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INFLUX THEORY AND SIZE OF POTASSIUM AND RUBIDIUM POOLS IN THE MIDGUT OF *HYALOPHORA CECROPIA*

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SUMMARY

1. The midgut contains a 'pool' which must mix with tracer added to the blood-side, before the transported potassium reaches a steady specific activity. The size and mixing time of the pool can be deduced from the time-course of rate of appearance of tracer on the lumen-side, the tracer influx. The theory has been extended to cover cases where the pool-size and the transport rate vary with time.

2. By using ^{42}K and ^{86}Rb together, it is shown that the midgut treats potassium and rubidium differently, rubidium being transported nine-tenths as fast as potassium, when both are present in equal concentration. The influx pool for potassium is one-third larger than that for rubidium, but mixes four-fifths as fast with blood-side tracer.

3. The size of the potassium pool decreases with time, but that for rubidium does not, and the ratios of mixing times and transport rates are constant.

4. The implications of the results are discussed, both in terms of the accuracy of previous investigations and with respect to the probable intracellular location of part of the influx pool.

INTRODUCTION

Proper understanding of transepithelial active transport requires that the pathway followed by the substance during transport be known. In general, this pathway will contain at least one compartment or 'pool', within which the material undergoing transport becomes mixed. Clearly, the size of such a 'pool' will be an important clue to its possible location. For example, in the frog skin (Koefoed-Johnsen & Ussing, 1958; Ussing & Windhager, 1964), if the pool in the pathway for sodium transport is intracellular, then its size will depend on the intracellular sodium concentration. If on the other hand the transport pathway is extracellular, as suggested by Cereijido & Rotunno (1968; see also Zerahn, 1969), then the pool must also be extracellular, and its size will depend on the 'pond-side' external sodium concentration. Generally speaking, extracellular pools are expected to show different kinetics than intracellular ones (Harvey & Zerahn, 1969).

The effective size and mixing time of a pool can be determined from the time-course of movement of a radioactive tracer across the epithelium (Wood & Harvey, 1975). If label is added at a known time to the input side, a delay will ensue while

the label becomes mixed with the pool, before the specific activity of the transported material attains a steady value ('tracer steady-state'). The time-course of appearance of the label on the output side will thus indicate the size of the pool, provided the rate of transport is known. A pool determined in this way will be referred to as a kinetic pool. The theory and method for measurement of a kinetic *influx* pool is developed here, 'influx' in this context referring to the flux from blood-side to lumen side, J_{BL}, which is the larger, 'active' unidirectional flux. With minor changes the theory and method can be used to deduce the size of an *efflux* pool as well. No assumptions are required about the structure of the epithelium, so that the information can be used freely to construct models (see also Wood, 1972; Harvey & Wood, 1972, 1973).

The theory of Wood & Harvey (1975) is subject to a number of limitations. In particular, it requires that the tissue be in a steady-state, so that the rate of transport and the size of the pool do not change appreciably during the period of the measurements, and that the transport rate be measurable, independently of the tracer kinetics. The latter requirement was met in the original application by using the short-circuit current as a measure of net ion transport, and correcting for the passive efflux on the assumption that it was constant. In the present paper it will be shown that the theory can be generalized to allow for variation in the transport rate, provided that an empirical equation can be found for its time-course. An attempt was also made to circumvent the use of short-circuit current as a measure of transport rate, with its associated conditions on the passive efflux, by using a dual-tracer technique with potassium and rubidium. Although this attempt was not successful, it has provided some interesting information on the differences between potassium and rubidium, with respect to the ability of the midgut to transport them, and to the relative sizes of the influx 'pools' for the two ion-species.

THEORY

As was shown by Wood & Harvey (1975), the size of the pool which must become labelled before a 'tracer steady-state' (see Introduction) is attained is given by the area between the curves representing the time-course of the steady-state influx, J_i^s, and the time-course of labelling the transported material, J_i^t (see Fig. 1). It is found that, for the midgut, the time-course of the influx can be represented by a simple exponential decay (Wood & Moreton, 1978):

$$J_i^s = J_i^0 \exp(-\beta t), \tag{1}$$

and that labelling of the transport pool also follows an exponential time-course, so that the tracer-measured influx varies according to:

$$J_i^t = J_i^0 [1 - \exp(-\alpha t)] \exp(-\beta t). \tag{2}$$

The size of the influx pool, P_i, is found by integrating the difference between the steady-state flux and the labelling of the flux, so that

$$P_i = \int_0^\infty \{J_i^0 \exp(-\beta t) - J_i^0 [1 - \exp(-\alpha t)] \exp(-\beta t)\} dt$$

$$= \frac{J_i^0}{\alpha + \beta}.$$

Here J_i is the influx with superscripts referring to time, 'o' being the time of addition of tracer and 's' being the time when the tracer steady-state is attained. α and β are the time constants for tracer mixing and transport decay, respectively.

