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UPTAKE AND EFFLUX OF ^{86}RUBIDIUM BY HUMAN BLOOD PLATELETS

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SUMMARY

1. The Na^+ and K^+ concentrations and the influx and efflux kinetics of $^{86}Rb^+$ in human blood platelets have been studied.

2. The passive membrane permeability of these platelets was studied in platelet-rich plasma by means of a dialysis technique, thus avoiding harmful washing, centrifugation and resuspension procedures.

3. In platelets from platelet-rich plasma $^{86}Rb^+$ influx and efflux are in equilibrium, while in platelets from concentrated suspensions the $^{86}Rb^+$ influx is decreased, while the efflux is increased. This is correlated with a K^+ loss of 24% during a 1-h stay of platelets in concentrated suspensions.

4. These observations indicate that platelets from platelet-rich plasma are in a better condition compared to platelets from concentrated suspensions, which are leaky and functionally inferior.

5. The dialysis method described offers a new tool in the study of platelet function and possibly also of other single cell systems.

INTRODUCTION

The hemostatic properties of blood platelets appear to be related to a functional lability, being able to adhere, aggregate, undergo fusion and release substances. All these functions can be shown to be intimately related to their membrane characteristics[1–3]. Therefore, a sensitive measurement of transport and diffusion across their membranes may offer additional information about platelet function and viability. However, the investigation of functional properties is often hampered by the relative insensitivity of the methods used, as well as by the occurrence of non-specific release processes induced merely by the separation and concentration procedures.

In this study, which deals with the measurement of platelet cation content, active transport into, as well as passive diffusion out of, human blood platelets of the radioisotope $^{86}Rb^+$, a method is described in which handling of the platelets is reduced as much as possible.

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The passive cation permeability of the platelet membrane for $^{86}Rb^+$ has been measured by means of a dialysis technique, originally devised for application to liposomes (phospholipid micelles) by Bangham *et al.*[4]. This technique permits the use of platelet-rich plasma, instead of isolated platelets, concentrated in an artificial medium. Comparison of the properties of both preparations reveals the superiority of the more physiological platelet-rich plasma preparation.

$^{86}Rb^+$ was used to simulate K^+ in these experiments, since it behaves similarly, but has a longer half-life than $^{42}K^+$ (refs 5, 6).

METHODS

Platelet preparations

Human blood (8.5 ml) was obtained by venipuncture using disposable needles (internal diameter 1.5 mm) and was collected in polypropylene tubes containing 1.5 ml acid–citrate–dextrose solution (N.I.H., formula A). Platelet-rich plasma was obtained by centrifugation of the blood for 90 s at $1500 \times g$. After removal of the supernatant plasma, the remaining cell suspension was centrifuged again and the resulting supernatant was combined with the previous one. The entire procedure was carried out at room temperature. Normally, red cell contamination was less than five erythrocytes per 1000 platelets.

The platelet-rich plasma ($0.3 \cdot 10^9$–$0.4 \cdot 10^9$ platelets per ml) was used either unmodified (plasma K^+ concentration 3.1–4.1 mM) or after dialysis in order to obtain varying plasma K^+ concentrations. Dialysis was performed at room temperature by dialysing 1 vol. of platelet-rich plasma against 10 vol. of a buffered saline solution (Tris–HCl, 10 mM; glucose, 11.1 mM; NaCl, 137 mM; final pH 7.3). After 60 min of dialysis the plasma K^+ concentration varied between 0.4 and 0.7 mM. Intermediate K^+ concentrations were obtained by varying the dialysis period.

The concentrated platelet suspensions were obtained by centrifugation of platelet-rich plasma for 15 min at $1500 \times g$. The packed platelets were washed in a K^+ free Krebs–Ringer buffer solution (composition in mM: Na^+, 145; Cl^-, 118; HCO_3^-, 23; HPO_4^{2-}, 1.62; $H_2PO_4^-$, 0.39; glucose, 11.1; EDTA, 10; pH 6.5–6.7). After centrifugation, the platelets were resuspended in a small volume of a similar buffer (EDTA being omitted, glucose being 22.2 mM) with a pH of 7.3–7.5. Platelet concentrations were usually 10^9–$2 \cdot 10^9$ per ml of buffer.

