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# Rubidium Uptake in Single Cells

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**Summary.** Rubidium uptake was measured in single erythroid and myeloid cells of rabbit by means of X-ray microanalysis. It was found in the nucleated bone marrow cells that after incubation in rubidium the sums of potassium and rubidium concentrations were similar to the original potassium concentrations, indicating that there was one-to-one replacement of potassium by rubidium. Although the nuclear potassium and rubidium concentrations were higher than those in the cytoplasm, the nuclear and cytoplasmic ratios of K/Rb were similar. This implies that the potassium in both compartments exchanged freely with rubidium. In the erythroid line of cells there was a continuous reduction of potassium transport activity during the maturation process as indicated by the decrease in rubidium uptake rates. The uptake was measured in seven groups of cell types that could be distinguished on the basis of morphology and chemical composition. The order of the groups from high to low rubidium uptake were: esosinophilic myelocyte > early erythroblast and thin-rimmed erythroblast > late erythroblast > early bone marrow red cell > late bone marrow red cell > peripheral blood red cell. Thus, there is a continuous decrease in rubidium transport as the erythroid cells mature.

**Key Words** rubidium uptake · X-ray microanalysis · bone marrow · red blood cells · transport · membrane · erythropoiesis · hemoglobin · blood

## Introduction

The study of ion transport in cells of the bone marrow is complicated by the heterogeneity of the hematopoietic cells. Without physical separation of the cells into distinct homogeneous populations, the conventional methods for studying transport cannot be applied. Energy-dispersive X-ray microanalysis of thin cryosections of quench-frozen specimens circumvents this difficulty. Our previous studies have shown that X-ray microanalysis is a suitable technique for *in situ* quantitation of the changes from high potassium erythroblast to low potassium red blood cells that occur during erythropoiesis in the dog bone marrow (Kirk, Lee & Tosteson, 1978; Kirk, Andrews & Lee, 1983; Lee & Kirk, 1982). In these studies, changes in membrane transport activity can be inferred from measurements of sodium and potassium concentrations, but the kinetics of ion transport cannot be determined directly because there are no net ionic changes in steady-state systems. Since rubidium is not normally present in cells, and since the transport characteristics of rubidium resemble those of potassium (Beauge & Adragna, 1971; Beauge & Ortiz, 1971), the determination of rubidium concentrations as a function of time in single cells can provide an estimate of potassium uptake rates in these cells. This paper presents the results of our study of rubidium uptake in single cells of rabbit bone marrow. These results describe: (i) the distribution of potassium and rubidium between nuclear and cytoplasmic pools of nucleated erythroid and myeloid cells, (ii) an estimate of the relative potassium uptake rate for erythropoietic cells *in situ*, and (iii) how this rate changes with development.

## Materials and Methods

Rabbit bone marrow cells were obtained from the femurs by flushing the bone marrow cavity with an ice-cold solution consisting of equal volumes of saline (0.155 M NaCl) and plasma. The bone marrow clumps were broken and the cells dispersed by repeated withdrawing and expelling the suspension with a Pasteur pipette. One ml of this suspension was added to 9 ml of rabbit plasma/RbCl solution (87.5% rabbit plasma, 12.5% 0.16 M RbCl solution, and 0.1% glucose). The final rubidium concentration was 18 mM. Plasma was used here not only to protect the cells during incubation, but also to reduce damage during freezing and to hold sectioned cells together after freeze-drying. The bone marrow cell suspension was incubated in rubidium-containing solution at 37°C for 15 and 30 min; a 1-ml sample of cell suspension was removed at each time point and quickly centrifuged (30 sec, Beckman microfuge). The supernatant solution was removed, and the cells were deposited on pieces of wooden dowels (3–5 mm in length, 1.5–2.0 mm in diameter) and rapidly frozen in Freon-22 which had been pre-cooled by liquid nitrogen. Sections were cut with glass knives at approximately −100°C using a cryokit (LKB, Bromma, Sweden). These procedures for

