more than 2 min.

Tissue that had been loaded with ^{42}K in shaken tubes for about 8 hr at STVR = 150 was used in elution experiments, following the methods of MacRobbie and Dainty (1958) and Pitman (1963). Solutions were completely replaced at intervals of 5 min initially, the intervals being increased gradually to 1 hr after the first 8 hr of elution. During elution the STVR was correspondingly increased from 20 to 60. At the end of the experiment, the tissue was weighed and its residual activity determined. Cellular membrane potential differences were measured as previously described (Scott, Gulline, and Pallaghy 1968). The rate of oxygen uptake by tissue segments was determined by a standard Warburg technique.

Symbols used for ^{42}K Tracer Exchange Studies

A	time (hr) for which tissue has been excised and equilibrated with the bathing medium before loading with isotope
T	period of loading of tissue with isotope (hr)
s_o	specific activity of the external solution (counts/min/m-equiv. of potassium)
Y_S, Y_L	the extrapolated short-term and long-term components of the activity of the tissue at commencement of elution (counts/min/kg fresh weight of tissue)
k_S, k_L	the rate constants for isotope exchange of the short-term and long-term component respectively (hr^{-1})
Q_c	calculated amount of potassium in the cytoplasmic phase (m-equiv/kg of tissue)
Q_v	calculated amount of potassium in the vacuolar phase (m-equiv/kg of tissue)
ϕ_{oc}	flux of potassium from free space to cytoplasmic phase (m-equiv/kg/hr)
ϕ_{co}	flux of potassium from cytoplasmic phase to free space (m-equiv/kg/hr)
ϕ_{cv}	flux of potassium from cytoplasmic phase to vacuolar phase (m-equiv/kg/hr)
ϕ_{vc}	flux of potassium from vacuolar phase to cytoplasmic phase (m-equiv/kg/hr)

III. Results

(a) *Ion Content of Root Tissue Segments*

Figure 1 gives the potassium and sodium content of tissue as a function of age of tissue (measured from time of excision and immersion in the growth medium). It is seen that there are initial rises in both sodium and potassium levels, but thereafter

these remain steady, at least up to the fifth day. The mean value during this steady period is 78·5 m-equiv/kg for potassium and 20·5 m-equiv/kg for sodium. The observation of the ionic state of the tissue has been confirmed in four experiments involving analyses of the ion concentrations in the bathing medium. During the first 12 hr, there was a net influx into the tissue for both ions, having peak values

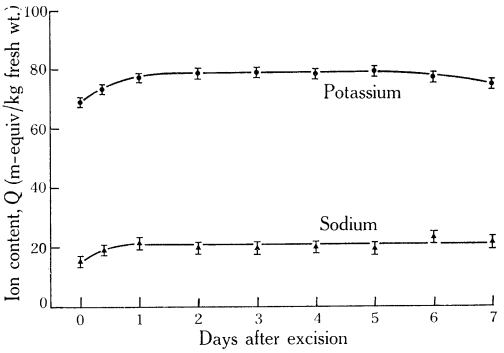

Fig. 1.—Potassium and sodium content of cortical tissue of broad bean roots that have been excised and aged in aerated growth medium (see Section II) at 23°C. The limits indicate the standard error of the mean.

of 0·6 m-equiv/kg/hr for potassium and 0·2 m-equiv/kg/hr for sodium. After 12 hr, there was no detectable net flux of potassium (the limit of detection being about 0·05 m-equiv/kg/hr.) There appeared to be a small continuing net influx of sodium, but its mean magnitude (0·05 m-equiv/kg/hr) was of the same order as the noise level in the flame-photometer.

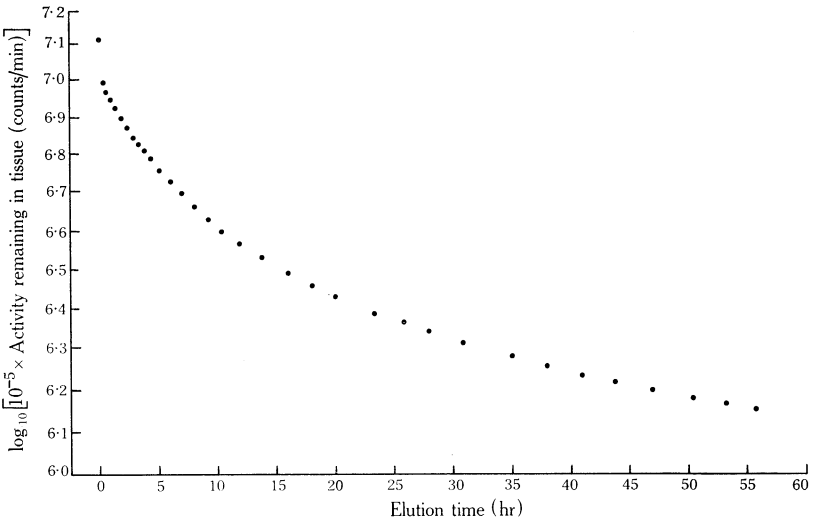

Fig. 2.—Typical semi-logarithmic plot of ^{42}K activity in root cortical tissue during elution. The tissue had been aged in non-radioactive solution for 6 hr before loading with isotope for 9 hr. Note that the graph is still curving 50 hr after elution commenced.