Na^+ and K^+ determinations

The Na^+ and K^+ content of the platelets was determined in platelets of platelet-rich plasma as well as in platelets of concentrated platelet suspensions. Platelet K^+ was measured immediately after preparation of the platelets and after 1 h at 22 °C. In view of the relatively low platelet concentrations in platelet-rich plasma, special centrifugation tubes were designed, in accordance with those which were used by Born[7]. A 50-mm long precision capillary of 0.58-mm internal diameter was fused onto a 55 mm × 7 mm glass tube. The other end of the capillary was sealed. Into these tubes 1.5 ml platelet-rich plasma or 0.5 ml concentrated platelet suspension was pipetted and the tubes were centrifuged in a suitable centrifugation adaptor for 30 min at $1500 \times g$. Sometimes occasional platelet clumping was observed on the shoulder of the cytocrit tubes, where the glass tube merges into the capillary tube.

In those instances the preparations were discarded. From the resulting packed platelet column, contained in the narrow capillary, a known length was cut off and the contents were transferred into cold double-distilled water. After freezing and thawing twice, the mixture was centrifuged for 15 min at $10000 \times g$ and the supernatant was analysed for Na^+ and K^+ by means of an Eppendorff flame photometer. The apparatus was calibrated with standard solutions containing equimolar concentrations of Na^+ and K^+ in the range 0.1–0.8 mM. The contamination of trapped plasma was corrected for by means of parallel determinations in the presence of 0.05 μCi [^{14}C]inulin. In this case, after centrifugation, a known length of the platelet column was cut off and the content of the tube transferred to a liquid scintillation vial containing 1 ml 0.5% Triton X-100 solution. After thorough mixing of the content of the vial, the latter was filled with 9 ml Instagel (Packard Instrument Cy) and the material was counted in a Packard Tri-Carb Liquid Scintillation Counter. Measurements of the extracellular Na^+ and K^+ concentrations were made on appropriate dilutions of platelet-poor plasma or suspending medium.

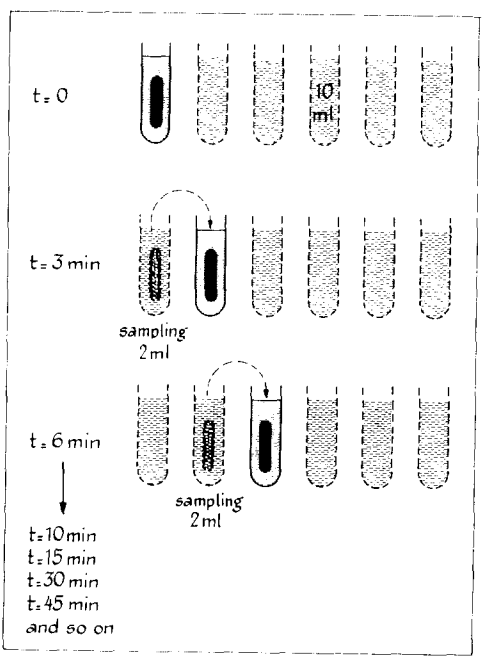

Fig. 1. Centrifugation tube with packed platelet sediment contained in the narrow capillary.

Fig. 2. Scheme of the dialysis and sampling procedure of the $^{86}Rb^+$ efflux measurements.

$^{86}Rb^+$ influx measurements

^{86}RbCl was obtained in aqueous solution with a specific activity of 3–5 Ci/g Rb (Radiochemical Centre, Amersham, England). Influx kinetics were only studied with platelet-rich plasma platelets, since experiments with platelets in concentrated suspensions failed to give reproducible results. After addition of 2.5 μCi $^{86}Rb^+$ per ml of unmodified platelet-rich plasma the mixture was incubated at 37 °C. After various time intervals ranging from 0 to 240 min, 1-ml portions were pipetted into cytocrit tubes as described in the previous section. Each tube was centrifuged for 15 min at $2000 \times g$ and the radioactivity of the resulting platelet sediment and the supernatant was determined by counting known aliquots of each in Philips PW 4138 counting tubes by means of a Philips PW 4251 counter. Correction for trapped plasma between the packed platelets was made by carrying out parallel experiments in the presence of [^{14}C]inulin as described before.

