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The Comparative Transport of K^+ and Rb^+ in Normal and Malignant Rat Tissues *in vivo* and in Liver Slices, Diaphragm, and Tumor Slices *in vitro*

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Summary. The selectivity in the steady state uptakes of Rb^+ and K^+ has been studied in a number of normal and malignant rat tissues. The selectivity is minimal in erythrocytes and the two fastest-growing of four transplantable tumors, in which there is little discrimination between the two ions, and ranges upwards to a maximum Rb^+ uptake in liver. In each tissue, the selectivity is independent of Rb^+ concentration or of K^+ deficiency (except in skeletal muscle). In liver slices *in vitro*, reduction of energy metabolism by lowering the temperature or by the addition of metabolic inhibitors reduces the Rb^+:K^+ discrimination proportionately much more than K^+ transport. Diaphragm and slices of a transplantable tumor give similar results. With temperature reduction, there is a logarithmic relation between the Rb^+:K^+ discrimination ratio and the respiration rate of liver slices. The results are quantitatively accounted for by simultaneous diffusion and metabolically coupled transport across a homogeneous membrane in which Rb^+ transport is more closely coupled than that of K^+ to a metabolic flux across the membrane. There is evidence that the tissue differences in Rb^+:K^+ selectivity originate in the different levels of the coupling metabolic flux in different cell types and thus of the energy expenditure on ion transport. In contrast to the differences in steady state selectivity between Rb^+ and K^+, the initial ratio of uptakes of trace ^{43}K and ^{86}Rb, in otherwise steady state conditions, is close to unity in both liver and tumor slices, in agreement with theoretical calculations.

The interpretation of the selectivity shown by biological membranes toward the alkali metal cations (Diamond & Wright, 1969) has received a physical basis from the work of Eisenman (1962) on the ion-exchange properties of glass electrodes, and it has been possible to predict likely sequences in the relative uptakes of the ions; e.g., $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$ is such a sequence which occurs in a number of biological systems. Since ion transport in the cell membrane, unlike that in a glass membrane, is dependent on metabolic energy, the question arises of the interrelationship of selectivity and energy supply. The pair of ions, Rb^+ and K^+, is of interest

from this point of view since, in spite of their close physical and chemical similarity, considerable differences in their relative uptake have been reported in different mammalian tissues (Love, Romney & Burch, 1954; Kilpatrick, Renschler, Munro & Wilson, 1956; Mabille, Martin & Burg, 1961; Skul'skii & Burovina, 1966). The differences are proportionately greater than the differences in K content of the various tissues and can also be demonstrated *in vitro* (Müller, 1965), implying that the differences originate at the cellular level. In other systems, e.g., amphibian muscle (Sjodin, 1961), quantitative differences in the rates of Rb^+ and K^+ transport are well established. There has been no attempt, however, to explain the tissue differences in the relative uptakes of Rb^+ and K^+. The present work aims to study this problem and to extend previous work to certain pathological, particularly malignant, tissues.

In normal tissues, the highest Rb^+ concentrations are generally reported in liver, and the lowest (in the more readily K^+-exchanging tissues) in erythrocytes where both the transport properties and steady state distribution of Rb^+ are very nearly equal to those of K^+ (Love & Burch, 1953; Tyor & Eldridge, 1956). Since different investigators have used different doses of stable Rb^+, often of a similar order of magnitude to the normal whole body content, and the highest Rb^+ uptake has been reported in rat muscle in K-deficient conditions (Relman, Lambie, Burrows & Roy, 1957), possible effects of K-deficiency and of the Rb^+ dosage have been looked for in the range of normal tissues studied.

Identification of the mechanism of $Rb^+ : K^+$ discrimination needs detailed studies that are more readily carried out *in vitro*. In view of the high uptake by liver, this tissue has been chosen for a fuller *in vitro* study of $Rb^+ : K^+$ discrimination. Steady state ion distributions similar to those *in vivo* have been found, confirming that the discriminative processes originate in the liver cells themselves.

The effects of metabolic inhibition by physical means (i.e., at lower temperatures) and by chemical inhibitors have been studied. For comparison, similar experiments have also been carried out on a fast-growing transplantable rat tumor, showing a $Rb^+ : K^+$ discrimination ratio near unity, and on isolated diaphragm which has *in vivo* a $Rb^+ : K^+$ discrimination intermediate between liver and the tumor.

The main conclusion is that selectivity in the steady state cellular distributions of Rb^+ and K^+ is increased by an increasing level of energy metabolism (linked to ion transport), and there is evidence that the tissue differences originate in the same way. The results can be quantitatively described in terms of simultaneous diffusion and metabolically coupled

transport in a homogeneous membrane, in which Rb^+ transport is more closely coupled than K^+ to a metabolically dependent flux across the membrane.

Materials and Methods

The animals used were male brown-hooded rats of the "August" strain, of about 120-g body weight at the beginning of each experiment, and maintained normally on standard M.R.C. diet No. 41 B, with a K content of 4.8 g/Kg. Potassium deficiency, when required, was induced by feeding, for 2 weeks before ^{86}Rb administration and during dietary addition of the isotope, a synthetic diet based on casein, glucose and corn oil (Cotlove, Holliday, Schwartz & Wallace, 1951), but with K salts omitted. As made up, this diet had a K content of 4.4 mg/Kg. Protein deficiency was induced by feeding a similar diet but with glucose substituted for the casein.

The transplantable tumors were of four lines maintained in this Institute: a mammary tumor and a fibrosarcoma in "August" strain rats (tumors BICR/A1 and /A2, respectively) and a mammary tumor and a fibrosarcoma in "Marshall" strain rats (BICR/M1 and /M2, respectively). Cell kinetic and other data for earlier transplants of two of these tumors have been reported (Steel, Adams & Barrett, 1966). The approximate weight-doubling times given in Table 1 refer to the transplants used and the two neighboring transplants.

^{86}Rb was obtained from Radiochemical Center, Amersham, and had a specific activity either of about 0.05 or 2 c/g. The contribution of the possible contaminant ^{134}Cs to the count rate was examined and found to be negligible by counting a solution after decay of the ^{86}Rb activity, and also by γ-spectrometry. The Rb content of these solutions was determined by flame photometry. Carrier-free ^{43}K was supplied by the Medical Research Council Cyclotron Unit, Hammersmith (whom I thank for their help).

^{86}Rb, with or without added carrier, was administered to the animals in their drinking water for a period of 4 to 12 days and, in certain experiments, followed by a further period on normal drinking water. Blood samples were obtained by cardiac puncture. Gut samples were cleared of their contents and rinsed with distilled water. Tissue samples were wet-ashed with HNO_3. The total activity ingested by each animal was 150 to 300 μc, permitting accurate radio-assay of the same tissue solutions which were used for K determinations. This activity by itself, i.e., at low concentrations of stable Rb, was found to have no discernible effect on the growth rate of the animals.