(*b*) *Potassium Movement in Recently Excised Tissue*

Figure 2 shows a typical semi-logarithmic elution graph for tissue that had been excised and equilibrated with the bathing medium for $A = 6$ hr before loading

for a further period of $T = 9$ hr. The graph is of the usual form, except that at no stage does it become linear. Linearity after the initial period of adjustment would be expected if the tissue were behaving as a simple system of compartments in series. Slow curving of the semi-logarithmic elution graph has been noted for *Nitella* by MacRobbie (1962) and for pea root tissue by Etherton (1967). In each of the present experiments, it was found possible to separate satisfactorily a short-term and a very short-term compartment in the manner of MacRobbie and Dainty (1958), provided that the slope of the long-term component (i.e. k_L) was taken at a time 12–15 hr after commencement of elution. The justification of this somewhat arbitrary choice of k_L is discussed in Section IV. The upper part of Table 1 is a summary of data

TABLE 1

QUANTITIES MEASURED IN ^{42}K ELUTION EXPERIMENTS, AND VALUES OF POTASSIUM FLUXES AND POTASSIUM CONTENT OF COMPARTMENTS ASSUMING STEADY-STATE CONDITIONS

See section (*a*) of Appendix for mathematical analysis

A (hr)	T (hr)	Y_S/s_o (m-equiv/kg)	Y_L/s_o (m-equiv/kg)	k_S (hr^{-1})	$10^3 k_L$ (hr^{-1})	ϕ_{oc} (m-equiv/kg/hr)	ϕ_{vc}	Q_c (m-equiv/kg)	Q_v
10	9·0	0·11	3·73	0·30	3·91	0·46	4·08	17·1	91
12	8·1	0·21	2·82	0·52	3·65	0·46	15·20	3·7	93
12	9·2	0·14	3·82	0·25	3·70	0·45	3·52	18·5	93
13*	9·1	0·067	1·76	0·26	3·96	0·27	1·60	7·9	42
18	8·5	0·068	1·35	0·34	3·95	0·16	1·07	3·9	37
24	8·6	0·058	1·09	0·28	3·42	0·144	1·07	4·3	36
24	5·8	0·055	0·53	0·46	3·18	0·118	0·38	1·1	29
36	8·9	0·038	0·89	0·26	3·27	0·097	0·85	3·6	27
37*	8·1	0·042	0·60	0·27	2·72	0·079	0·48	2·1	25
61	8·2	0·040	0·70	0·31	3·68	0·086	0·52	2·0	20
72	5·8	0·046	0·31	0·40	3·45	0·069	0·19	0·7	15
85*	8·2	0·045	0·27	0·35	2·66	0·048	0·10	0·4	12
96	5·9	0·037	0·13	0·36	4·31	0·034	0·06	0·3	4·9

* Indicates experimental data further analysed in Table 2.

obtained in five experiments following this procedure. No experiments were performed on more recently excised tissue in view of the initial net influx of potassium (Fig. 1). Tissue from about 5–10 roots is used in each experiment. Data for the very short-term compartment (assumed to be due to ion exchange with the free space of the tissue) are not included in this table. The time of half-exchange with this compartment is about 9 min.

The data in the lower part of Table 1 can be applied to a model of the system for which the vacuolar and cytoplasmic components of the tissue are each regarded as single compartments in series with one another and with the external medium, being separated by the tonoplast and plasmalemma respectively. If there is no net flux in this simple model, it is possible to solve explicitly for the ion content of the vacuole, Q_v, and cytoplasm, Q_c, and the ion fluxes across the tonoplast, ϕ_{cv}, and plasmalemma, ϕ_{oc}, in terms of the measurable parameters Y_S, Y_L, k_S, k_L, T (the time of loading), and s_o (the specific activity of the external medium) [see section (*a*) of the Appendix]. Previous workers considering this model have used approxima-

tions which, though normally adequate, cannot always be assumed to apply. Furthermore, they have often made use of measurements of ion content obtained in chemical analyses of bulk tissue in the calculation of the fluxes (e.g. Pitman and Saddler 1967). In the methods described here, chemical analysis can be used as an independent check of the validity of the model. Calculated values of Q_v, Q_c, ϕ_{cv}, and ϕ_{oc} are included in the upper part of Table 1. The estimate of the total ion content of the two compartments (Q_v+Q_c) can be compared with the estimate obtained through chemical analysis (78·5 m-equiv/kg).

(c) *Effects of Aging of Tissue on ^{42}K Analysis*

The persistent curving of the semi-logarithmic graph suggested that the characteristics of the tissue were changing with time. This was examined further in a series of experiments in which the uptake and elution of ^{42}K were followed in tissue that had been excised for different periods of time.

(i) *Uptake Experiments*

A typical example of time course of ^{42}K uptake by root cortical tissue is shown in Figure 3. It is noted that after the initial rapid build-up of activity in the short-term compartments of the root the activity continues to increase steadily but the rate of increase is not constant, falling by about 30% during the period 10–45 hr.