Clearly, if two isotopes are available which can be distinguished by the experimenter, but not by the tissue, then J_i^o and β can be measured by adding the first isotope, allowing it to reach a tracer steady-state, and following the time-course of its influx. The second isotope is then added, and its influx followed until it, too, reaches a tracer steady-state. By subtracting the influx as indicated by the second isotope from that given by the first (with due allowance for their relative specific activities on the input side of the epithelium), and plotting the result semi-logarithmically against time, the mixing time-constant, α, can be calculated from the slope, and the pool size, P_i, deduced.

For the midgut (Wood & Harvey, 1975) a complication arises in that exponential labelling of the transport pool begins not immediately, but after a delay, so that the label in the pool varies according to $\exp(-\alpha t+\gamma)$ instead of $\exp(-\alpha t)$. The integral in eqn. (3) thus becomes

$$P_i = \frac{J_i^o \exp \gamma}{\alpha+\beta} \tag{4}$$

and the semi-logarithmic plot of the difference between the two isotope fluxes will intersect the line $J = 0$ at a time $t = \gamma/\alpha$, different from zero. However, this delay was considered by Wood & Harvey (1975) to be due to non-instantaneous mixing of tracer with the saline in the chamber. Its contribution to the apparent pool size is thus artifactual, the true size being given by eqn. (3), as has been assumed in the present investigation.

The above theory should be of general application in the study of epithelial transport. Unfortunately, it has been found in the present investigation that the two isotopes chosen, ^{42}K and ^{86}Rb, are not indistinguishable by the midgut. It was therefore necessary to rely on the short-circuit current, together with calculations of the passive efflux, which was assumed to be constant for a given preparation. The justification for this procedure has been discussed by Harvey & Wood (1975). Since under short-circuit conditions we know that the passive influx and efflux are equal, the active component of the influx is found by adding the efflux, J_e, to the net flux which is equivalent to the short-circuit current, I_{sc}/F:

$$J_i = \frac{I_{sc}}{F} + J_e. \tag{5}$$

(F is the Faraday.) Conversely, if J_i and I_{sc} are measured under 'tracer steady-state' conditions, J_e can be deduced. By taking it as constant, the efflux thus found can be added to I_{sc}/F to find values of J_i during the tracer equilibration period.

In the presence of two transported substances, one more piece of information is needed, namely the ratio of their active fluxes when both are present at the same concentration. Defining

$$p = \frac{J_{iRb}}{J_{iK}} \tag{6}$$

eqn. (5) may be re-written for each ion-species as

$$J_{iK}(1+p) = \frac{I_{sc}}{F} + J_{eK}, \tag{7}$$

$$J_{iRb}\left(1+\frac{1}{p}\right) = \frac{I_{sc}}{F} + J_{eRb}. \tag{8}$$

Provided that the effluxes are fairly small, the ratio p can be found with sufficient accuracy, by simply taking the ratio of total influxes of the two tracers, when both have reached 'tracer steady-states'. The effluxes can then be obtained from eqns. (7) and (8).