Incubations of isolated tissue preparations were carried out in 3 × 17-cm boiling tubes, each with a glass capillary sealed to the base through which a stream of 95 % O_2 — 5 % CO_2, saturated with water vapor, was passed. The tubes were sealed with wax-coated rubber bungs with gas outlets through small wash bottles. The incubation medium finally used was close to Krebs-Ringer-phosphate (medium III of Krebs, 1950), pH 7.5, but with the addition of 5 mmole/liter of glucose and 20 g/liter of insulin. The median and left lateral lobes of livers of male "Marshall" strain rats of 110-g body weight were sliced in a McIlwain chopper. About 0.5 to 1 g of slices was incubated in 100 ml of medium. Rapid stirring was essential, and significant improvements in the K^+ and dry-matter content of the slices followed the exclusion of light. On removal from the medium, slices were quickly drained on coarse sintered glass discs before weighing. In experiments with diaphragm, 4 hemi-diaphragms were incubated and treated similarly. The transplantable tumor (BICR/M1) was sliced and incubated similarly. Respiration measurements were carried out by the standard Warburg method: the O_2 consumption was measured as the linear slope over 1 to $1\frac{1}{2}$ hours incubation.

Potassium analyses were carried out flame-photometrically using blanks prepared similarly to the samples, and standards in solution of equal acidity. In experiments at high Rb^+ loadings, the Rb content of samples was calculated from the count rate, and the K emission corrected for the contribution from Rb present in the sample. In the experimental conditions, the emissions of the two ions were found to be additive, with an error in the least favorable samples of not more than 1.6%, and for most samples considerably less than this. Natural levels of Rb were determined using a wave-length scanning flame photometer and the 780-nm emission line. (I am grateful to Dr. J. G. Hollins for these analyses.)

Samples were assayed for radioactivity in an automatic well-type γ-spectrometer; or, if samples contained both isotopes, they were counted, after dissolution in concentrated HNO_3, using a 3-inch flat NaI crystal in conjunction with a 100-channel analyzer. A correction was made for the ^{86}Rb contribution to the ^{43}K peak.

Results

Normal Tissue Rb Contents

Before investigating effects of different body levels of Rb, it was necessary to know the normal tissue concentrations of Rb. Analyses of three samples of rat liver gave a mean Rb content of 25 ± 2.5 (SD) μg/g wet weight, equivalent to 0.29 mM/Kg wet weight or about 0.3% of the K content on a mole for mole basis. The results to be described show that, of the tissues studied, liver takes up the greatest concentration of dietary Rb and that the Rb concentration in the other tissues can be derived from this value together with the tissue K content. This value, bearing in mind the dietary dependence, is reasonably close to the 0.195 mM/Kg wet weight of rat liver determined by a mass spectrometric method (Skul'skii & Burovina, 1966).

The Steady State Rb^+: K^+ Discrimination Ratios

It was the aim in this series of experiments to use conditions in which the distribution of administrated Rb^+ between the tissues studied and the extracellular fluid had reached a steady state (as had that of K^+). The results of Richmond (1958), in accord of those of Mabille *et al.* (1961), show a constant (percentage-wise) tissue distribution of ^{86}Rb 1 day after administration of the isotope to rats, with the possible exceptions of skin, testes and brain. It was confirmed that steady state conditions were attained in the present experiments since the distribution ratios of ^{86}Rb between plasma and all the tissues studied were not significantly different after allowing a further period of 7 to 8 days without isotope administration. The results are presented in terms of the $Rb^+ : K^+$ discrimination ratio, used by Relman, Lambie, Burrows and Roy (1957) in a study of ^{86}Rb uptake by skeletal muscle, and by Skul'skii and Burovina (1966) in a study of the natural Rb

Table 1. *Steady state Rb^+:K^+ discrimination ratios in normal and pathological rat tissues in vivo*[a]

Sample	No. of animals	Rb^+:K^+ discrimination ratio
Tissue		
Liver	29	2.27±0.21
Kidney	24	1.97±0.16
Skeletal muscle (K-deficient)	8	1.68±0.16
Skeletal muscle (normal)	16	1.37±0.17
Lung	24	1.41±0.10
Small intestine	24	1.38±0.08
Spleen	24	1.37±0.16
Diaphragm	3	1.31±0.02
Erythrocytes	44	1.04±0.07
Plasma	—	1.00 (by definition)
Transplantable tumors		
"Marshall" (BICR/M2) fibrosarcoma (9 days)	5	1.32±0.16
"August" (BICR/A2) fibrosarcoma (5.4 days)	5	1.20±0.03
"August" (BICR/A1) mammary tumors (1.1 days)	5	1.13±0.02
"Marshall" (BICR/M1) mammary tumors (0.9 days)	5	1.09±0.03
Liver: of protein-deficient rats	5	1.61±0.11 ($P<0.001$)
of fasted rats	5	2.30±0.17

[a] Duplicate or triplicate samples were taken of all tissues. For the normal tissues no significant dependence on plasma K^+ or Rb^+ concentrations was found by partial correlation analysis except for skeletal muscle and plasma K^+. The approximate weight-doubling times of the tumors during exponential growth are given.

levels in tissues of a number of mammalian and other species. This ratio is

$$R = \frac{[Rb^+]_{tissue}}{[K^+]_{tissue}} \Big/ \frac{[Rb^+]_{plasma}}{[K^+]_{plasma}}$$

(the concentration being conveniently in terms of wet weight of tissue and volume of plasma) and would have the value unity if there were no selectivity between the two ions. The ratio has been calculated for each tissue (Table 1). Since it was apparent that any effects of K^+ deficiency or of Rb^+ loading on the discrimination ratio were mostly slight, this question was studied by (linear) multiple-correlation analysis of R with respect to plasma K^+ and Rb^+ concentrations. The ranges of plasma concentrations were $2.2 \leqq K^+ \leqq 5.7$ mM/liter and (in terms of administered Rb^+) $0.35 \leqq Rb^+ \leqq 300$ μM/liter, i.e., from a "tracer" dose to a level about 50 times

normal. Within these ranges, no significant correlations with R could be detected with the single exception of a negative correlation between R in skeletal muscle and the plasma K^+ concentration ($P \simeq 0.01$). Graphical examination of the muscle results showed that R varied in a nonlinear manner with the plasma K^+ concentration, tending to rise sharply near the lowest experimental plasma K^+ concentrations. Two values of R for this tissue are therefore given in Table 4, referring to animals on a normal K^+ diet and on a K^+-deficient diet. This is in accord with the exceptionally high Rb^+ uptakes reported by Relman *et al.* (1957) in K^+-deficient skeletal muscle.