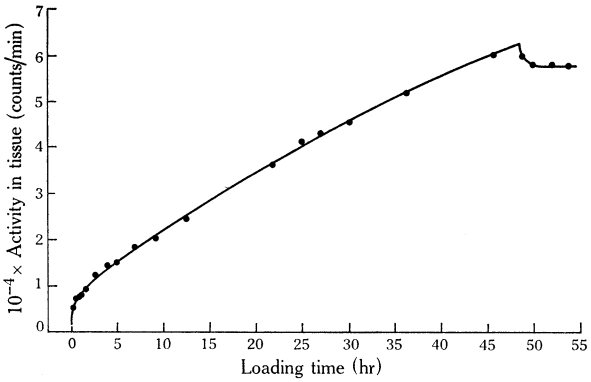

Fig. 3.—Typical example of ^{42}K uptake by 0·182 g of cortical root tissue segments at 23°C. The tissue had been aged for 48 hr in non-radioactive solution before loading commenced. ϕ_{in} is given by the slope of the graph after the initial transient ($s_o = 1{\cdot}34\times10^{11}$ counts/min for each equivalent of potassium). The graph also shows wash out from the short-term compartments after 47 hr of loading.

Curving of the graph would be expected if there were an appreciable efflux of isotope from the long-term compartment, which would occur if the specific activity of the vacuole became significant. This cannot be the explanation in the present experiments since the mean specific activity of the tissue was never greater than 3% of the specific activity of the loading medium. Furthermore the apparent influx per unit mass of tissue, ϕ_{in}, which is obtained from the slope of the graph [see section (*b*) of the

Appendix] was independent of the degree of loading of the tissue but depended only on the age of the tissue after excision. Values of ϕ_{in} obtained for tissues of various ages and in various stages of loading are shown in Figure 4.

(ii) *Elution Experiments*

Elution experiments as described for recently excised tissue in Section III(*b*) were repeated for root tissue that had previously been excised and stored in aerated medium for various aging periods (A) before loading. As before it was found possible to make a satisfactory compartmental separation provided that the slope of the long-term compartment was taken in each case about 15 hr after elution commenced. Values of Y_S/s_o, Y_L/s_o, k_S, and k_L are given in the lower part of Table 1. It will be noted that the only major effect of aging on these observations is in the value of Y_L/s_o, i.e. accumulation of isotope in the long-term compartment falls progressively with age. This fall is consistent with the steady fall in apparent influx (Fig. 4). The

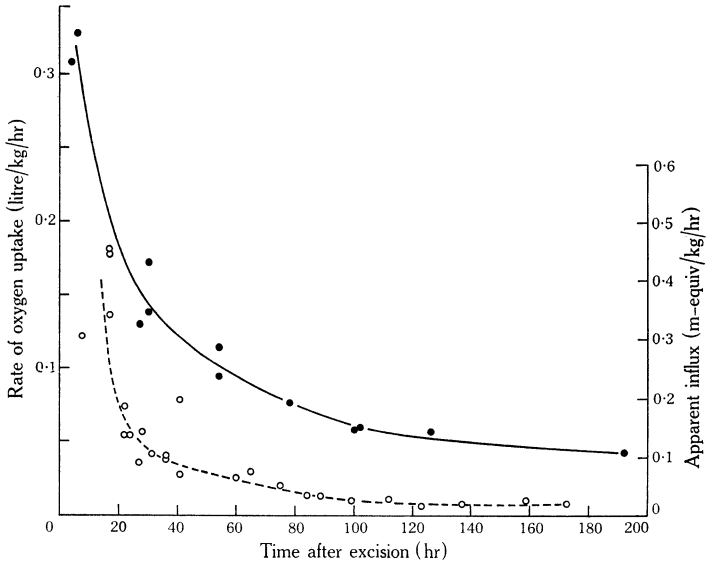

Fig. 4.—Time course of apparent influx ϕ_{in} (see text) of ^{42}K (○) and rate of oxygen uptake (●) by cortical root tissue at various times after excision.

values of Q_c, Q_v, ϕ_{oc}, and ϕ_{vc} calculated on the assumption that steady-state conditions apply are all found to fall as the tissue ages. In particular the value of $Q(= Q_v+Q_c)$ becomes much less than the steady value of 78·5 m-equiv/kg during this period of aging which is found by chemical analysis.

The discrepancy is so large that it clearly demonstrates that the tissue cannot be regarded as being in the steady-state condition that the method of analysis requires. It is therefore necessary to consider to what extent the calculations are invalidated under non-steady conditions.

One possible explanation for the discrepancy is that it is due to an undetected net flux of potassium in the tissue. This possibility can be tested using the analysis described in section (*b*) of the Appendix, in which the effects of changing fluxes are

also taken into account. Typical samples from Table 1 have been re-examined by these methods in Table 2. The analyses 1 and 2 differ because different estimates of ϕ_{in} have been used. In analysis 1, ϕ_{in} is estimated for each sample during the loading period (i.e. $\phi_{in} = Y_L/s_oT$), whereas in analysis 2 ϕ_{in} is taken from the uptake data in Figure 4 at a time 4 hr after elution commenced which more nearly corresponds to the time at which the other quantities are estimated [see discussion in section (*b*) of the Appendix].