METHODS

Midguts were isolated from chilled, fifth-instar larvae of *Hyalophora cecropia* (L.) fed on synthetic diet (Riddiford, 1968). Each was mounted as a flat sheet in a chamber (Wood & Moreton, 1978), and bathed in a solution containing (mM) KCl, 16; RbCl, 16; $MgCl_2$, 5; $CaCl_2$, 5; Trizma base, 5; HCl, 1·5 (pH 8·3); and sucrose 166. The midguts were continuously short-circuited using a three-bridge system and a negative feedback device (Wood & Moreton, 1978), and the short-circuit current was monitored on an ammeter and recorded on a Servoscribe recorder. The short-circuit current decays on a double-exponential time-course, so measurements were delayed until after 90 min, by which time the fast component had effectively decayed away (see Wood & Moreton, (1978), fig. 5). ^{42}K and ^{86}Rb were obtained from New England Nuclear, Boston, Mass., and were added to the blood-side of the chamber in 5 μCi amounts. Samples 1 ml in volume were removed from the lumen-side at 2, 4 or 10 min intervals, and standards, 0·05 ml in volume, were taken from the blood-side. Radioactivity in the samples was counted by the Čerenkov effect in a liquid scintillation counter.

The samples were counted immediately, then again about 10 days later when essentially all of the ^{42}K had decayed. The ^{86}Rb counts remaining were corrected for decay back to the original counting period using a standard containing only ^{86}Rb, and subtracted from the original counts to give the counts due to ^{42}K. These in turn were corrected for decay during the initial counting period, and the corrected counts were used to calculate the influx as indicated by each of the two tracers. Calculations were performed on a Monroe Model 1785 programmable calculator.

The efflux of each ion-species was calculated from eqns. (7) and (8) above, using the determinations of influx and short-circuit current which were made after it was judged that a 'tracer steady-state' had been reached. The results were averaged to give mean efflux figures for each experiment.

The efflux values (Table 1) should be regarded with caution as being indirectly obtained. Furthermore, the present experiments were completed before the discovery that magnesium (Wood, Jungreis & Harvey, 1975) and calcium (Wood & Harvey, 1976) are actively transported from lumen- to blood-side of the midgut. This transport, by reducing the net short-circuit current by about 10%, will make the apparent effluxes slightly too large. However, neither magnesium nor calcium transport affect the time-course of the short-circuit current or the determination of pool sizes and mixing times because, unlike the potassium transport, they are time-independent.

Table 1. *Summary of data from three types of experiment, representing different orders of addition of the tracers. 'Initial' fluxes were obtained by extrapolation to the time of addition of the tracer, and 'apparent' effluxes were calculated from equations* (7) *and* (8). *Symbols are those used in the Theory section.*