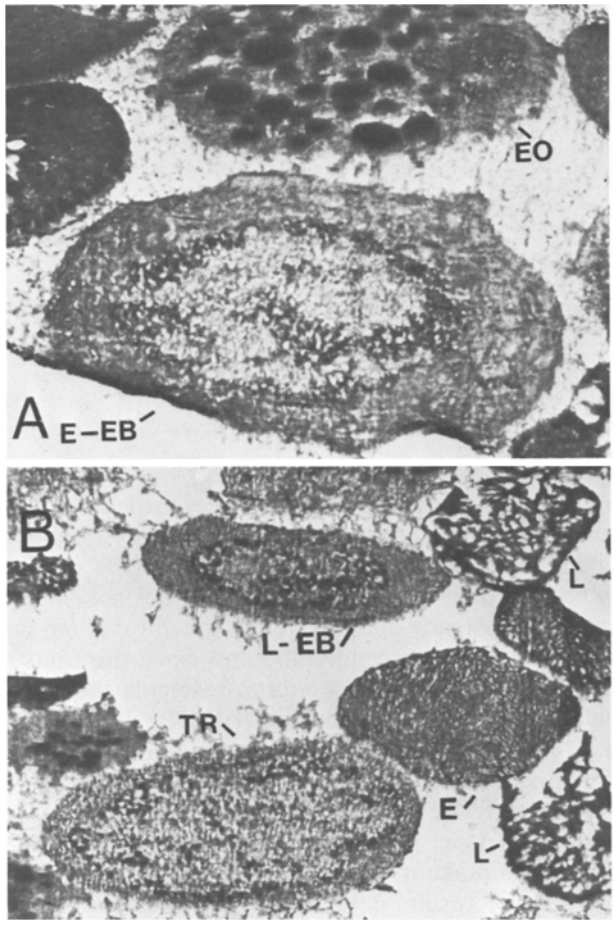


**Fig. 1.** Transmission electron micrographs of freeze-dried cryosections of rabbit bone marrow cells. (*A*): *EO*, eosinophilic myelocyte; *E-EB*, early erythroblast. (*B*): *TR*, thin-rimmed intermediate erythroblast; *L-EB*, late erythroblast; *E*, early red blood cell; *L*, late red blood cells

freezing, cryosectioning, and analyzing samples are basically similar to the methods of Somlyo, Somlyo and Shuman (1977) and have been verified for our laboratory use (Andrews, Kirk & Mazurkiewicz, 1983). Sections were freeze-dried on carbon foil-coated copper grids in a vacuum evaporator (Denton Vacuum, Cherry Hill, NJ). During freeze-drying, samples were protected in small brass containers which had been precooled to liquid nitrogen temperatures. Containers were placed on a cold block within the vacuum evaporator. Samples were allowed to warm to room temperature overnight in the high vacuum ($10^{-5}$ to $10^{-6}$ Torr) and then carbon coated. A small electrical heater in the support block was used to warm the samples (30 to 40°C) to prevent rehydration. Dry nitrogen was vented into the evaporator and the samples were transferred to a glass dessicator that had been prefilled with dry nitrogen. Prefilling was accomplished by evacuating the dessicator with a mechanical vacuum pump followed by venting with dry nitrogen. Samples were carried to the electron microscope room in the nitrogen-filled dessicator and transferred to the microscope carbon grid holder in the dessicator in the dry nitrogen atmosphere.

X-ray spectra were obtained using a 30-mm$^2$ energy-dispersive detector (Kevex Si (Li)) and a Kevex 7000 series X-ray spectrometer (Kevex Corp.) interfaced to a PDP 11V03-L computer (Digital Equipment Corp., Maynard, MA). This spectrometer was used in conjunction with a JEOL 100-CX electron microscope. Spectra were acquired from specimens at ambient temperature, accelerating voltage of 80 kV, and a beam current of 1 nA for 100 sec. The multiple least squares (ML) method (Schamber, 1977; Shuman, Somlyo & Somlyo, 1976) was used for the quantitation of the energy dispersive spectroscopy (EDS) data. The concentrations were calculated as mmol/kg dry wt from the peak/continuum ratios, according to Hall (1971).

## Results

Freeze-dried cryosections of rabbit bone marrow tissue provide sufficient morphological details for discrimination of the cells of the erythroid series from the myeloid series. A useful feature for identification of the erythroid cells is the absence of cytoplasmic granules as shown in Fig. 1.