If flux equality is assumed (i.e. if ϕ is put equal to zero in the values listed in Table 2) it will be noted that there is reasonable agreement between these estimates and those in Table 1 despite the approximations and assumptions that have been used. If there is a net influx, ϕ_{vc}, Q_c and Q_v would have been overestimated by the

TABLE 2

DATA FOR THREE EXPERIMENTS ANALYSED BY THE METHODS OF SECTION (*b*) OF THE APPENDIX

A (hr)	ϕ_{oc} (m-equiv/kg/hr)	ϕ_{vc} (m-equiv/kg/hr)	Q_c (m-equiv/kg)	Q_v (m-equiv/kg)	ϕ^* (m-equiv/kg/hr)
			Analysis 1		
13	0·21	2·31–12·2ϕ	9·9–47ϕ	39–207ϕ	−0·12
37	0·08	0·55– 7·5ϕ	2·4–28ϕ	25–340ϕ	−0·14
85	0·05	0·10– 3·1ϕ	0·4– 8·9ϕ	14–434ϕ	−0·15
			Analysis 2		
13	0·17	1·43– 9·5ϕ	6·2–37ϕ	32–217ϕ	−0·12
37	0·09	0·65– 8·2ϕ	2·7–30ϕ	26–338ϕ	−0·13
85	0·04	0·07– 2·6ϕ	0·3– 7·4ϕ	11–437ϕ	−0·15

* Value of ϕ necessary to make $Q_c+Q_v = 78·5$ m-equiv/kg.

methods of section (*a*) of the Appendix. Values of ϕ necessary to make Q equal to 78·5 m-equiv/kg are included in Table 2. It will be seen that net effluxes within the range 0·12–0·15 m-equiv/kg/hr would be required. The possibility of this will be considered in Section IV.

(*d*) *Oxygen Uptake by Root Tissue*

Figure 4 also shows the rapid decline in rate of oxygen uptake by root cortical tissue segments after excision. The fall is most marked in recently excised tissue. It will be observed that the fall in oxygen consumption is closely correlated with ϕ_{in}.

(*e*) *Potential Differences across Cellular Membranes*

Measurements of the electric potential relative to the normal bathing medium were made with microelectrodes inserted into individual cells of cortical tissue segments at 23°C. The internal potential had a value of $-138\pm1·4$ mV 8 hr after excision. During the next 16 hr the magnitude of the potential was observed to fall but after the first day it had reached a steady level of $-112\pm1·4$ mV which was maintained for at least the next 4 days. It was not possible to measure separately the potential differences across the outer and inner cytoplasmic boundaries.

IV. Discussion

Excised tissue segments were used in the experiments described in this paper in an endeavour to avoid the complexities of analysis of ion movements in the cells of growing broad bean roots. The results have instead indicated complexities of a different kind, associated with the aging of excised tissue. The marked fall in oxygen consumption (Fig. 4) is a clear indication that the tissue is far from being in a steady-state condition, in at least this aspect, despite its near-constant ionic content (Fig. 1). Reduced oxygen uptake could have arisen through the death of cells, but this would have led to loss of potassium and sodium by the tissue and this was not observed in the first 100 hr of aging. No attempts were made to maintain the metabolic activity of the tissue by the addition of sugar to the medium, as was done in recent experiments by Lüttge and Laties (1967), as it was felt that this might enhance bacterial contamination. Some experiments were performed at 2°C in an endeavour to slow down the aging process, but these were discontinued as the tissue did not appear to be in a state of flux equality under these conditions.

In contrast to the constant ionic content found by chemical analysis, the experiments using ^{42}K show an apparent fall in the potassium content of the compartments and in the fluxes between compartments during the aging period. While some of the potassium detected by this means might have been bound in sites not accessible to isotope exchange, this could not account for the much lower tracer estimate of Q nor for its decrease during aging. Estimates of Q by the two methods could be reconciled had there been a net efflux from the tissue of 0·12–0·15 m-equiv/kg/hr during the whole period covered by the elution experiments [from 15–96 hr after excision—Section III(*c*)]. While this net flux is small, its presence would have been detected in chemical analysis of the external solution, or of the tissue whose potassium content would have decreased by at least 10 m-equiv/kg during this period. An assumption made in the theoretical analysis is that specific activity is uniform throughout each compartment. This assumption would be invalid if non-mixing or slow diffusion within a compartment led to the specific activity adjacent to a boundary being appreciably different from its value in the compartment as a whole. Under these circumstances, values of flux and ion content calculated by the methods given in the Appendix would underestimate the true values. However, it is unlikely that this effect (due to an unstirred boundary layer) would become more marked as the tissue ages. It is more likely to be a factor in recently excised, rapidly exchanging tissue. A further possible source of discrepancy arises from the somewhat arbitrary choice of a time in the range 12–15 hr after elution commenced to give the slope of the long-term component (i.e. k_L). A time in this range was chosen because it gave the most satisfactory separations of two more rapid exponential components. However, the effect of choosing other times for the estimation of k_L was examined in a typical case (Table 3). It will be seen that the estimates of ion content are greater if later times are chosen (although no reasonable choice would bring them into line with the values obtained by flame-photometry). It is important to note, however, that the choice of time has little effect on the estimates of flux. This can also be seen from the approximate formulae in section (*b*) of the Appendix since the product $k_S\ Y_S$ is almost unaffected by the choice of k_L.