Date (1974)	Wet wt* (mg cm^{-1})	I_{sc}^{205}/F Short-circuit current at 205 min (μ-equiv cm^{-2} h^{-1})	Rb-influx/K-influx (%)	J_{eK} Apparent K-efflux (μ-equiv cm^{-2} h^{-1})	J_{eRb} Apparent Rb-efflux (μ-equiv cm^{-2} h^{-1})	J_{iK}° Initial K-influx (μ-equiv cm^{-2} h^{-1})	β_K K-influx decay constant (h^{-1})	$(\gamma/\alpha)_K$ K-delay (min)	α_K K-mixing time-constant (h^{-1})	P_{iK} K-influx pool (μ-equiv cm^{-2})	J_{iRb}° Initial Rb-influx (μ-equiv cm^{-2} h^{-1})	β_{Rb} Rb-influx decay constant (h^{-1})	$(\gamma/\alpha)_{Rb}$ Rb-delay (min)	α_{Rb} Rb-mixing time-constant (h^{-1})	P_{iRb} Rb-influx pool (μequiv cm^{-2})	Rb-mixing time/K-mixing time (%)	Rb-pool/K-pool (%)	K-pool/wet wt (μ-equiv g^{-1})
A. ^{42}K at 90 min; ^{86}Rb at 150 min																		
20 Mar.	140	14·6	91	8·0	6·0	29·8	0·125	1·4	4·48	6·47	22·8	0·107	−2·0	5·60	4·00	125	62	46
10 Apr.	80	14·2	98	2·1	1·9	25·3	0·219	1·4	5·95	4·10	19·2	0·212	1·0	6·70	2·78	113	68	51
11 Apr.	62	19·5	85	2·7	−0·4	23·9	0·104	1·1	5·09	4·60	20·6	0·130	1·0	5·78	3·48	114	76	74
17 Apr.	52	14·7	91	3·7	2·2	22·5	0·104	0·9	6·16	3·59	18·5	0·128	0·6	7·45	2·44	211	68	69
24 Apr.	—	12·7	90	1·6	0·4	19·0	0·197	1·1	5·41	3·39	15·2	0·236	0·6	5·87	2·49	108	73	—
2 May	62	15·1	92	5·2	3·8	25·5	0·161	1·0	4·33	5·68	21·6	0·210	1·1	6·81	3·08	157	54	92
Mean			91													123	67	66
			±2													±7	±3	±8
B. ^{86}Rb at 90 min; ^{42}K at 150 min																		
8 May	80	9·3	100	1·7	1·7	13·1	0·243	−0·3	8·80	1·45	15·9	0·194	1·1	9·92	1·57	113	108	18
9 May	78	16·5	90	2·8	1·0	21·9	0·168	0·5	3·97	5·29	23·5	0·161	0·4	7·05	3·26	178	62	68
15 May	64	17·2	92	1·5	0·3	21·5	0·211	0·5	10·06	2·09	26·6	0·175	0·5	9·28	2·81	92	134	33
16 May	110	16·9	93	0·3	−0·8	19·7	0·200	0·5	8·17	2·35	20·7	0·167	0·7	8·27	2·45	101	104	21
Mean			94													121	102	35
			±2													±19	±15	±11
C. ^{42}K at 90 min; ^{86}Rb at 90 min																		
22 May	134	14·1	91	4·7	3·0	22·6	0·106	1·0	3·59	6·11	20·9	0·115	1·1	3·90	5·20	109	85	46
5 June	84	13·2	87	6·9	4·3	22·8	0·070	0·6	4·80	4·68	20·1	0·080	0·4	6·70	2·96	140	63	56
5 June (2)	96	14·8	92	3·6	2·2	30·0	0·276	1·0	5·95	4·82	28·5	0·295	1·2	7·35	3·73	124	77	50
6 June	108	15·2	88	4·1	1·8	26·3	0·177	0·5	4·42	5·72	24·0	0·197	0·6	6·38	3·65	144	64	53
7 June	66	16·3	89	4·4	2·1	28·3	0·179	1·1	6·50	4·24	26·0	0·197	1·0	7·24	3·50	111	82	64
Mean			89													126	74	54
			±1													±7	±4	±3
Combined mean	**87** ±7	**15·0** ±0·6	91 ±1	**3·6** ±0·5	**2·0** ±0·5			**1·0** ±0·2					**0·6** ±0·2			**123** ±6		

* Not corrected for adhering solution.

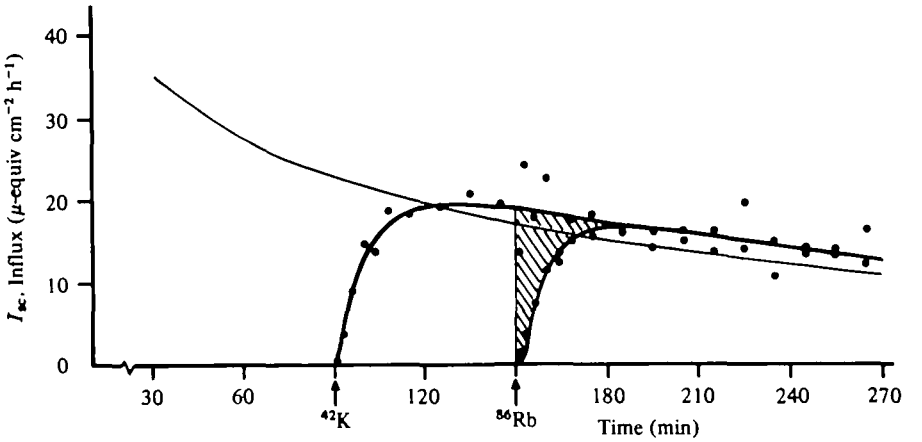

Fig. 1. A typical experiment (10 April 1974) showing the time-course of the short-circuit current (I_{sc}, thin curve), the time-course of the ^{42}K-measured K+Rb influx (○), and the time-course of the ^{86}Rb-measured K+Rb influx (●). The lines are least-square fits as projected by a computer. The size of the pool is given by the area (shaded) between the steady-state influx curve (^{42}K) and the labelled influx curve (^{86}Rb).