Rb^+: K^+ Discrimination in Transplantable Rat Tumors

The discrimination ratio was similarly determined in four transplantable rat tumors chosen to cover a range of growth rates (Table 1). It will be seen that the Rb^+: K^+ discrimination ratios tend to be below those of the normal tissues studied (excepting erythrocytes), and that the two fastest-growing tumors have discrimination ratios near unity.

Rb^+: K^+ Discrimination in Rat Liver in Protein Deficiency and Fasting

In a study of trace metal concentrations in livers of normal and protein-deficient rats, Gaudin-Harding, Robin and Pinta (1966) reported that the only change (apart from a small reduction in molybdenum content) was a threefold reduction in Rb content. This rather surprising finding was confirmed qualitatively using ^{86}Rb (Table 1). It is possible that the quantitative difference is due to the prolonged period of protein depletion in the work of Gaudin-Harding *et al.* (1966): although animal weights and weight losses were matched, the diet in the present experiments was completely protein-free and the weight losses were correspondingly more rapid. To explain this finding, the energy metabolism of the tissue is relevant (the theoretical basis is put forward later in this paper), and the respiration rate of liver from rats made protein-deficient in the same conditions was determined in separate experiments. The respiration rate of the protein-deficient slices was found to be lowered to about 70% of controls [9.4 ± 1.8 (SD) μliters O_2/mg dry wt per hr; 18 samples]. Quantitative treatment of these results is presented in the theoretical section. As a control experiment, the effect of fasting on Rb^+ uptake by rat liver *in vivo* was explored (Table 1). No effects were detected.

Rb^+ and K^+ in Liver Slices Incubated in vitro: K^+ and Dry-Matter Content

Under the experimental conditions, the liver slices accumulated K^+ from the medium to 80 to 100% of normal *in vivo* wet-weight concentrations

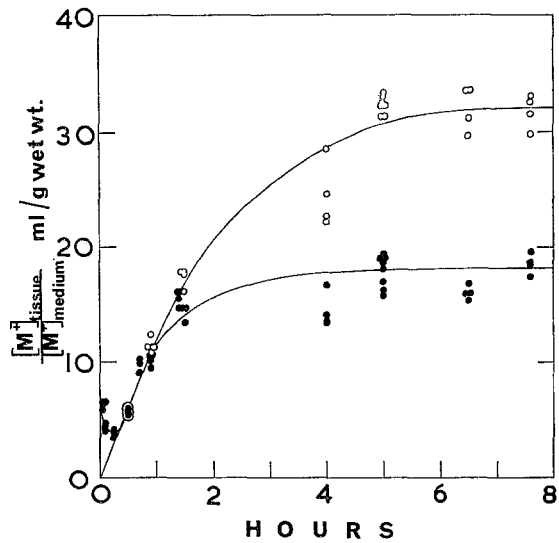

Fig. 1. Uptake of ^{86}Rb and K^+ by freshly sliced rat liver on incubation at 38° C. •——•, K^+; ○——○, Rb^+

during the first 2 hr of incubation and maintained this level for a further 5½ hr (Fig. 1). The initial loss of K^+ during the first few minutes of incubation is a usual finding with liver preparations, attributable to the effects of partial anoxia during dissection. The dry-matter content of the slices after K^+ reaccumulation was within the normal *in vivo* range (27.4 ± 0.8% compared to 27.6 ± 0.4% in normal liver).

^{86}Rb Uptake by Liver Slices

^{86}Rb was accumulated from the incubation medium initially at a rate close to that of K^+, but the slices continued to accumulate ^{86}Rb after net K^+ uptake ended, and a steady state ^{86}Rb distribution was not reached until after 5-hr incubation (Fig. 1). The ratio of slice/medium ^{86}Rb content was then nearly twice that of K^+. In all control experiments, the mean steady state Rb^+: K^+ discrimination ratio was 1.87 ± 0.11 (SD; mean of 96 samples). The discrimination ratio *in vitro* is thus within the normal *in vivo* range (*cf.* Table 1).

Relative Rates of ^{43}K and ^{86}Rb Uptakes

These rates were studied by addition of a mixture of the two isotopes to suspensions of liver slices preincubated for a time sufficient for both K^+ and Rb^+ distributions to have reached a steady state (i.e., about 5½ hr).

Table 2. *Relative rates of uptake of ^{43}K and ^{86}Rb by liver slices at 38° C*[a]

Time	$\frac{[^{43}K]_{tissue}}{[^{43}K]_{medium}}$ (ml/g wet wt)	$\left(\frac{^{86}Rb}{^{43}K}\right)_{tissue} / \left(\frac{^{86}Rb}{^{43}K}\right)_{medium}$
0 (extrapolated)		0.998 ± 0.085
15 min	5.01 ± 0.17	1.088 ± 0.033
20 min	7.05 ± 0.85	1.178 ± 0.084
30 min	8.35 ± 0.74	1.216 ± 0.119

[a] Each point represents the mean of four samples. The extrapolation is linear by the least squares method.

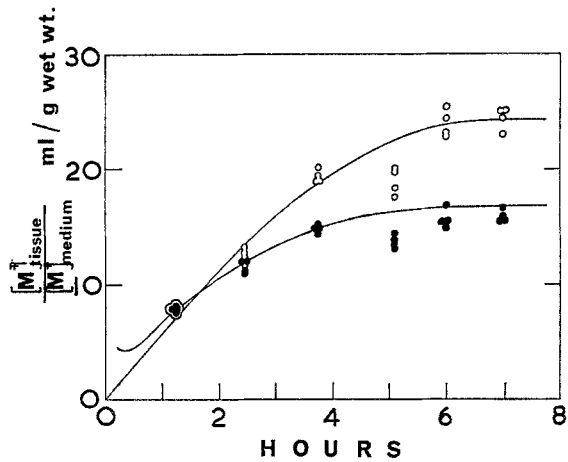

Fig. 2. Uptake of ^{86}Rb and K^+ by freshly sliced rat liver on incubation at 26° C. •——•, K^+; ○——○, Rb^+

The results are given in Table 2. Under these conditions, the initial rates of uptake of the two ions are nearly identical. Assuming a linear change in discrimination ratio during the first half-hour of incubation, a least-squares calculation yields a zero-time uptake ratio of 1.00 ± 0.08.