$^{86}Rb^+$ efflux measurements

The efflux of $^{86}Rb^+$ was determined by the procedure schematically presented in Fig. 2. 1 ml of platelet-rich plasma or concentrated platelet suspension, which had previously been incubated for 120 min at 37 °C with 2.5 or 0.5 μCi $^{86}Rb^+$, respectively, was transferred into a dialysis bag. The bag, containing the isotope-loaded platelet preparation, was dialysed in the first of a series of test tubes, each containing 10 ml of a modified Krebs-Ringer buffer solution (composition in mM: Na^+, 142; K^+, 3; Cl^-, 118; HCO_3^-, 23; HPO_4^{2-}, 1.62; $H_2PO_4^-$, 0.39; glucose, 22.2; pH 7.3–7.5). Dialysis was performed by vertically rotating the tubes at 30 rev./min. After 3 min, the dialysis bag was transferred to the second tube. Transferring to the third, fourth and fifth tube occurred at times 6, 10 and 15 min, respectively. Thereafter, the dialysis bag was transferred to each following tube every 15 min. All efflux experiments were carried out at room temperature (22 °C). The radioactivity in the dialysate was measured by counting 2-ml samples in a Philips PW 4251 counter. The radioactivity in the dialysis bag at the beginning of the efflux period was calculated by adding the total counts in each of the test tubes to the activity in the dialysis bag at the end of the experiment. Subtracting the counts in test tube 1 from the total activity gave the activity remaining in the dialysis bag at the end of the first dialysis period of 3 min; subtracting the sum of the counts of test tubes 1 and 2 gave the remaining activity after 6 min and so on. In this way the activity remaining in the dialysis bag after each transfer to the next tube could be calculated.

RESULTS

Platelet cation concentrations

The Na^+ and K^+ concentrations were determined in plasma and in platelets of 8 different healthy persons. The platelets were prepared as described under Methods (platelet-rich plasma and concentrated platelet suspension). As is shown in Table I, the K^+ concentration in platelets in concentrated suspensions tended to be lower than in platelets in platelet-rich plasma, whereas the reverse was found for the sodium concentration. Clear deviations in potassium concentration existed in samples which were left for 1 h at 22 °C. Under this condition, the K^+ loss in concentrated platelet suspensions amounted to 24.5%, whereas the platelets in platelet-rich plasma hardly lost K^+.

TABLE I

Na^+ AND K^+ CONCENTRATIONS IN PLASMA AND PLATELETS OF PLATELET-RICH PLASMA AND CONCENTRATED PLATELET SUSPENSIONS

Values for platelets are expressed in mmoles/l platelets (± S.E.) and for plasma as mM (± S.E.).

Sample	K^+	Na^+	*No. of expts*
Platelets in platelet-rich plasma	99.1 ± 4.8	45.8 ± 5.0	8
Platelets in platelet-rich plasma after 1 h stay	95.6		1
Platelets in concentrated suspensions	92.3 ± 7.1	49.1 ± 5.3	8
Platelets in concentrated suspensions after 1 h stay	69.7		1
Plasma	3.6 ± 0.2	168.8 ± 2.2	11

Influx measurements

The $^{86}Rb^+$ concentration in the platelets was calculated from the radioactivity in the platelets and the plasma and from the K^+ concentration in the plasma, assuming that at equilibrium the $^{86}Rb^+/K^+$ ratio is the same in plasma and platelets. This assumption is based on the experimental evidence that Rb^+ behaves identically to K^+[5,6]. The concentration is expressed as the 'apparent Rb^+ concentration' in mmoles/l of platelets (Fig. 3). The 'apparent Rb^+ concentration' of 88 mmoles/l of platelets agrees fairly well with the mean platelet K^+ content of 99 mmoles/l as determined directly.

Equations for the interpretation of influx measurements were given by Duncan[8]: when $\log(1-[Rb]_t/[Rb]_\infty)$ is plotted as a function of time (Fig. 4), where $[Rb]_t$ is the Rb^+ concentration at the sampling time t and $[Rb]_\infty$ the rubidium concentration at equilibrium, the resulting straight line indicates that the $^{86}Rb^+$ uptake process obeys first-order rate kinetics. Assuming that at $t=0$, $\log(1-[Rb]_t/[Rb]_\infty)$ is unity, it is possible to correct for the uptake which takes place during the time