It is therefore concluded that the apparent fall in ion fluxes in tissue is a real effect and not an artifact due to an oversimplified model. The fall in flux is closely correlated with the decrease in respiration.

It would not seem unreasonable to expect a general fall in membrane permeability (and hence in the value of ϕ_{in}) as metabolic activity decreases. However, it would be erroneous to conclude that the observations indicate that the fluxes are falling in a similar manner in all cells in the tissue segments, for in this case there would be no decrease in the estimates of Q_c or Q_v. If the permeability of the cellular membranes of *all* cells were decreasing with time, the observed rate constants k_S and k_L in elution experiments would be less in aged tissue, and this is not observed (Table 1). These rate constants can only remain unchanged if the cells in aged tissue that are losing isotope during elution have membrane fluxes similar to those for cells in fresher tissue. The difference between the aged and fresh samples appears to be that fewer cells are accessible to isotope exchange in the aged tissue. This would account for the falling value of ϕ_{in} estimated for the bulk tissue.

TABLE 3

EFFECT OF CHOOSING DIFFERENT TIMES FOR THE ESTIMATION OF k_L IN A TYPICAL CASE

$A = 24$ hr, $T = 5{\cdot}8$ hr

Time of Analysis* (hr)	Y_S/s_o (m-equiv/kg)	Y_L/s_o (m-equiv/kg)	k_S (hr^{-1})	$10^3 k_L$ (hr^{-1})	ϕ_{oc} (m-equiv/kg/hr)	ϕ_{vc} (m-equiv/kg/hr)	Q_c (m-equiv/kg)	Q_v (m-equiv/kg)	ϕ_{oc}/ϕ_{vc}
6·5	0·051	0·54	0·64	4·73	0·13	0·35	0·7	19·5	2·69
15	0·055	0·53	0·46	3·18	0·12	0·38	1·1	28·5	3·17
25	0·066	0·52	0·41	2·35	0·12	0·37	1·2	37·6	3·08

* Indicates choice of time after elution commenced to give the slope of the long-term component (i.e. k_L).

The following model is therefore postulated. It exhibits characteristics similar to those of bean root tissue that has been excised and stored as described. Each cell in a tissue segment is assumed to be in one of two states. In recently excised tissue, most cells are exchanging potassium freely with the environment. After a period of aging, the cells switch to a state for which potassium exchange virtually ceases. The time at which this switch occurs varies from cell to cell, and the fraction still exchanging after various periods of aging can be found from the calculated values of Q (Table 1). These values of Q indicate the ion content of the exchanging cells per kilogram of tissue. For example, after 100 hr of aging only about 7% are still exchanging, but each of these cells is exchanging at about the same rate (seen from the rate constants) as the much more numerous exchanging cells in a freshly excised sample. In the real biological situation, cells may not switch suddenly from one state to the other as postulated in this model, and some more gradual fall to a state of low potassium permeability at a rate which varies markedly from cell to cell might equally well fit the observations.

A model of this kind accounts for the decreasing slope of the uptake graph (i.e. ϕ_{in}), without corresponding falls in the early slopes of the semi-logarithmic elution graphs (i.e. in the values of k_S and k_L). The persistent curving of the elution graph would be due to cells which had accumulated isotope during loading subsequently ceasing to exchange with the external medium later during elution, and hence retaining their isotopic content.

Since the calculated bulk tissue values of both Q_c and Q_v associated with exchanging cells fall almost proportionally as the tissue ages, the permeability change can be assumed to take place at the plasmalemma.

It should be pointed out that there is little independent confirmation of this model. No differences indicative of two cell types could be found through visual inspection, or in membrane potential measurements. There was some indication that cells on the outer surface of the tissue may cease exchanging first. This possibility was suggested in a few experiments in which it was found that ^{42}K uptake fell off more rapidly in tissue from thin roots than from thick roots which had a smaller surface area per unit mass. As a further test of this possibility, tissue was loaded with ^{42}K for 8 hr, after which thin longitudinal slices were removed from the outer surfaces containing cells from only the outer few cortical layers. It was found that ^{42}K concentration in these cells was $31 \pm 7\%$ less than in the remaining tissue, which would be consistent with the suggestion that fewer of these cells had accumulated isotope.

Much attention has been given by Laties and co-workers to effects of aging of storage tissue. Tissue that has been excised and aged in 0·5 mM $CaSO_4$ before being transferred to a KCl solution has a higher respiration rate than fresh tissue and an enhanced capacity to absorb K^+ and Cl^- (Laties 1967; Osmond and Laties 1968). A recent study on excised root tissue (Lüttge and Laties 1967) has indicated similar ion-absorption properties for isolated stele, and to a lesser extent for isolated root cortex, although these results for cortical tissue in the two experiments performed were not clear cut.