The Effect of Temperature on the Relative Accumulation of K^+ and ^{86}Rb by Rat Liver Slices

At 26° C liver slices reaccumulated K^+ from the incubation medium but more slowly than at 38° C (Fig. 2). A steady state K^+ concentration (of about 90% that at 38° C) was reached after about 4-hr incubation and of Rb^+ after 6 to 7 hr. The final Rb^+: K^+ discrimination ratio was considerably lower at the lower temperature. At 20° C, a steady state discrimination ratio was established after 6½ to 7½ hr of incubation. Over

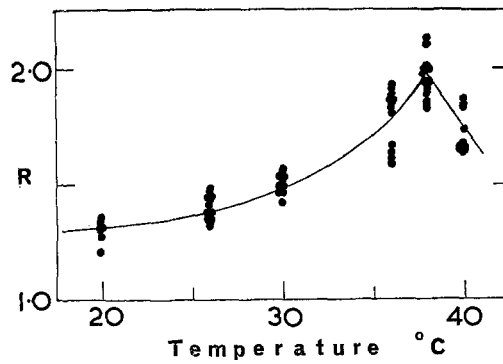

Fig. 3. Variation of steady state Rb^+:K^+ discrimination ratio in rat liver slices with temperature of incubation

the range 38 to 20° C, the fall in the steady state discrimination ratio was progressive with the temperature drop (Fig. 3). A peak in the discrimination ratio was shown at 38° C; at higher temperatures there was a reduction.

To investigate the effects of temperature on the relative rates of influx of tracer K^+ and Rb^+ in steady state conditions, experiments similar to those described above were carried out at 38 and 26° C but using ^{42}K and ^{86}Rb. Although a small systematic counting error of about 15% was subsequently discovered (apparently due to greater self-absorption of the high energy ^{42}K β-emission in the concentrated HNO_3 used to disolve the tissue samples compared with the less-dense samples of incubation medium), the results are considered valid for comparison only. There was no significant difference in the relative rates of intake of the tracers at the two temperatures (the ratio of the two was 0.98 ± 0.05). This result contrasts with the differences in steady state discrimination ratio (Figs. 3 & 4).

Rb^+: K^+ Discrimination Ratio and Liver Slice Respiration Rate

In rat liver, the energy required for alkali metal ion transport is derived almost entirely from respiration (Elshove & Van Rossum, 1963). On theoretical grounds, discussed in a later section, a relationship is expected between the Rb^+: K^+ discrimination ratio and the respiration rate at the various temperatures. The respiration rate of liver slices was therefore determined, in separate experiments, at the same temperatures as the discrimination ratios. In Fig. 4, the function $RT \log R$ (where R is the Rb^+: K^+ discrimination ratio) is plotted against the slice respiration rates at the corresponding temperatures.

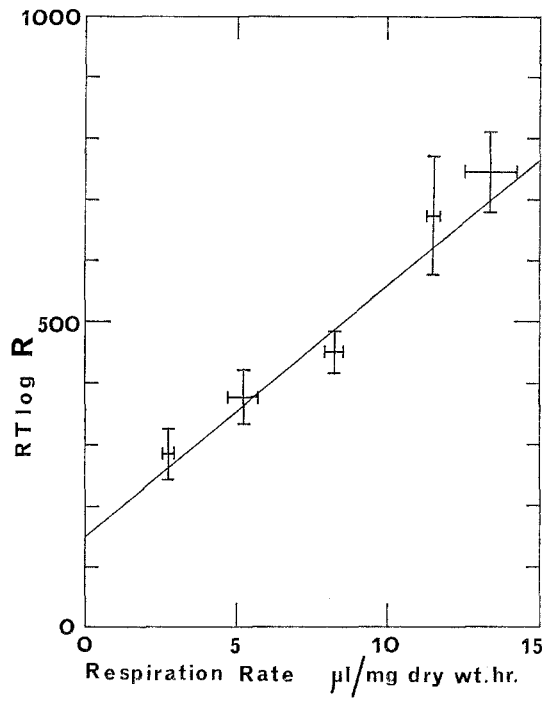

Fig. 4. Plot of $RT \log_{10} R$ where R is the $Rb^+:K^+$ discrimination ratio in rat liver slices as a function of slice respiration rate. The point at 40° C has been omitted as discussed in the text

The Effects of Metabolic Inhibitors on Rb^+ and K^+ Uptake by Rat Liver Slices

The addition of inhibitors of respiration or of oxidative phosphorylation to the incubation medium reduced the transport of both K^+ and Rb^+ (Fig. 5). In the experimental conditions, the effects of iodoacetate, dinitrophenol (DNP), cyanide and arsenate were similar to those of complete anoxia: K^+ transport was greatly reduced, and the Rb^+: K^+ discrimination ratio was near 1.0 (i.e., Rb^+ was distributed across cell membranes in a ratio near that of K^+). Ouabain, azide, and oligomycin in the concentrations used had much smaller effects on K^+ transport but large effects on Rb^+: K^+ discrimination. A smooth curve could be drawn through these inhibitor results and the controls, implying that the variation in the controls could be interpreted as owing to small degrees of inhibition perhaps by trace contaminants. The discrimination ratio peaks sharply as slice K^+ concentrations approach 0.13 mole/kg slice water. Partial metabolic inhibition caused by temperature reduction gave results falling on the same curve

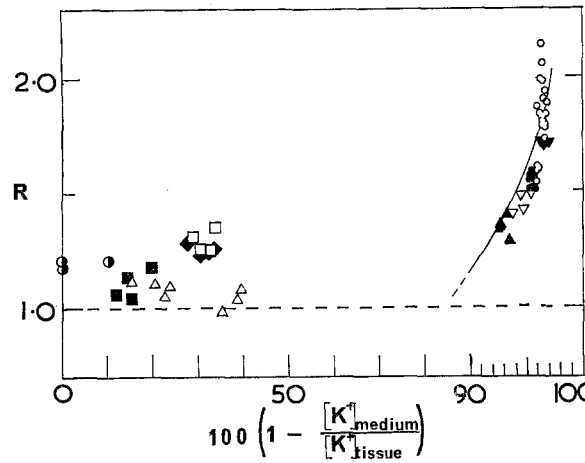

Fig. 5. Plot of steady state Rb^+:K^+ discrimination ratio in rat liver slices *in vitro* against the proportion of actively transported K^+ in the tissue in control slices and in the presence of metabolic inhibitors: ○, controls; ▼, oligomycin; ●, 26° C; ▽, ouabain; ▲, azide; □, cyanide; ◆, arsenate; △, DNP; ■, anoxia; ◐, iodoacetate. The line has been calculated theoretically as described in the text. Tissue concentrations have been calculated in terms of slice water. Medium K^+ concentrations were approximately constant for all results plotted ($4.1 < [K^+] < 4.7$ mM/liter)

(Fig. 5). From Fig. 5, it can be seen that considerable reductions in Rb^+ uptake can occur with only small reductions in tissue K^+ concentration; e.g., the fall in slice K^+ brought about by azide is only about 3.5%, but slice Rb^+ has fallen by about 30%.