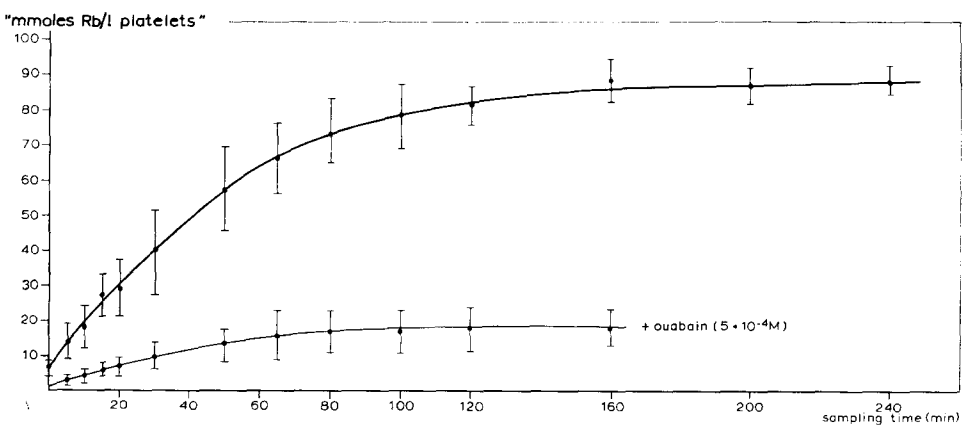

Fig. 3. $^{86}Rb^+$ uptake into human blood platelets. Apparent Rb^+ concentrations (± 2 S.E.) were calculated from the T/M ratios observed and from the plasma K^+ concentration.

period of centrifugation and separation of the platelets from the plasma. The half-time for the active uptake deduced from Fig. 4, by taking the time at which $\log(1-[Rb]_t/[Rb]_\infty)=0.5$, is 33 min, which is equivalent to a rate constant, k_{influx}, of $2.1\cdot10^{-2}$ min^{-1}. Using the uptake data from Figs 3 and 4, an initial influx velocity of 110 (S.E. 11) mmoles Rb^+ per l of platelets per h at 37 °C can be calculated. The enzyme system, now generally accepted to be responsible for active cation transport in cells, is the $(Na^+ + K^+)$-activated ATPase. The presence of this enzyme system in the platelet membrane has been established by several investigators[3,9,10]. It is therefore not surprising that parallel experiments in the presence of ouabain, a specific inhibitor of the $(Na^+ + K^+)$-activated ATPase system, revealed a marked inhibition of the $^{86}Rb^+$ uptake (Fig. 3).

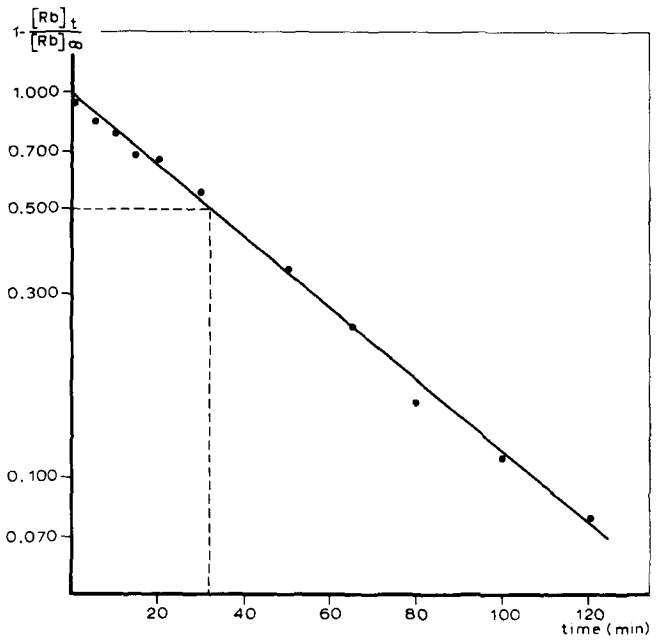

Fig. 4. Demonstration of first-order rate kinetics for the active uptake of $^{86}Rb^+$ by platelets. Use is made of the data of Fig. 3.