The differences between these results and those reported in this paper may be attributed to the difference in treatment of the tissue during aging. In the present experiments, the tissue was not deprived of potassium during aging, the same medium being used throughout for growth, aging, and experimental treatment. The results of Osmond and Laties (1968) suggest that the enhanced uptake of ^{42}K by aged beet tissue is severely reduced by pretreatment with solutions containing potassium. Furthermore, Lüttge and Laties (1967) added sucrose to the aging medium for root tissue. Its absence in the present experiments may account for the fall in respiration rate and in bulk fluxes.*

* *Note added in proof.*—A possible explanation for the effects of aging of tissue on the uptake and elution of ^{42}K described in Section III may be salt accumulation by the formation of minivacuoles from the endoplasmic reticulum (MacRobbie, *Abh. dt. Akad. Wiss. Berl.*, **4a**, 179–86, 1965). If potassium influx were linked to the formation of minivacuoles, the results shown in Table 1 and Figure 4 would suggest that the activity of this process falls markedly with aging of the tissue, while the rate constant for ^{42}K elution remains unchanged. Preliminary computer analyses based on such a model have simulated ^{42}K uptake and elution resembling those obtained experimentally. The possibility that potassium influx in bean root cells is a one-way process of salt accumulation in minivacuoles, whereas potassium leakage is by passive diffusion, will be given further consideration.

Values of the potential inside the cell can be used to calculate the ratio of the passive inward and outward fluxes of potassium, using the familiar Ussing–Teorell equation

$$\phi_{out}/\phi_{in} = a_i[\exp(zFE/RT)]/a_o,$$

where a_o and a_i are the external and internal activities of potassium. Difficulties in applying this equation to the available data are discussed in Scott, Gulline, and Pallaghy (1968). In the present experiments net fluxes of water were very small: 24–120 hr after excision, when the internal potential is −112 mV and the value of a_i is assumed to be 78 mM, a flux ratio of 1·05 is calculated, which is in good agreement with the observed state of flux equality; 8 hr after excision, the potential is −138 mV and a_i is 74 mM. The calculated flux ratio is now 3·1. Since the net inward flux at this time is about 0·6 m-equiv/kg/hr [Section III(*a*)], the separate influx and efflux would be 0·9 and 0·3 m-equiv/kg/hr respectively. These values do not appear to be out of line with tracer estimates of apparent influx and efflux. It is therefore concluded that passive fluxes can account for a major part of the potassium movement, and any active transport of potassium would appear to make no more than a minor contribution. A similar conclusion was reached for vacuolated cells of growing roots (Scott, Gulline, and Pallaghy 1968). Pitman and Saddler (1967) find that there is a fall in potassium influx in barley roots in the presence of the inhibitor carbonyl cyanide 3-chlorophenylhydrazone (CCCP), and this observation may indicate an inward pump. It is necessary to check that the inhibitor has not reduced membrane potential, which would also have the effect of reducing influx. Pallaghy (unpublished data) found that 10^{-4}M azide causes a fall of about 9 mV in the magnitude of the potential in bean root cells in the presence of divalent cations.

The mean value of Q in recently excised tissue is 81 m-equiv/kg (upper part of Table 1) which is in good agreement with the estimate from chemical analysis (78·5 m-equiv/kg). The later large variation could have arisen because of two counteracting factors: (1) aging which reduces the estimate, and (2) some net influx during the loading period which leads to an enhanced estimate. Uptake of potassium has been observed in the first 12 hr after excision [Section III(*a*)].

In all cases the tonoplast flux is much greater than the plasmalemma flux, the ratio being about 12 in recently excised tissue and falling gradually with aging. The finding that the plasmalemma is the main barrier to potassium movement in cortical cells in a medium 1 mM with respect to KCl is not inconsistent with the few observations obtained on root cells by other workers. Lüttge and Laties (1967) also find larger fluxes at the tonoplast in maize root cortex in 0·2 mM KCl at 0·5°C. At 2·5 mM K^+ at 5°C, the fluxes across the two membranes of barley cells are comparable (Pitman and Saddler 1967), whereas in a medium containing 10 mM K^+ at 25°C, Etherton (1967) finds the flux at the plasmalemma in pea root cells to be five times greater than at the tonoplast. The change to a rate-limiting tonoplast at high external concentrations (system 2) is well known (Torii and Laties 1966).

The results indicate that potassium fluxes in excised tissue (even when non-exchanging cells are allowed for) are much less than in mature cells of growing roots. In these cells the apparent efflux (which approximates to ϕ_{co} since the plasmalemma is the main barrier) is about 2·5 m-equiv/kg/hr (Scott, Gulline, and Pallaghy 1968).

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Appendix

Analysis of the three-compartment system

A system in which an isotope tracer is used for ion kinetic analysis is illustrated in Figure 5. The system is divided into three compartments in series O, C, and V by

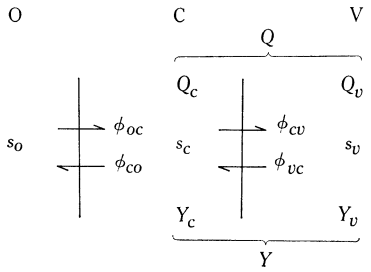

Fig. 5.—The three-compartment system analysed in the Appendix.