Myeloid cells are characterized by the presence of numerous granules. The most abundant myeloid cells found in rabbit marrow were the eosinophilic myelocytes which contain larger granules than those found in the polymorphonuclear myelocytes. The early erythroblastic cells are larger, have higher cytoplasmic-to-nuclear volume ratios than the later erythroid cells, and never have detectable iron. The intermediate stage erythroid cells are characterized by their small cytoplasmic-to-nuclear volume ratios, which give them a thin-rimmed appearance in cryosectioned samples. These thin-rimmed cells are smaller than the early erythroblasts and larger than the late erythroblasts. The smaller late erythroblasts also have higher levels of iron than the thin-rimmed intermediate and earlier cells (Fig. 2). The red cells found in the bone marrow samples were classified into two types: early and late. The late red cells contained more iron and less phosphorus than the early red cells (Fig. 2 and Table 1), and in addition they appeared lacy in the cryosectioned samples (Fig. 1).

In Table 1 the cytoplasmic and nuclear concentrations of potassium, chloride, and phosphorus under control conditions were compared in myeloid and erythroid cells. The myeloid cells always contained granules with high levels of sulfur (Fig. 3*C*). In the nucleus of both cell types the potassium and phosphorus concentrations were found to be elevated, whereas the chloride concentrations were somewhat lower. These trends were similar after the cells had been incubated in plasma containing 18 mM rubidium for 30 min (Table 2, and Figs. 2 and 3). Note that the sum of potassium and rubidium (Table 2) was similar to the original potassium concentra-