Steady-state values for the influx were deduced from the efflux and the measured short-circuit current (eqn. (5) above). Influx values deduced from the tracer measurements were subtracted from these, and the logarithm of the result plotted against time, with the origin at the time of addition of the tracer to the blood-side compartment (cf. fig. 2 of Wood & Harvey, 1975). Best-fit lines were found by inspection, since a least-squares fit requires that all the points be equally weighted. The slope was used to give the mixing time-constant for the pool (α), and the intercept on $J_t = 0$ to give the delay before mixing begins (γ/α).

The order of addition of the tracers to the blood-side was varied, to give separate information on the behaviour of potassium and rubidium in the midgut.

RESULTS

The results of a typical experiment are shown in Fig. 1. The time-course of the I_{sc} is shown by the thin line. ^{42}K was added to the blood-side compartment at time 90 min, and the time-course of the ^{42}K-measured influx is shown by the open circles, the points having been scaled according to eqn. (7) of the Theory section. ^{86}Rb was added to the blood-side compartment at time 150 min, and the time-course of the ^{86}Rb-measured influx (eqn. (8)) is shown by the closed circles.

Results from this and five other similar experiments are summarized in Table 1 A. The principal conclusions are that, in the presence of equal concentrations of the two ion-species, potassium is transported across the midgut slightly faster than rubidium, the mean ratio J_{iRb}/J_{iK} being $0{\cdot}91 \pm 0{\cdot}02$ (S.E.M.; $n = 6$); the influx pool for rubidium appears smaller than that for potassium ($P_{iRb}/P_{iK} = 0{\cdot}67 \pm 0{\cdot}03$), but equilibrates more rapidly with blood-side tracer ($\alpha_{Rb}/\alpha_K = 1{\cdot}23 \pm 0{\cdot}07$).

Since the short-circuit current decreases by *ca.* 20% over the period between 90 min (when the ^{42}K was added) and 150 min (when the ^{86}Rb was added), it is possible that these results arise solely from a decrease in the ion content of the tissu

over that period. To test this, the tracers were added in reverse order: the results of four experiments are summarized in Table 1 B. The discrepancy in transport rates remains the same ($J_{iRb}/J_{iK} = 0{\cdot}94 \pm 0{\cdot}02$), as does the ratio of mixing times ($\alpha_{Rb}/\alpha_K = 1{\cdot}21 \pm 0{\cdot}19$). The ratio of pool sizes ($P_{iRb}/P_{iK} = 1{\cdot}02 \pm 0{\cdot}15$), however, is now close to unity, instead of being reversed, which suggests a real difference in the pool sizes for the two ion-species.

These results were confirmed by adding the two tracers simultaneously (Table 1 C), and the difference between rubidium and potassium can be seen directly: $J_{iRb}/J_{iK} = 0{\cdot}89 \pm 0{\cdot}01$; $\alpha_{Rb}/\alpha_K = 1{\cdot}26 \pm 0{\cdot}07$; and $P_{iRb}/P_{iK} = 0{\cdot}74 \pm 0{\cdot}04$).

Of the relationships between the various parameters of the midgut, the following general statements may be made. The pool sizes measured at 90 min for potassium ($r = 0{\cdot}80$; $n = 10$) and rubidium ($r = 0{\cdot}57$; $n = 9$) correlate reasonably well with the wet weight of the tissue. The potassium pool at 90 min contains (60 ± 5) μequiv g^{-1} wet wt (S.E.M.; $n = 10$), decreasing to (35 ± 11) μequiv g^{-1} wet wt ($n = 4$) at 150 min. The rubidium pool under the same conditions is (36 ± 3) μequiv g^{-1} wt wt ($n = 9$) at 90 min, and (43 ± 5) μequiv g^{-1} wet wt ($n = 5$) at 150 min. The short-circuit current at 205 min was not significantly correlated with the weight, in contrast to a previous finding for the current at 60 min (Wood & Moreton, 1978). The time-course of the current from 90 to 205 min could be fitted to within 5% by a simple exponential decay (eqn. (1)).

The efflux figures for potassium and rubidium showed no correlation with the short-circuit current, but were strongly correlated with each other ($r = 0{\cdot}92$; $n = 15$).

Rubidium equilibrates more rapidly with the transport pool than does potassium, as expressed by the ratio $\alpha_{Rb}/\alpha_K = 1{\cdot}23 \pm 0{\cdot}06$ ($n = 15$). The mixing time-constants were not significantly correlated with the influx for either ion-species.