Efflux measurements

The efflux experiments were started after an initial loading period with $^{86}Rb^+$ of 2 h. This time period was chosen because the influx experiments (Fig. 3) indicate that at that time the equilibrium level has been reached. In Fig. 5, the efflux resulting from $^{86}Rb^+$-loaded platelets in platelet-rich plasma is presented as a function of time. The efflux of $^{86}Rb^+$ from the dialysis bag is biphasic and reveals that the $^{86}Rb^+$ is distributed between two compartments. These compartments presumably correspond to the extracellular space, which is responsible for the initial fast efflux, and the intracellular platelet space, from which $^{86}Rb^+$ is released at a much slower rate. When the radioactivity of the slow efflux component (extrapolated to $t=0$), at any time during the first 50 min, is subtracted from that in the whole dialysis bag, then the $^{86}Rb^+$ content of the extracellular fast-efflux compartment is obtained. Plotting the

log of this activity as a function of time, this fast efflux again represents an exponential process with a half-efflux time of about 5 min (Fig. 5, inset). This is in good agreement with the efflux kinetics of experiments in which a dialysis bag with $^{86}Rb^+$ without platelets is dialysed according to the same procedure (Fig. 5, inset).

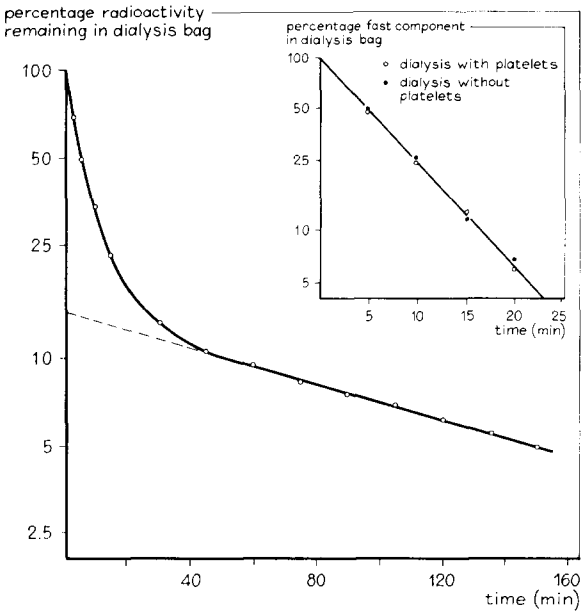

Fig. 5. Efflux of $^{86}Rb^+$ from platelet-rich plasma ($0.27 \cdot 10^9$ platelets per ml), previously incubated for 120 min at 37 °C with 2.5 μCi $^{86}RbCl$ per ml platelet-rich plasma.

The linearity of the log of the $^{86}Rb^+$ concentration as a function of time after 50 min indicates that the intracellular $^{86}Rb^+$ space apparently behaves like one single 'Rb^+/K^+' compartment. The half-efflux time determined on experiments with platelet-rich plasma of 15 different healthy persons is 98 min, equivalent to a mean efflux rate constant, k_{efflux}, of 0.71 (S.E. 0.03)$\cdot 10^{-2}$ min^{-1}.

The degree of uptake of $^{86}Rb^+$ into the platelets after the loading period can also be determined by analysis of the efflux kinetics. Extrapolation of the slow intracellular efflux component to $t=0$ (Fig. 5) reveals the percentage of $^{86}Rb^+$ initially present in the platelets. If this value is divided by the percentage of $^{86}Rb^+$ which would be present in the platelets under conditions of passive distribution of the $^{86}Rb^+$ between the extracellular and intracellular space, a tissue/medium (T/M) ratio is revealed which is indicative of the Rb^+ gradient generated during the initial loading period. Variation of the plasma potassium concentration between 0.3 and 4 mM through pre-dialysis of platelet-rich plasma against K^+-free buffer solutions, permits the study of the relation between the T/M ratio and the plasma K^+ concentration. Fig. 6 shows that at higher K^+ concentrations the $^{86}Rb^+$ uptake is reduced, which is understandable in view of the competition between K^+ and Rb^+ (ref. 11). Inserting the appropriate plasma K^+ concentrations in the values for T/M, the absolute accumulation of isotope, expressed as the 'apparent rubidium concentration', can be

calculated (Fig. 7). At 3.5 mM, a level of 95 mmoles 'tissue rubidium' per l of platelets is reached. This value agrees well with the value determined by the $^{86}Rb^{+}$-uptake method and with the K^{+} concentration in the platelets. This implies that complete exchange of K^{+} for isotope is obtained at equilibrium.