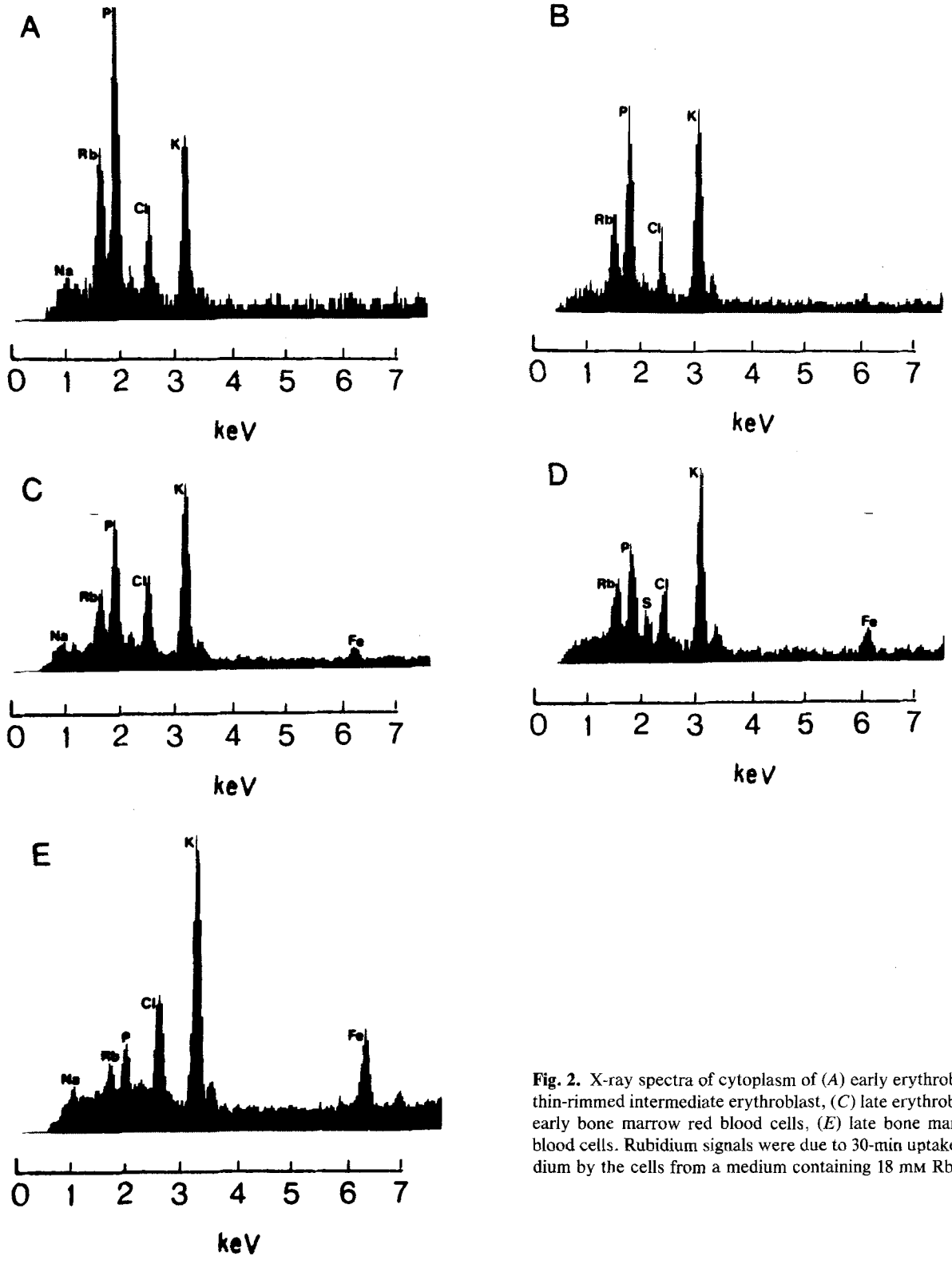


**Fig. 2.** X-ray spectra of cytoplasm of (*A*) early erythroblast, (*B*) thin-rimmed intermediate erythroblast, (*C*) late erythroblast, (*D*) early bone marrow red blood cells, (*E*) late bone marrow red blood cells. Rubidium signals were due to 30-min uptake of rubidium by the cells from a medium containing 18 mM RbCl

tions (Table 1), consistent with a one-to-one replacement of potassium by rubidium. The ratios of rubidium to potassium in the nuclei and cytoplasm were similar, indicating that potassium and rubidium in both pools were exchangeable.

The relative uptake of rubidium in various cell types in the bone marrow and the red cells in the peripheral blood are presented in Fig. 4. The order of cells, from high to low rubidium uptake, were: eosinophilic myelocytes > early erythroblast, thin-