two membranes. The symbols Y, Q, and $s = Y/Q$ refer respectively to the amount of isotope, the total amount of ion, and the specific activity in each compartment, and ϕ is the flux through each membrane (not flux density). Uniform specific activity throughout each compartment is assumed. Under these conditions

$$\mathrm{d}Y_c/\mathrm{d}t = \phi_{oc}s_o+\phi_{vc}s_v-(\phi_{co}+\phi_{cv})s_c. \tag{1}$$

$$\mathrm{d}Y/\mathrm{d}t = \phi_{oc}s_o-\phi_{co}s_c. \tag{2}$$

Provided that s_o and the fluxes are not functions of time, Y_c, s_c, and s_v can be eliminated from these equations, leading to the following differential equation for Y, the total amount of isotope in compartments C and V:

$$\frac{\mathrm{d}^2Y}{\mathrm{d}t^2}+\frac{\mathrm{d}Y}{\mathrm{d}t}\left[\frac{\phi_{oc}+\phi_{vc}}{Q_c}+\frac{\phi_{vc}}{Q_v}\right]+Y\frac{\phi_{co}\phi_{vc}}{Q_cQ_v}=\phi_{oc}s_o\left[\frac{\phi_{oc}-\phi_{co}+\phi_{vc}}{Q_c}+\frac{\phi_{vc}}{Q_v}\right]. \tag{3}$$

(a) *Flux Equality*

Equation (3) can be solved explicitly only if there is flux equality, i.e. $\phi_{oc} = \phi_{co}$ and $\phi_{vc} = \phi_{cv}$. Under these conditions Q_c and Q_v are not functions of time and

$$Y = Y_S \exp(-k_S t) + Y_L \exp(-k_L t) + Y_o,$$

where

$$k_S = \left(\frac{\phi_{oc}+\phi_{vc}}{2Q_c} + \frac{\phi_{vc}}{2Q_v}\right) + \left[\left(\frac{\phi_{oc}+\phi_{vc}}{2Q_c} + \frac{\phi_{vc}}{2Q_v}\right)^2 - \frac{\phi_{oc}\phi_{vc}}{Q_c Q_v}\right]^{\frac{1}{2}},$$

$$k_L = \left(\frac{\phi_{oc}+\phi_{vc}}{2Q_c} + \frac{\phi_{vc}}{2Q_v}\right) - \left[\left(\frac{\phi_{oc}+\phi_{vc}}{2Q_c} + \frac{\phi_{vc}}{2Q_v}\right)^2 - \frac{\phi_{oc}\phi_{vc}}{Q_c Q_v}\right]^{\frac{1}{2}},$$

and Y_S, Y_L, and Y_o are dependent on the initial or final conditions. Thus during loading, $Y = 0$ and $\mathrm{d}Y/\mathrm{d}t = \phi_{oc} s_o$ at $t = 0$, and $Y = s_o Q$ at $t = \infty$. The equation for Y then becomes

$$Y = \frac{s_o}{k_S - k_L}\left\{(\phi_{oc} - k_L Q)[1-\exp(-k_S t)] + (k_S Q - \phi_{oc})[1-\exp(-k_L t)]\right\}.$$

After loading for a time T suppose s_o is made zero (time being now measured from this instant). The equation for elution becomes

$$Y = Y_S \exp(-k_S t) + Y_L \exp(-k_L t),$$

where

$$Y_S = \frac{s_o}{k_S - k_L}(\phi_{oc} - k_L Q)[1-\exp(-k_S T)],$$

and

$$Y_L = \frac{s_o}{k_S - k_L}(k_S Q - \phi_{oc})[1-\exp(-k_L T)].$$

In an elution experiment the data can frequently be separated into a long-term component and a short-term component by semi-logarithmic plotting, and k_S, k_L, Y_S, and Y_L can be obtained directly from the slopes, and intercepts with the ordinate.

The unknowns ϕ_{oc}, ϕ_{vc}, Q, Q_c, and Q_v ($= Q - Q_c$) can now be expressed without approximation in terms of these observed quantities and s_o and T.

Writing $Y_S/[1-\exp(-k_S T)] = S$ and $Y_L/[1-\exp(-k_L T)] = L$, it is readily shown that

$$\phi_{oc} = (1/s_o)(k_S S + k_L L),$$

$$Q = (1/s_o)(S + L),$$

$$Q_c = \frac{1}{s_o}\left[\frac{(k_S S + k_L L)^2}{k_S^2 S + k_L^2 L}\right],$$

$$\phi_{vc} = \frac{k_S k_L}{s_o}\left[SL(k_S S + k_L L)\right]\left[\frac{(k_S - k_L)^2}{(k_S^2 S + k_L^2 L)^2}\right].$$

(b) Non-steady-state Conditions

If there is a net flux through the membranes or if the fluxes change with time no general solution of the differential equation for Y can be obtained. An approximate solution can be found by treating separately the long-term and short-term components of Y. During the transient changes which result from an abrupt change in s_o, it is reasonable to assume that s_v remains constant, and the fluxes also, unless they are changing very rapidly with time. Except during these transient phases, the specific activity of the middle compartment will assume a quasi-steady value for which $\mathrm{d}Y_c/\mathrm{d}t \simeq 0$, i.e. from equation (1)

$$s_c \simeq (\phi_{oc}s_o+\phi_{vc}s_v)/(\phi_{co}+\phi_{cv}). \tag{4}$$

Under these quasi-steady conditions equation (2) gives the rate of change of the total activity in compartments C and V