In the presence of equal concentrations of potassium and rubidium, the relative affinity of the transport system for the two ion-species is expressed by the overall mean of $J_{iRb}/J_{iK} = 0{\cdot}91 \pm 0{\cdot}01$ ($n = 15$).

DISCUSSION

The results show clearly that rubidium and potassium are not treated alike by the Cecropia midgut, so that the use of rubidium as a tracer for potassium is not justifiable, except for semi-quantitative work. The ratio $J_{iRb}/J_{iK} = 0{\cdot}91$ agrees with the previous figure for Cecropia (Nedergaard & Harvey, 1968), and is close to that of 0·93 found by Wood (1972) for the closely related species *Antheraea pernyi* (G-M).

The difference in size and equilibration time of the transport 'pools' for the two ion-species is a new finding. That the rubidium pool is both smaller and more rapidly equilibrated than that for potassium, suggests either that, under the present conditions the tissue contains more potassium than rubidium, or that blood-side potassium can exchange with some additional tissue compartment which is not accessible to rubidium. In the former case, one would also need to postulate a lower permeability of the blood-side barrier to potassium, to account for its slower equilibration with the pool. Also, if the relative concentrations of potassium and rubidium in the pool at time 90 min are in the ratio of the pool sizes, i.e. 60:36 (Table 1), then to account for the ratio of their active fluxes would require a pump with a somewhat higher affinity for rubidium than potassium.

A second compartment for potassium, on the other hand, would explain the data if it were more slowly exchanging than the non-specific compartment. Evidence for such a second slowly-exchanging compartment for potassium is given by Harvey & Wood (1972, their fig. 11) and by Zerahn (1975, his fig. 1, curve *b*). If potassium in the slowly-exchanging compartment were at the same time not directly available to the active transport mechanism, then the relatively high affinity of the system as a whole for rubidium would be explained. Of course, the time-course of labelling of a dual potassium pool would be expected to be complex: but it is doubtful whether the accuracy of the present data would be sufficient to show this.

A third possibility is that the pathways for potassium and rubidium across the epithelium are totally distinct, involving separate compartments and active transport mechanisms. The data could be accounted for very easily by such a system, but it is difficult to see why the midgut should possess a separate and powerful mechanism for transporting a completely foreign cation. Moreover competition between the transport of the alkali metal ions is well documented (Harvey & Zerahn, 1972).

Finally, recent work from this laboratory deserves mention. When a midgut is isolated from a larva fed on artificial diet and is short-circuited, as in the present study, there are two inputs to the potassium transport pool, one across the goblet cell basal membrane and another across the lateral membranes from a potassium pool in the columnar cells (Blankemeyer, 1976; Blankemeyer & Harvey, 1977, 1978). A lower permeability of these lateral membranes for potassium than for rubidium would account for the slower potassium mixing time.

Whatever model is favoured, it is clear that the transport pool for potassium decreases substantially in size during the course of an experiment. This decrease is presumably connected in some way with the progressive decay of the short-circuit current, the cause of which is not yet known. The pool-size for rubidium apparently remains much more constant, so that by time 150 min the two pools are almost identical in size. The discrepancy in equilibration times remains, however, and the ratio of active fluxes of the two ions is constant. Clearly the behaviour of the tissue is complex, and any attempt at explanation requires more detailed information on the total ion content under the present experimental conditions.

One obvious implication is that the assumption of Wood & Harvey (1975) that the pool-size remained constant, was not valid. The present results suggest that the pool-size declines by some 20% over the period of their measurements, so that their figure is likely to be in error by about half this amount. Bearing this in mind, the present finding of 60 μequiv g^{-1} wet wt agrees quite well with their value of 80·5 μequiv g^{-1} wet wt, for the potassium pool at time 90 min, confirming that the influx pool constitutes a substantial fraction of the total tissue potassium.

The influx pool, and therefore the transport pathway, for potassium across the midgut is thus almost certainly intracellular, which supports the model proposed by Harvey & Wood (1972), in which passive permeation through the basal cell membranes is followed by active transport across the apical membrane of the goblet cells, into the lumen. An intracellular location for the pool is further confirmed by the observation that it is similar in size to the intracellular, exchangeable potassium fraction demonstrated by Harvey & Wood (1973).

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