Rb^+: K^+ Discrimination in Rat Diaphragm in vitro and the Effects of Metabolic Inhibitors

Experiments were also carried out with rat hemi-diaphragms. In preparations of this type, a proportion of the cells is sectioned, and the total K^+ content after incubation is about half that of the fresh tissue owing to the decrease in intracellular space. In preliminary experiments similar to those with rat liver slices, it was found that steady state Rb^+: K^+ discrimination ratios were established after 4 to 5 hr of incubation. The results obtained in control incubations and in the presence of metabolic inhibitors are given in Fig. 6. The plot is of the same form as Fig. 5, but the "peaking" of the discrimination ratio with an increasing proportion of actively transported K^+ is less sharp owing to the greater extracellular space. The controls are more widely scattered than the liver controls, probably due to variability of dissection, but the control discrimination

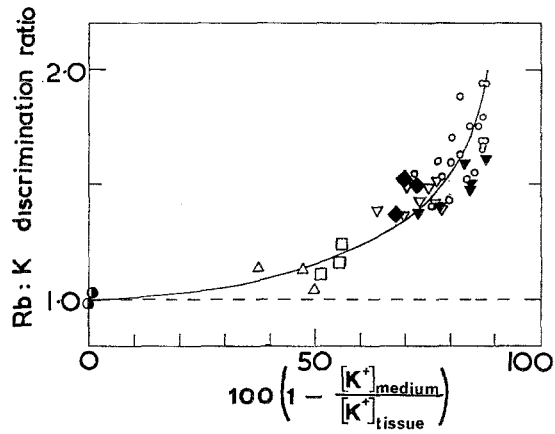

Fig. 6. Plot of steady state $Rb^+:K^+$ discrimination ratio in rat hemi-diaphragms *in vitro* against the proportion of actively transported K^+ in the tissue in control preparations and in the presence of metabolic inhibitors: ○,controls; ▽, oligomycin; ▼, ouabain; ◆, arsenate; □, cyanide; △, DNP; ◑, iodoacetate. Concentrations are as in Fig. 5

ratios are all higher than the *in vivo* values (Table 1) and overlap the liver values. In comparison with the liver slice results, the inhibitors tend to reduce K^+ transport less, possibly due to the higher glycolytic energy metabolism in diaphragm. K^+ transport is completely abolished by the (glycolytic) inhibitor iodoacetate, but appreciable transport still occurs in the presence of respiratory inhibitors (cyanide) or uncoupling agents (DNP).

Rb^+: K^+ Discrimination in Tumor Slices in vitro

For comparison, the effects of metabolic inhibitors on Rb^+ and K^+ uptake in tumor slices, in which the Rb^+: K^+ discrimination ratio is near unity, were investigated. In preliminary experiments, it was found that, in contrast with liver slices and diaphragm, little loss of K^+ occurred during early incubation of the slices. Rb^+ uptake occurred at a slower rate than in liver slices, but a steady state was established between $4\frac{1}{2}$ to $6\frac{1}{2}$ hr of incubation. The *in vitro* discrimination ratio (1.21 ± 0.07; 32 control samples) was higher than the *in vivo* value (Table 1), with $P < 0.001$. The addition of metabolic inhibitors caused a reduction in Rb^+: K^+ discrimination along with a fall in K^+ transport in a similar pattern to that found in liver slices and in diaphragm (Fig. 7). The effect of individual inhibitors were dissimilar; e.g., alkali metal ion transport systems in this tumor were resistant to cyanide inhibition. An unexpected finding was that actinomycin D in low concentrations caused appreciable inhibition of K^+ transport. The inhibition

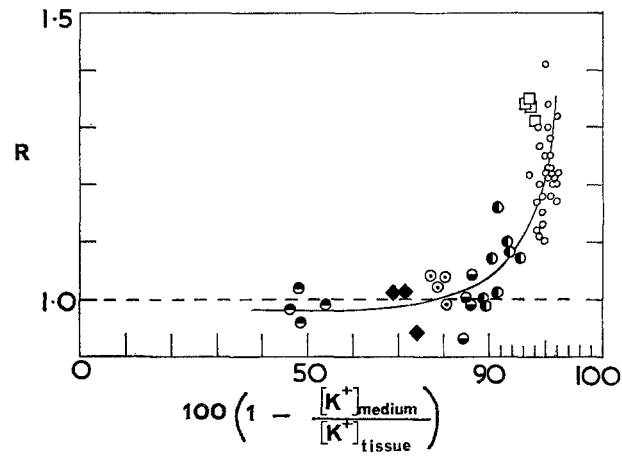

Fig. 7. Plot steady state $Rb^+:K^+$ discrimination ratio in M.1. tumor slices *in vitro* against the proportion of actively transported K^+ in the tissue in control preparations and in the presence of metabolic inhibitors: ○, controls; □, cyanide; ◐, actinomycin D (two concentrations); ◒, arsenite; ⊙, EDTA; ◆, arsenate; ◓, fluoride. Tissue concentrations are in terms of wet weight

Table 3. *Relative rates of uptake of ^{43}K and ^{86}Rb by tumor (M.I.) slices at 38° C*[a]

Time	No. of samples	$\frac{^{43}K_{tissue}}{^{43}K_{medium}}$ (ml/g wet wt)	$\left(\frac{^{86}Rb}{^{43}K}\right)_{tissue} / \left(\frac{^{86}Rb}{^{43}K}\right)_{medium}$
0 (extrapolated)	—	—	0.965 ± 0.019
14 min	8	2.84 ± 0.31	1.015 ± 0.042
50 min	4	7.23 ± 0.61	1.096 ± 0.021

[a] The extrapolation is linear by the least squares method.

was progressive with increasing dose over the range 0.3 to 5 μg/ml and was accompanied by a rise in the dry-matter content of the tumor slices [from 15.1 ± 0.4 to 18.0 ± 0.8 ($P < 0.001$) at zero and maximal dose, respectively]. This increase is presumably due to loss of intracellular water.

Relative Rates of ^{43}K and ^{86}Rb Uptake in Tumor Slices

Uptake in tumor slices was determined in a manner exactly similar to that used for liver slices. The results are shown in Table 3. There was a significant increase in the apparent R between 14 and 50 min after addition of the isotopes, analogous to that found in liver, and the ratio of uptake

extrapolated to zero time was very close to unity (0.97 ± 0.02) and to the ratio found in liver, in contrast to the greatly differing steady state distributions.