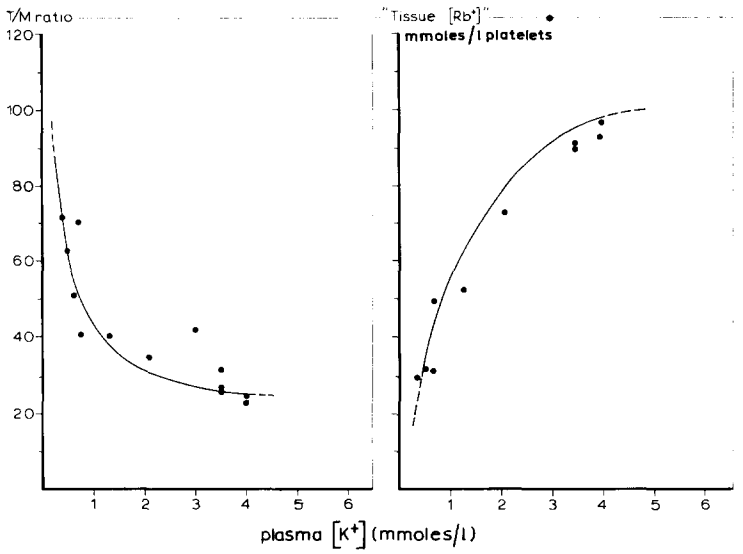

Fig. 6. Dependence of the $^{86}Rb^{+}$ accumulation ratio on the plasma K^{+} concentration.

Fig. 7. 'Tissue-rubidium' concentration as a function of the plasma K^{+} concentration, as calculated from the data of Fig. 6.

Comparison of platelet-rich plasma with concentrated platelet suspension

It appeared that the use of concentrated platelet suspensions showed several serious disadvantages over the use of platelet-rich plasma. The preparations of concentrated platelet suspensions often failed to give smooth suspensions and the experimental results from those preparations were not reproducible. Even in the absence of visible clumping, the results obtained with concentrated platelet suspensions tended to vary to a greater extent than did the results of experiments with platelet-rich plasma. Apart from these facts, the platelets in concentrated suspensions, diluted to the same platelet count with autologous platelet-poor plasma, failed to aggregate upon addition of ADP as measured according to the method of Born[12]. Table II presents a comparison between several data obtained for both preparations. As already shown in Table I, the platelets in concentrated suspensions lose considerably more K^{+} than platelets in platelet-rich plasma (24.5 *versus* 3.5% K^{+} loss during 1 h at room temperature, respectively). This finding is also reflected by the much higher efflux rate of $^{86}Rb^{+}$ from platelets in concentrated suspensions than from platelets in platelet-rich plasma (k_{efflux} $1.20 \cdot 10^{-2}$ *versus* $0.71 \cdot 10^{-2}$ min^{-1}, comparable to half-efflux times of 58 and 98 min, respectively). As mentioned before, technical difficulties made it impossible to obtain direct influx data for the active $^{86}Rb^{+}$ uptake into platelets in concentrated suspensions. However, indirect determination of the T/M ratio after a 120-min uptake period prior to the efflux measurements

reveals a markedly lowered $^{86}Rb^+$ uptake into the platelets in concentrated suspensions. The platelets in concentrated suspensions, being suspended in K^+-free buffer, cannot generate a T/M ratio over 29, whereas platelets in platelet-rich plasma reach much higher values at the lower K^+ concentrations (Fig. 6).

TABLE II

UPTAKE AND EFFLUX KINETICS OF PLATELETS FROM PLATELET-RICH PLASMA AND CONCENTRATED PLATELET SUSPENSIONS

Averages are given with S.E.

	Platelet-rich plasma	*Concentrated platelet suspension*	*No. of expts*
Influx rate constant at 37 °C ($\cdot 10^{-2}$ min^{-1})*	2.1 ± 0.3		8
Initial influx rate at 37 °C (mmoles Rb^+/l platelets/h)*	110 ± 11		8
Influx rate constant at 22 °C ($\cdot 10^{-2}$ min^{-1})**	0.74		
Efflux rate constant at 22 °C ($\cdot 10^{-2}$ min^{-1})*	0.71 ± 0.02	1.20 ± 0.07	15
T/M ratio***	58.5 ± 4.1	29 ± 7.5	15
Efflux at 22 °C (mmoles Rb^+/l platelets/h)	40.0	55.4	
% K^+ loss during 1 h stay at 22 °C	3.5	24.5	1

* Directly measured

** Calculated from the value at 37 °C, using a Q_{10} of 2.

*** Values obtained after 120 min incubation at 37 °C with $^{86}Rb^+$. The value for platelet-rich plasma is based on a plasma K^+ concentration of 0.5 mM.