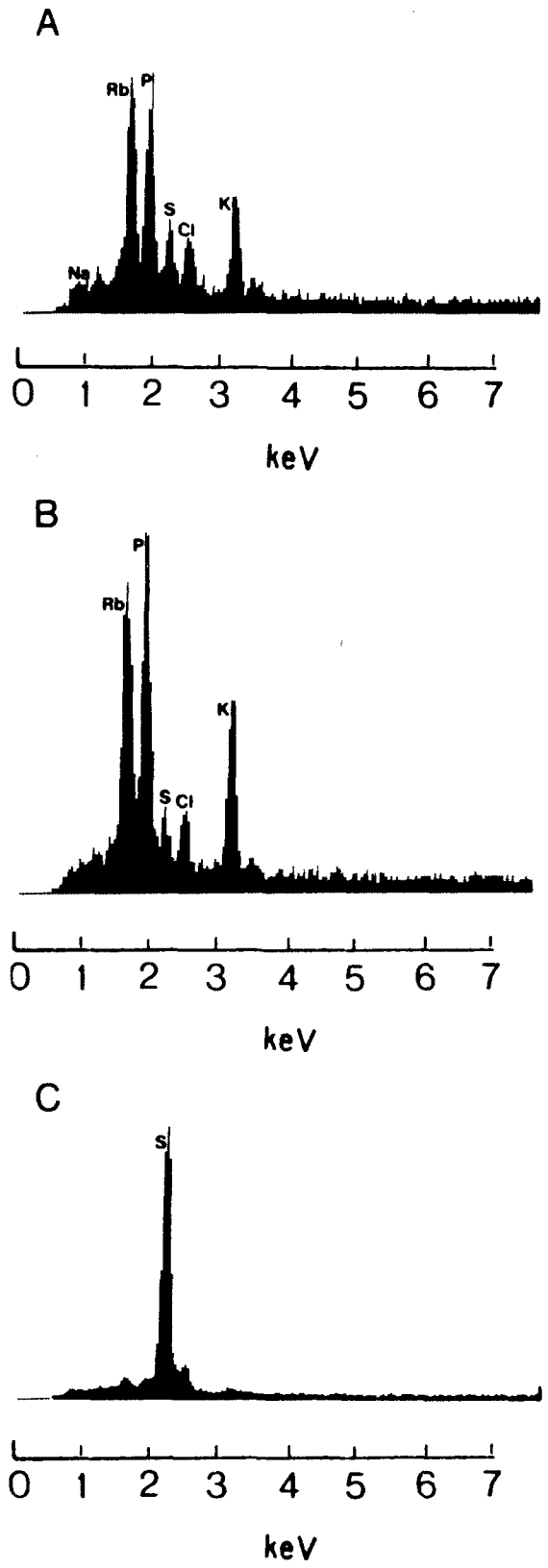


**Fig. 3.** X-ray spectra of a eosinophilic myelocyte from rabbit bone marrow. Rudidium signals from (*A*) cytoplasm, (*B*) nucleus, and (*C*) granule were due to 30-min uptake of rubidium by the cells from a medium containing 18 mM RbCl

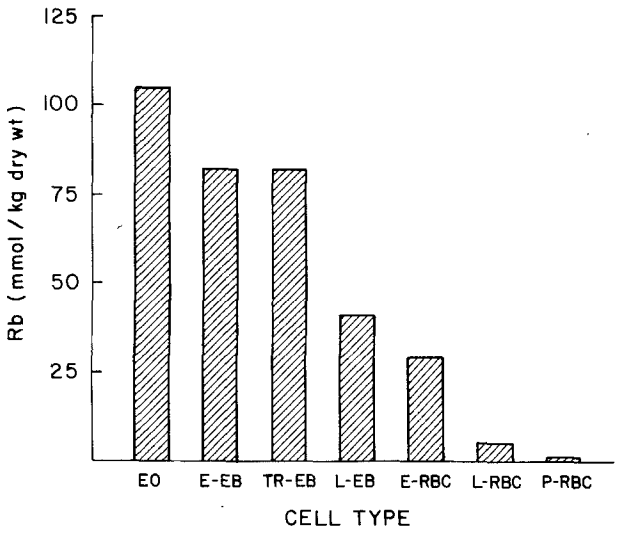


**Fig. 4.** Rubidium concentrations in the cytoplasm of various types of rabbit bone marrow cells after 30-min exposure to 18 mM RbCl. The peripheral red blood cell rubidium concentration was calculated from a 10-hr incubation period. *EO*, eosinophilic myelocyte; *E-EB*, early erythroblast; *TR-EB*, thin-rimmed intermediate erythroblast; *L-EB*, late erythroblast; *E-RBC*, early marrow red blood cells; *L-RBC*, late marrow red blood cells; *P-RBC*, peripheral red blood cells

**Table 1.** Distribution of elemental concentrations in rabbit bone marrow cells

| | P | Cl | K |
|---|---|---|---|
| | (mmol/kg dry wt) | | |
| Erythrocytes | | | |
| Late ($n$ = 40) | 90 ± 3 | 154 ± 7 | 308 ± 8 |
| Early ($n$ = 20) | 146 ± 11 | 167 ± 14 | 350 ± 17 |
| Erythroblasts | | | |
| Late ($n$ = 21) | | | |
| Nucleus | 636 ± 48 | 130 ± 7 | 433 ± 24 |
| Cytoplasm | 398 ± 40 | 169 ± 13 | 378 ± 22 |
| Thin-rimmed ($n$ = 32) | | | |
| Nucleus | 686 ± 47 | 124 ± 7 | 465 ± 22 |
| Cytoplasm | 396 ± 39 | 176 ± 11 | 394 ± 23 |
| Early ($n$ = 8) | | | |
| Nucleus | 571 ± 86 | 125 ± 7 | 509 ± 34 |
| Cytoplasm | 472 ± 52 | 158 ± 26 | 495 ± 18 |
| Eosinophilic myelocytes | | | |
| All ($n$ = 30) | | | |
| Nucleus | 565 ± 29 | 115 ± 6 | 429 ± 12 |
| Cytoplasm | 383 ± 34 | 150 ± 13 | 357 ± 19 |

Data are given as the mean ± SEM and $n$ is the number of cells analyzed.

rimmed intermediate erythroblasts > late erythroblasts > early bone marrow red cells > late bone marrow red cells > peripheral blood red cells. This indicates that there is a continuous decrease in rubidium transport as the erythroid cells develop into