Discussion

A theoretical treatment of the present results must: (1) explain the direct correlation between the Rb^+: K^+ discrimination ratio in a tissue and the supply of metabolic energy (Fig. 4, and also implied in Figs. 5–7); (2) explain why the rates of influx of tracer K^+ and Rb^+ in otherwise steady state conditions are nearly equal and have no apparent relation to the steady state discrimination ratios in different tissues (Tables 1–3) or to the energy supply (with temperature change); and (3) provide some physical basis for considering the tissue differences in Rb^+: K^+ discrimination (Table 1). It follows from the first and second of these findings that some K^+ transport process as well as the influx must vary with the supply of metabolic energy. This conclusion seems incompatible with a membrane model containing independent active transport and passive "leak" channels, since if discrimination originated in the "leak" channels of such a system it would be independent of metabolism (at least in conditions not too far from normal), and if the leak channels vary in relative permeability with the degree of active transport the two sets of channels are no longer independent. However, if the assumption of independence of the active transport and "leak" channels is discarded, [i.e., that interaction is permitted between actively transported cations (inward) and freely diffusing ions (outward)] movements of ions in each direction (or of tracer ions if the system is "saturated") are metabolically linked but in opposite senses. It will be shown that a simple countercurrent system of this type quantitatively predicts the results obtained. Moreover, the system is physically meaningful and can be treated analogously to an artificial membrane system with a maintained solvent flow (e.g., Meares & Ussing, 1959). A quantitative consequence of the theory is that the major part of Rb^+: K^+ discrimination originates in the relative tracer effluxes. This is a mathematical consequence of the present results, since in steady state conditions the absolute numbers of any ionic species entering the cells must equal the number leaving, whatever mechanisms operate. It is, however, technically difficult to demonstrate this directly in steady state conditions, particularly for tissues such as those near the top of Table 1, where the experiment would have most meaning, owing to the rapidity of K^+ loss and accompanying pH changes, on dissection or transfer of tissue slices; a comparable situation has been shown directly

in frog muscle (*compare*, e.g., Lubin & Schneider, 1957, and Ling & Schmolinske, 1954; Sjodin, 1961).

Steady State Theory of Simultaneous Diffusion and Metabolically Coupled Flux

In this treatment, it is assumed that the cell membrane is homogeneous and that alkali cations can traverse the membrane either as free or solvated ions or coupled to a flux of a metabolite, e.g., a carrier molecule such as the macrotetralides studied in model systems (Ciani, Eisenman & Szabo, 1969). The mechanism of coupling may be frictional as in the quasi-thermodynamic treatment of Nims (1962), but the evidence seems to favor a more specific type of coupling, e.g., complex formation, as in the hypothetical scheme treated from a thermodynamic viewpoint by Katchalsky and Spangler (1968). The total flux of K^+ is then the sum of a diffusive flux, given by a form of Fick's Law (*see*, e.g., Meares & Ussing, 1959), and a metabolically maintained flux as indicated by the two terms of Eq. (1):

$$J_K = -u_K \left(c_K \frac{\partial \tilde{\mu}_K}{\partial x} - k_K \phi c_K \right) \tag{1}$$

where u_K is the mobility, C_K the concentration, $\tilde{\mu}_K$ the electrochemical gradient, ϕ the flux of coupling metabolite, and k_K a coupling coefficient. Eq. (1) is mathematically analogous to that used by Meares and Ussing (1959), but the coupling flux of metabolite ϕ replaces the solvent flow in their system. Interaction between the fluxes is permitted by assuming the concentration C_K in each term on the right-hand side to be identical and by solving the equation as it stands without separating the terms. In the present experiments, two different steady state conditions have been studied in which $\partial c/\partial t = 0$ within the membrane (in the tracer influx experiments, this is a limiting approximation). Therefore, using $\partial c_K/\partial t = -(\partial/\partial x) J_K$ and expanding

$$\tilde{\mu}_K = \mu_K^0 + RT \ln c_K + F\psi ,$$

where ψ is the electrical potential:

$$\frac{\partial c_K}{\partial t} = u_K \left(RT \frac{\partial^2 c_K}{\partial x^2} + F \frac{\partial \psi}{\partial x} \cdot \frac{\partial c_K}{\partial x} + F c_K \frac{\partial^2 \psi}{\partial x^2} - k_K \phi \frac{\partial c_K}{\partial x} \right) = 0 . \tag{2}$$

Approximating ψ as a linear function of x, $\partial \psi/\partial x = \psi_m/d$, where ψ_m is the membrane potential and d is the membrane thickness:

$$u_K \left(\frac{d^2 c_K}{dx^2} + \frac{1}{RT} (F \psi_m/d - k_K \phi) \frac{dc_K}{dx} \right) = 0 . \tag{3}$$

This has a general solution of exponential form, and using the boundary conditions $c_K = c_K^i$, $x = d$; $c_K = c_K^0$, $x = 0$:

$$c_K = \frac{c_K^i[1-\exp(-wx)] - c_K^0[\exp(-wd)-\exp(-wx)]}{1-\exp(-wd)} \tag{4}$$

where:

$$w = \left(\frac{F\psi_m}{d} - k_K\phi\right)\Big/ RT. \tag{5}$$

The flux of K^+ is then obtained from Eq. (1): two conditions are of interest, firstly that in which $J_K = 0$, the steady state distribution, when

$$c_K^i/c_K^0 = \exp[(k_K\phi d - F\psi_m)/RT]. \tag{6}$$

Similarly under these conditions, if Rb^+ transport is coupled to the same metabolite flux,

$$c_{Rb}^i/c_{Rb}^0 = \exp[(k_{Rb}\phi d - F\psi_m)/RT]. \tag{7}$$

Therefore:

$$\begin{aligned} RT\ln(c_{Rb}^i/c_{Rb}^0) - RT\ln(c_K^i/c_K^0) &= RT\ln(c_{Rb}^i c_K^0/c_{Rb}^0 c_K^i) \\ &= RT\ln R = (k_{Rb}-k_K)\phi d. \end{aligned} \tag{8}$$

Thus, if the coupling coefficients and the physical properties of the membrane are constant, $RT \log R$ is a linear function of the metabolic flux ϕ only. Fig. 4 is an experimental verification of Eq. (6), showing additionally under these conditions that the metabolite flux ϕ represents a nearly constant proportion of the total respiratory energy available. The low discrimination at 40° C (Fig. 3) is attributable to irreversible cell damage at this temperature in the longer incubation times used in the determination of the ion uptake, not always found in the shorter incubations used in the determination of the respiration rate (Brauer, Balam, Bond, Carroll, Grisham & Pessotti, 1963).

Coupling of K^+ and Rb^+ transport to a respiratorily dependent flux also accounts, quantitatively, for the reduced Rb content in the livers of protein-deficient rats (Gaudin-Harding *et al.*, 1966; this paper). At the respiration rate of slices of livers from protein-deficient rats, an interpolated $Rb^+ : K^+$ discrimination ratio at 38° C is calculated from Fig. 5 of 1.6 ± 0.2, in agreement with the value found of 1.66 ± 0.11 (Table 1).