DISCUSSION

A rather wide range is noticed in data of the normal platelet Na^+ and K^+ concentrations reported by various authors (Table III). This is probably due to the difficulties of keeping platelets intact during isolation and concentration procedures. Drastic treatments in order to remove the last traces of trapped plasma from between the platelets will cause damage, resulting in a loss of K^+ and a gain of Na^+. The finding that the K^+ concentration in platelets in platelet-rich plasma tends to be higher than in platelets of concentrated platelet suspensions (99 *versus* 92 mmoles/l of platelets), whereas the reverse is true for the Na^+ content (46 *versus* 49 mmoles/l of platelets), may support this explanation. From the various data presented in Table III one may conclude that the true platelet K^+ concentration will not be far from 99 mmoles/l of platelets, as determined by us.

Quantitative evaluation of the $^{86}Rb^+$ uptake of platelets in platelet-rich plasma, either determined directly (Fig. 3) or by analysis of the efflux kinetics (Fig. 5), indicates that, at equilibrium, complete exchange of K^+ for $^{86}Rb^+$ can be obtained. This finding is in accordance with the results of Gorstein *et al.*[17]. Both our influx and efflux studies revealed first-order rate kinetics for the $^{86}Rb^+$ movements. Therefore, strong evidence is obtained for the existence of only one intracellular K^+ compartment.

In spite of the fact that ouabain in a concentration of $5 \cdot 10^{-4}$ M completely

TABLE III

MEAN PLATELET Na^+ AND K^+ CONTENT; COMPARISON OF DATA FROM PRESENT STUDY WITH OTHER PUBLISHED DATA

The data are recalculated into mmoles/l platelets either by using data reported by the authors or using a mean platelet water content of 80% (w/v) and a mean platelet cellular volume of $7.7 \cdot 10^{-15}$ l (Baadenhuijsen[9]).

Reference	K^+ *(mmoles/l platelets)*	Na^+ *(mmoles/l platelets)*
Hartmann *et al.*[13] (1958)	69	27
Zieve and Solomon[14] (1966)	75	
Born[15] (1967)	96	
Cooley and Cohen[16] (1967)	95	
Gorstein *et al.*[17] (1967)	88	29
Aledort *et al.*[10] (1968)	88	13
Hess and Mohler[18] (1968)	105	48
Present study*	99	46

* Values represent those of platelets in platelet-rich plasma.

inhibits the $(Na^+ + K^+)$-activated ATPase in the enzyme assay[3,18], the $^{86}Rb^+$ uptake into the platelets was not completely inhibited by ouabain in this concentration (Fig. 3). This might be explained by the presence of morphological cavities and tubules (surface-connected tubular system), which prevents the ouabain from reaching all pump sites.

The efflux technique used in this study has enabled us to work with platelets without previous concentration and therefore, it was possible to compare the functional characteristics of the platelet membrane in two different conditions: platelets of the relatively undisturbed platelet-rich plasma and platelets of concentrated platelet suspensions. From Table II it is clear that the use of concentrated platelet suspensions has several disadvantages. The platelets of concentrated platelet suspensions show a serious loss of K^+ when kept at room temperature. Previous work of Cooley and Cohen[16] also shows such a high rate of K^+ loss from platelets of concentrated suspensions in the absence of extracellular K^+.

Fig. 6 shows that for platelet-rich plasma the T/M ratio increases with decreasing extracellular K^+ concentration with a maximal T/M value of 70 at 0.4 mM K^+. Platelets in concentrated suspensions, however, suspended in a K^+-free $^{86}Rb^+$-containing medium take up isotope to a T/M ratio of only 29. Even in the case that at the highest platelet concentration used ($2 \cdot 10^9$ platelets in concentrated suspension per ml buffer) all intracellular K^+ would have been lost, the extracellular K^+ concentration would be lower than 1.5 mmole/l. Since with a similar extracellular K^+ concentration the platelets in platelet-rich plasma reach a T/M value of about 35, this indicates that the Na^+ pump works less well in the platelets in concentrated suspensions than in platelets in platelet-rich plasma.