**Table 2.** Distribution of elemental concentrations in rabbit bone marrow exposed to 18 mM RbCl for 30 min

| | P | Cl | K | Rb | K + Rb | K/Rb |
|---|---|---|---|---|---|---|
| | | | (mmol/kg dry wt) | | | |
| Erythrocytes | | | | | | |
| Late ($n$ = 35) | 95 ± 4 | 131 ± 5 | 349 ± 17 | 5 ± 2 | 354 | 70 |
| Early ($n$ = 21) | 196 ± 10 | 148 ± 8 | 373 ± 14 | 29 ± 4 | 402 | 13 |
| Erythroblasts | | | | | | |
| Late ($n$ = 13) | | | | | | |
| Nucleus | 861 ± 78 | 110 ± 10 | 491 ± 43 | 44 ± 6 | 535 | 11 |
| Cytoplasm | 468 ± 67 | 116 ± 13 | 465 ± 45 | 41 ± 5 | 506 | 11 |
| Thin-rimmed ($n$ = 19) | | | | | | |
| Nucleus | 770 ± 54 | 97 ± 9 | 310 ± 34 | 104 ± 10 | 414 | 3.0 |
| Cytoplasm | 446 ± 72 | 141 ± 13 | 308 ± 35 | 82 ± 11 | 390 | 3.8 |
| Early ($n$ = 12) | | | | | | |
| Nucleus | 655 ± 65 | 121 ± 17 | 382 ± 29 | 114 ± 16 | 496 | 3.4 |
| Cytoplasm | 688 ± 84 | 133 ± 26 | 370 ± 47 | 82 ± 17 | 452 | 4.5 |
| Eosinophilic myelocytes | | | | | | |
| All ($n$ = 18) | | | | | | |
| Nucleus | 553 ± 45 | 102 ± 8 | 266 ± 20 | 126 ± 8 | 392 | 2.1 |
| Cytoplasm | 409 ± 41 | 116 ± 10 | 236 ± 25 | 110 ± 10 | 346 | 2.1 |

Data are given as the mean ± SEM and $n$ is the number of cells analyzed.

mature red cells. In addition the rubidium uptake was measured for a 15-min incubation period. Results for 15-min and 30-min incubation periods are compared in Fig. 5. In both cases the highest rubidium uptakes were observed in eosinophilic myelocytes and the lowest were found in the late red blood cells.

## Discussion

In order to determine at what stage rubidium uptake rates are reduced during maturation of the erythroid cells, it was essential to identify the various cell types in cryosections. The early and late erythroid cells could be characterized morphologically and confirmed by their iron concentrations. But without appropriate fixing and staining, it is not easy to make fine distinction between the various erythroblastic cells. It is possible, however, to identify the erythroid cells observed in thin cryosections on the basis of features similar to those seen in conventional electron microscopic sections (*see* Tanaka & Goodman, 1972). Presumably the early erythroid cells described here were the proerythroblastic and basophilic erythroblasts, and late erythroid cells were the polychromatophilic erythroblasts and normablasts. The intermediate (thin-rimmed) erythroid cells were likely to comprise mainly the late basophilic erythroblastic and/or polychromatophilic erythroblasts. The reticulocytes, the immature red blood cells, are normally identified by the presence

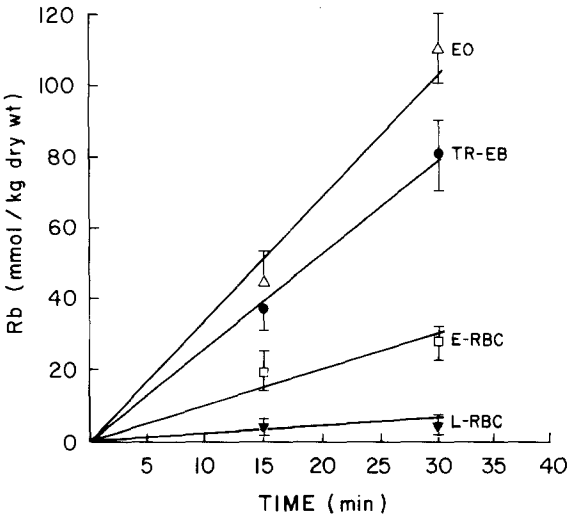


**Fig. 5.** Comparison of 15-min and 30-min cytoplasmic rubidium uptake levels in eosinophilic myelocyte (*EO*), thin-rimmed intermediate erythroblast (*TR-EB*), early bone marrow red blood cells (*E-RBC*), and late bone marrow red blood cells (*L-RBC*)