Eq. (6) is of similar form to that derived by Nims (1962) for the steady state distribution of the ions K^+ and Na^+, partly based on the thermodynamics of irreversible processes and confirmed experimentally by Thurber

and Thompson (1967) in studies of the Na^+ and K^+ distributions in erythrocytes over a range of temperatures. In this predominantly glycolyzing system, it was found that the steady state K^+ and Na^+ distribution could be described by an equation similar to Eq. (8) if the coupling metabolite flux or fluxes were taken as proportional to the rate of lactate production.

The effect of chemical inhibitors of metabolism on K^+ and Rb^+ uptake can be interpreted analogously as due to a reduction in the coupling flux ϕ, and it is possible to derive the form of the plot in Fig. 5 from Eqs. (6) and (7). The mean membrane potential ψ_m of rat liver cells has been determined by Schanne and Coraboeuf (1966) and by Limberger (1963) as $-$ 50.7 mV. On the assumptions that relatively small changes of energy metabolism change only the coupling flux ϕ, and that the membrane potential remains approximately constant (support for this assumption comes from the findings of Schanne & Coraboeuf, 1966, that the liver membrane potential is relatively insensitive to changes in C_K^i/C_K^0 and that it drops by only about one half in the $\frac{1}{2}$ hr after the death of the animal), and taking the normal steady state ratios of C_K^i/C_K^0 and C_{Rb}^0/C_{Rb}^i as 28.6 and 54.3, respectively (in terms of slice water), it is possible to calculate R for any ratio C_K^i/C_K^0 from Eqs. (6) and (7). These calculation have been carried out, and the results are shown by the continuous line in Fig. 5. Although the range of this calculation is limited by the assumption of an approximately constant membrane potential, it includes the region of maximum variation in Rb^+: K^+ discrimination, and the agreement with experiment is excellent. A similar form of variation to Fig. 5 is expected in any system with $k_{Rb} > k_K$ and following Eqs. (6) and (7) in agreement with Figs. 6 and 7, but detailed comparison is hindered by the high extracellular space in the diaphragm preparation and by lack of knowledge of the tumor-cell membrane potential.

Theory of Comparative Tracer Fluxes of K^+ and Rb^+

The initial relative rates of uptake of tracer ^{86}Rb and ^{43}K by tissue slices in steady state conditions bear no apparent relation to the steady state Rb^+ and K^+ distributions. In fact, the initial influx ratio is close to unity in both liver slices (with a maximal Rb uptake) and tumor slices (Tables 2 & 3). A comparable discrepancy is found in frog muscle in which the relative influx of Rb^+ occurs at about half the K^+ rate, but the steady state accumulation ratio of Rb^+ is considerably greater than that of K^+ (Ling & Schmolinske, 1954; Lubin & Schneider, 1957; Sjodin, 1961). The same phenomenon is also shown in cardiac Purkinje fibers of the sheep in which Rb^+ is accumulated to about twice the K^+ accumulation, but the relative rate of Rb^+ influx is only about 2/3 that of K^+ (Müller, 1965). There may

thus be quantitative species differences in the cell membrane transport of Rb^+ and K^+, although the experimental conditions are not all exactly comparable. In the rat, and under the conditions described above, the equality of the initial tracer influxes in two tissues with very different Rb^+ uptakes suggests the possibility that the influxes may tend to be identically equal, i.e., that tissue parameters may tend to cancel in the equation for the relative rates of uptake.

From a thermodynamic viewpoint, the tracer isotopes must be regarded as distinct molecular species. Denoting tracer ions by asterisks and substituting Eq. (4) in Eq. (1), under conditions in which $C_K^{i*}/C_K^{0*} \ll \exp(-wd)$, which will apply at short times after addition of radiotracer:

$$J_K^*/c_K^{0*} = \frac{u_K w \cdot \exp(-w d)}{1-\exp(-w d)} . \tag{9}$$

The exponents in Eq. (9) have their normal steady state values which are known from Eq. (7), and unity in the denominator may be neglected (with an error of less than 4%) giving:

$$J_K^*/c_K^{0*} \simeq -u_K \left(\frac{F\psi_m}{d} - k_K \phi\right) . \tag{10}$$

Treating the ^{86}Rb influx similarly, the ratio of initial influxes is:

$$J_{Rb}^* c_K^{0*}/J_K^* c_{Rb}^{0*} \simeq \frac{u_{Rb}}{u_K} \cdot \frac{(k_{Rb}\phi d - F\psi_m)}{(k_K \phi d - F\psi_m)} . \tag{11}$$

In both aqueous and mixed aqueous-organic media, $u_{Rb}/u_K \simeq 1.0$, and for the purposes of calculation is taken as unity. The two quantities in parentheses are known from Eqs. (6) and (7) and the values given above for liver slices, and in normal conditions the ratio in Eq. (11) equals 1.19. [Allowance for the 25% extracellular water space of the liver slices in calculating the intracellular concentrations lowers this value by about 2%, and inclusion of the unity in the denominator of Eq. (9) and its analog by a further 2% bringing the ratio to about 1.15.] In tumor slices, a ratio of initial influxes of 1.04 is similarly calculated (the two corrections totalling 2%). These values may be compared with the experimental values in Tables 2 and 3: agreement with the first experimental points is within about 5%, but with the extrapolated zero time points it is less good. These discrepancies would both be resolved by putting $u_{Rb}/u_K \simeq 0.9$, but may be due to the approximate nature of the extrapolation; in particular, no allowance has been made for

the (presumably nondiscriminating) uptake of the two tracers in the extracellular space. In view of the approximations and assumptions made in the development of these equations, they provide a satisfactory account both of the steady state distributions and tracer fluxes of Rb^+ and K^+ and of the differences between them. The apparent discrepancy between the tracer fluxes and the steady state distribution is seen to be due to the reduction in the experimental conditions of the exponential term in Eqs. (6) and (7) to a linear function of its exponent in Eqs. (10) and (11).

A similar treatment of tracer efflux can readily be carried out, and it is found that, in contrast to the influx equation, the metabolic flux occurs exponentially in the denominator and no longer tends to cancel out.

Thermodynamics of the Steady State Rb^+ and K^+ Distributions

The energetics of the type of transport considered have been discussed from a thermodynamic point of view by Kedem and Caplan (1965) and Essig and Caplan (1968). The appropriate quantity is the rate of entropy production, and, in a system in which the ion distributions are maintained in a steady state by a coupled metabolite flux, the entropy production is the product of the flux and the chemical potential gradient down which it moves, i.e., $\phi \cdot X$. If, for example, a carrier recycles in two different forms, there will be two such terms. This quantity can be expressed in terms of the ionic distributions and the coupling coefficients (Kedem & Caplan, 1965). No data is available about the force term X, but it is reasonable to regard ϕ and X as positively correlated; thus, in a given cell type an increasing flux, ϕ an corresponds to an increasing entropy dissipation $\phi \cdot X$. Due to the relative coupling coefficients, a rise in the Rb^+: K^+ discrimination ratio is therefore a consequence of a rise in the entropy dissipation of K^+ transport processes as implied by Fig. 4. Coupling between K^+ and Na^+ transport would be expected to lead also to an increase of the K^+: Na^+ exchange ratio as found by Thurber and Thompson (1967), but this aspect has not been studied in the present experiments.