In the platelets in concentrated suspensions k_{efflux} is $1.20 \cdot 10^{-2}$ min^{-1}, while in the platelets in platelet-rich plasma this value is only $0.71 \cdot 10^{-2}$ min^{-1}. The efflux can be calculated by

$$J = k_{efflux}([K^+]_{in.av.} - [K^+]_{ex.})$$

in which J, the efflux, is expressed in mmoles K^+ per l of platelets per h, $[K^+]_{in.av.}$ is the average intracellular K^+ concentration, and $[K^+]_{ex}$ is the plasma K^+ concentration. $[K^+]_{in.av.}$ can be calculated according to Katchalsky and Curran[19] with the formula

$$[K^+]_{in.av.} = \frac{[K^+]_{t=0} - [K^+]_{t=60\ min}}{\ln \frac{[K^+]_{t=0}}{[K^+]_{t=60\ min}}}$$

This results in a value for J of 40.0 and 55.4 mmoles K^+ per l of platelets per h at 22 °C for platelets in platelet-rich plasma and platelets in concentrated suspensions, respectively, giving an extra efflux of 15.4 mmoles/l of platelets per h from the platelets in concentrated suspensions. This value is in reasonable agreement with the K^+ loss of 22.6 mmoles K^+ as determined by the direct non-isotope measurements (Table I). This calculation is based on the assumption that the influx and efflux in platelet-rich plasma are of the same order of magnitude. This is reasonable in view of the following calculation. Assuming a Q_{10} of 2 for the platelet $(Na^+ + K^+)$-activated transport ATPase (Bonting *et al.*[20] and Bakkeren and Bonting[11]), a rate constant of the $^{86}Rb^+$ influx of $0.74 \cdot 10^{-2}$ min^{-1} at 22 °C can be calculated. Comparison of this value with the value of k_{efflux} of platelets in platelet-rich plasma of $0.71 \cdot 10^{-2}$ min^{-1} reveals a close agreement between the two values, implying that the platelets in platelet-rich plasma show no net efflux of Rb^+. This means that the platelets in concentrated suspensions on the one hand have a lower pump capacity and on the other hand a higher efflux of isotope. This results in a net efflux, which is also reflected in the directly measured K^+ loss.

The values for both the Rb^+ influx and efflux can be compared with those obtained by other workers. Our results provide an initial influx velocity into platelets in platelet-rich plasma of 110 ± 11 mmoles/l platelets per h. This value is somewhat higher but in reasonable agreement with the values of Gorstein *et al.*[17] of 88 ± 5 mmoles K^+/l of platelets per h and of Born[15] of 86 ± 8 mmoles/l platelets/h. The small differences observed may be due to different experimental approaches. We believe that the fact that Cooley and Cohen[16] found a lower influx together with a net efflux for K^+ is in agreement with the evidence presented here, that platelets in concentrated suspensions are considerably less able to accumulate Rb^+ and also lose much more K^+ due to damage of the plasma membranes of the platelets in those concentrated preparations.

In an earlier study of Born and Gilson[21], dealing with the uptake of 5-hydroxytryptamine into blood platelets, an analogue technique was used in which the platelets were kept in a cellophane dialysis tube in their own plasma environment. In this technique samples were taken out of the bag at various times. It was concluded in their study that platelets, when maintained in their own plasma environment, could accumulate 1.5–2 times more 5-hydroxytryptamine than with a method in which platelets had been treated by ordinary techniques. As compared to the technique described by Born and Gilson[21], our technique has the additional advantage that the samples were taken out from the dialysis fluid which was regularly refreshed, leading to low backgrounds and avoiding a saturation effect, and making this method suitable for efflux studies.

Both the study of Born and Gilson[21] and the experiments described in this paper show that the centrifugation and washing procedures normally used in platelet studies are very harmful to the functional integrity of the platelet membrane. The use of this relatively mild dialysis technique has an additional advantage, since ionic movements occurring during the centrifugation and washing steps can influence the results, while in the dialysis method, values at each moment of the experiment can be obtained by extrapolation. The last advantage also makes this method suitable for the study of transport phenomena in other single cell systems like erythrocytes, yeast and bacteria.

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