of reticular structures after staining with new methylene blue. These structures are presumed to be present in the population called early red cells, which are characterized by a higher level of phosphorus and lower level of iron (hemoglobin) than in the late red cells. At the surface of the sample, the cells were best preserved by rapid freezing, and both the early and late red cells had differences in electron density and iron concentrations. The lacy

cells were found several microns from the outer surface of the sample. Here the late red cells appeared lacy, a differential effect presumably due to their high hemoglobin concentration and lower water content. Although the late red cells appeared lacy or smooth depending on their relationship to the sample surface, they all had similar X-ray spectra.

The uptake of rubidium measured by X-ray microanalysis represents the net influx of this ion during the period of measurement. No distinction has been made between active transport component and that due to passive diffusion. Nor can we tell the extent of back-flux when rubidium accumulation is high. Nevertheless, the incubations of the bone marrow cells give us a comparison of the exchange rates in the various cell types. Furthermore, since the sums of potassium and rubidium after incubation in rubidium were similar to the potassium concentration originally present in the cells, it appears that there is a one-to-one exchange of potassium with rubidium in the cell types examined. It should be noted that the changes in Rb are so small in the early and late erythrocytes that they provide little information on this point. Nevertheless, it seems reasonable to use rubidium as a marker for potassium in transport studies especially in the nucleated erythroid cells, which have high potassium turnover rates.

The nuclear potassium was significantly higher than the cytoplasmic potassium concentrations in both the erythroid and myeloid cells of the rabbits. This is in contrast to the observations in the dog bone marrow cells (Kirk et al., 1983) and some tissues (Beck et al., 1980; Andrews et al., 1983), where the nuclear and cytoplasmic potassium concentrations were similar. Higher nuclear potassium has been observed in other tissues (Gupta & Hall, 1982). The reason for these higher nuclear potassium concentrations in rabbit cells is not known.

A comparison of the relative rubidium uptake rates of the various bone marrow cells shows that the granulocytes had the highest exchange rate. This is not surprising since it is known that potassium transport in peripheral leukocytes is at least an order of magnitude faster than the peripheral red blood cells (Civivalli & Nathan, 1974; Naccache, Showell, Becker & Shaafi, 1977; Simchowitz, Spilberg & Bedewecr, 1982). In peripheral red cells of rabbit, the rubidium uptake was so slow that it was not possible to measure the accumulation of rubidium in an hour or two. Therefore, red cells were incubated for ten hours in order to have sufficiently high intracellular rubidium concentration for accurate quantitation of this element by X-ray microanalysis. This value was scaled so that it could best be compared with the 30-min values of the other cell types.

As the erythroid cells mature the rubidium uptake rate decreases. Thus, there is a loss of transport activity during the maturation process. This finding is consistent with that previously reported on dog erythroid cells which, in contrast to rabbit cells, have low intracellular concentrations of potassium (Lee & Kirk, 1982; Kirk *et al.*, 1983). In the dog a major reduction in K/Na ratio was seen before denucleation of the erythroid cells to form the reticulocytes. This was interpreted to indicate a reduction in K/Na active transport of the dog erythroid cells. In normal dog bone marrow, the change from high potassium to low potassium can be detected during the transition from basophilic erythroblast to polychromatophilic erythrobast or normablast. Apparently, these cells were already well past their early stage of differentiation when the change in membrane transport function occurs. In Friend erythroleukemic cells, there is evidence to suggest that the decrease in the rate of rubidium transport is associated with differentiation of the cells (Mager & Bernstein, 1978). The Friend cells also exhibit an increase in chloride transport and a decrease in sulphate transport during maturation induced by dimethylsulfoxide (Harper & Knauf, 1979). It is clear that in both rabbit and dog erythroid cells and the Friend cells the change in membrane transport function is associated with the maturation process. However, it is unlikely that induction of differentiation is the primary stimulus for the change in membrane function since membrane transport function change may occur early or late during the maturation process (Kirk et al., 1983).

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