The Origin of the Tissue Differences in Rb^+: K^+ Discrimination Ratios

Although the model of K^+ transport described can only be a simplification, its success in interpreting the liver slice results (Figs. 4 & 5) and the similarity of Figs. 5–7 imply that the same model may be applied to all three tissues, and suggest the possibility of explaining the tissue differences in Rb^+ and K^+ uptake (Table 1) in terms of this model. Eq. (6) contains two membrane parameters, ϕ and d, and a pair of coefficients, k_{Rb} and k_K,

which may vary between different cell types. The cell membrane thickness, d, however does not seem to vary sufficiently in the tissues studied to cause the wide dispersion of $Rb^+:K^+$ discrimination ratios, and is possibly approximately constant (e.g., Gaylarde & Sarkany, 1968). The flux term and coupling coefficients are inseparable in the equations, and it is difficult to obtain evidence from the present measurements about their relative importance as regards tissue variability. However, if it is justifiable to divide Eq. (6) into constant parameters (k_{Rb}, k_K and d) and a variable (ϕ), as has been assumed in part of the above treatment, the fact that in appropriate conditions the $Rb^+:K^+$ discrimination ratio of diaphragm can reach values found in liver tissue (Figs. 5 & 6) implies that the difference between the tissues is due to the variable rather than to the constants. The similarity in the shapes of Figs. 5–7 (making allowance for the high extracellular space in Fig. 6) supports this conclusion. A possible explanation of the enhanced $Rb^+:K^+$ discrimination ratio in diaphragm *in vitro* is that since it is then performing no work, energy is diverted to ion transport with a consequent rise in ion selectivity as discussed in the paragraph above.

There is also some direct evidence that, as between some of the tissues studied, the normal *in vivo* $Rb^+:K^+$ discrimination ratio is correlated with the degree of energy expenditure on K^+ transport and therefore with the flux ϕ. Estimates of the energy utilized for K^+ transport have been made from the component of ATP hydrolysis in erythrocytes which is eliminated by the omission of K^+ from the incubation medium, or by ouabain addition (Whittam & Ager, 1965), from the ouabain-sensitive component of respiration in rat kidney cortex (Whittam & Willis, 1963); a similar calculation is possible using the data of Elshove *et al.* (1963) for K^+ transport and K^+- and ouabain-sensitive respiration in rat liver. [The erythrocyte result is for human cells but, with reference to Rb^+ and K^+ uptakes, these are very similar to rat erythrocytes (Love & Burch, 1953; Tyor & Eldridge, 1956)]. Expressing the results in terms of the ATP production linked to K^+ transport (assuming 6 moles ATP/mole O_2), the results are: liver, 1.1×10^{-7}; kidney, 1.2×10^{-7}; erythrocytes, 1.4×10^{-10} moles ATP/g wet weight per sec. In addition, a ouabain-sensitive component amounting to about 12% of the total respiration has been reported in rat diaphragm (Nissau, Aviram, Czaczkes, Ullmann & Ullmann, 1966), equivalent to 1.0×10^{-8} moles ATP/g wet weight per sec. Differences in specific cellular surface area complicate comparison, but, to an approximation, the similar energy expended on K^+ transport in liver and kidney accords with the similar $Rb^+:K^+$ discrimination ratios. The energy expenditure is about a thousand times less in erythrocytes, which, if the logarithmic relation [Eq. (3)] holds and assuming linearity

between ATP production rate and the metabolite flux ϕ, would lead to a discrimination ratio of about 1.001, in agreement with the experimental value (Table 1); in diaphragm with an intermediate discrimination ratio, the energy expenditure is also intermediate in value. Thus, in these tissues the variation in energy supply to K^+ transport is in the order of the $Rb^+:K^+$ discrimination ratios and can account for the differences in the discrimination ratio without postulating different coupling coefficients in Eq. (3). In fact, an overall 50-fold reduction in energy expenditure from that in liver is sufficient to include the observed range of discrimination ratios (Table 1).

A possible argument against this explanation is that since the ratio C_K^i/C_K^0 does not vary by much in the different tissues, there should from Eq. (4) tend to be an inverse correlation between $(-\psi_m)$ and $k_K \phi d$ and therefore the discrimination ratio, which is not found. In the theoretical treatment, however, the cell membrane has been assumed to be homogeneous: any additional mode of ion transfer will add a term to the exponents of Eqs. (6) and (7). If such transfer is non-discriminative, the terms will cancel in Eq. (8). In particular this will apply to "leak" fluxes accompanying secretory or phagocytic activity, and, in comparing different tissues, Eq. (8) is likely to be more general than Eqs. (6) and (7). From a functional point of view, a higher energy expenditure on K^+ transport may thus be a response to a higher ion-leakage rate.

Some comment seems justified with respect to the results for the malignant tissues (Table 1). In summary, in four lines of transplantable tumors, the $Rb^+:K^+$ discrimination ratio was low and tended to unity with increasing growth rate. From the preceding discussion, it is at least possible that this indicates a low energy expenditure on K^+ transport which decreases with increasing growth rate. From the thermodynamics as discussed above, it does not seem possible to estimate the energy expenditure from tracer K^+ fluxes, but it is quantitatively plausible that a cell growth rate near that of the M.1. tumor should diminish the energy available for other processes (*see*, e.g., Kilburn, Lilly & Webb, 1969). It is possible that such a diminished energy expenditure on K^+ transport may lead to an increased intracellular Na^+concentration as shown in the work of Thurber and Thompson (1967) discussed above, in which case it is an intriguing speculation that intracellular metabolic processes in the fast-growing tumors may be more tolerant of higher Na^+ concentrations. Alternatively, "leak" permeabilities may tend to be low in the tumors, and in this connection it is of interest that relatively high cell membrane resistances have been reported in certain tumors (Loewenstein, 1967). Such effects, however, if they occur, may be the result of selection pressures during the numerous tumor transplants.

The enhanced $Rb^+:K^+$ selectivity in tumor slices *in vitro* (Figs. 6 & 7, Table 1) may conversely be due to an increased energy supply *in vitro* since, for example, protein synthesis is expected to be reduced in the presence of glucose as sole substrate.

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