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = \phi_{oc}s_o-\phi_{co}\left[\frac{\phi_{oc}s_o+\phi_{vc}s_v}{\phi_{co}+\phi_{cv}}\right],$$

$$= \phi_{\mathrm{in}}s_o-\phi_{\mathrm{out}}s_v, \tag{5}$$

where

$$\phi_{\mathrm{in}} = \phi_{oc}\phi_{cv}/(\phi_{co}+\phi_{cv})$$

and

$$\phi_{\mathrm{out}} = \phi_{co}\phi_{vc}/(\phi_{co}+\phi_{cv})$$

are frequently referred to as the apparent influx and apparent efflux respectively, the first being dominant during loading when $s_o \gg s_v$ and the second during elution when $s_o = 0$. At the commencement of elution s_o is made zero. Equation (1) now becomes

$$\mathrm{d}Y_c\Big/\left[Y_c-\frac{Q_c\phi_{vc}s_v}{\phi_{co}+\phi_{cv}}\right] = -\left[\frac{\phi_{co}+\phi_{cv}}{Q_c}\right]\mathrm{d}t = -k_S\,\mathrm{d}t,$$

where

$$k_S = (\phi_{co}+\phi_{cv})/Q_c. \tag{6}$$

This equation can be solved provided Q_c is constant. This requires that the same net flux ϕ passes through each membrane, i.e.

$$\phi = \phi_{oc}-\phi_{co} = \phi_{cv}-\phi_{vc}.$$

The solution of the equation, subject to condition (4) at the start of elution, is

$$Y_c = Q_c[\phi_{vc}s_v+\phi_{oc}s_o\exp(-k_St)]/(\phi_{co}+\phi_{cv}),$$

and hence

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = -\phi_{\mathrm{out}}s_v-\frac{\phi_{oc}\phi_{co}s_o}{\phi_{co}+\phi_{cv}}\exp(-k_St),$$

since $\mathrm{d}Y/\mathrm{d}t = -\phi_{co}s_c$ during elution.

Integrating the second term over a time long in comparison with $(1/k_S)$ gives Y_S, the short-term component of Y, i.e.

$$Y_S = \frac{s_o}{k_S}\left[\frac{\phi_{co}\phi_{oc}}{\phi_{co}+\phi_{cv}}\right] = \frac{\phi_{co}\phi_{oc}s_oQ_c}{(\phi_{co}+\phi_{cv})^2}. \tag{7}$$

After the initial transient $\mathrm{d}Y/\mathrm{d}t = -\phi_{\mathrm{out}}s_v$. Hence

$$k_L = -\frac{\mathrm{d}}{\mathrm{d}t}(\ln Y) = -\frac{1}{Y}\frac{\mathrm{d}Y}{\mathrm{d}t} = \frac{\phi_{\mathrm{out}}s_v}{s_cQ_c+s_vQ_v}$$

$$= \phi_{co}\phi_{vc}/[(\phi_{co}+\phi_{cv})Q-\phi_{oc}Q_c], \tag{8}$$

since the quasi-steady value of s_c during elution is $\phi_{vc}s_v/(\phi_{co}+\phi_{cv})$. The apparent influx

$$\phi_{\mathrm{in}} = \phi_{oc}\phi_{cv}/(\phi_{oc}+\phi_{cv}) \tag{9}$$

can be estimated either (1) from the long-term component of Y (i.e. $Y_L = \phi_{\mathrm{in}}s_oT$), or (2) from the slope of an uptake graph (such as Fig. 3). If the fluxes are changing with time, the usual method (1) may be unsatisfactory since Y_L gives the mean value of ϕ_{in} during the loading period, whereas the other quantities are estimated during elution. It may be more satisfactory to use method (2), taking the slope at a time more closely corresponding to the time at which the other quantities are estimated.

Equations (6), (7), (8), and (9) and the expression for ϕ_{in} can now be rearranged so that the unknowns ϕ_{oc}, ϕ_{vc}, Q_c, and Q are expressed in terms of the observable quantities s_o, k_S, k_L, Y_S, ϕ_{in}, and the net influx ϕ:

$$\phi_{oc} = (k_SY_S/s_o)+\phi_{\mathrm{in}},$$

$$\phi_{vc} = (1+\alpha)(\phi_{\mathrm{in}}-\phi),$$

$$Q_c = (1/k_S)(1+\alpha)[(k_SY_S/s_o)+\phi_{\mathrm{in}}-\phi],$$

$$Q = (1/k_L)(\phi_{\mathrm{in}}-\phi)+(Y_S/s_o)(1+\alpha),$$

where

$$\alpha = s_o\phi_{\mathrm{in}}/k_SY_S.$$

ϕ_{co} and ϕ_{cv} can of course be obtained from ϕ_{oc}, ϕ_{vc}, and ϕ. Note that these estimates are essentially the same as those employed by Pitman (1963) and Lüttge and Laties (1967), except that Q (the ion content of the non-free-space compartments) is estimated without resort to chemical analysis, and the possibility of the fluxes changing with time is taken into account, at least to some extent.