Potassium Uptake and Release by Human Blood Platelets

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Thrombin is known to reduce the K⁺ content of human platelets, but the subcellular origin of the lost K⁺ is not known. The effect of aggregating agents on K⁺ release was studied in platelets labeled in plasma by preincubation with ⁴²KCl. Platelets were separated from plasma by gel filtration through Sepharose 2B equilibrated with K⁺-free Tyrode's buffer. Platelet K⁺ was 116 nEq/10⁶ platelets, of which 23% was found to be extracellular immediately after gel filtration. K⁺ influx was 65 nEq/10⁶ platelets/hr at pH 7.5 and was more rapid at pH 7.9. About 70% of cell K⁺ exchanged with plasma in 4 hr with first-order kinetics, while a minor fraction of about 30% exchanged with a slower time course. This slowly exchanging fraction of platelet K⁺ was thought to arise from heterogeneity in the platelet population. Epinephrine and ADP aggregated gel-filtered platelets and released serotonin, but with loss of only 5%-10% of cell K⁺ and no β-glucuronidase. In contrast, thrombin released up to 30% of platelet K⁺, whether aggregation occurred or was prevented by not stirring the cells. The specific activity of K⁺ released by all aggregating agents was identical to the specific activity of total platelet K⁺. Thrombin (0.01–0.2 NIH U/ml) released serotonin and also β-glucuronidase (an enzyme of the α-granule), and there was a linear relation between release of K⁺ and this enzyme (r = 0.88). No lysis of platelets occurred, since lactic dehydrogenase was not detected. Pretreatment of platelets with aspirin in vitro inhibited thrombin-induced release of serotonin but had no effect on the loss of K⁺ or β-glucuronidase. In contrast, the ingestion of aspirin by mouth inhibited the release of serotonin, β-glucuronidase, and K⁺ by thrombin. The data suggested that the K⁺ loss induced by thrombin was primarily derived from release of α-granules and that these organelles contained about 20% of the total platelet K⁺ in a freely exchangeable and nonsequestered state.
contain the acid hydrolases of platelets. These release reactions have been termed release I for dense granules and release II for α-granules, since the two reactions can be separated by their different time courses and sensitivities to thrombin. Thus, thrombin releases serotonin more rapidly than β-N-acetylglucosaminidase, which is an enzyme of the α-granule. Low doses of thrombin, like ADP and epinephrine, can release serotonin in the absence of α-granule release.

This study utilized gel filtration to obtain a platelet suspension in which Li replaced Na and K as the extracellular cation. Total platelet K+, as well as the loss of platelet K+ to the Li+ tyrode medium, were measured both by flame photometry and by isotopic counting, and the effect of aggregating agents on this K+ loss was studied. The results showed that thrombin caused a major reduction (20%-30%) in the K+ content of gel-filtered platelets by a mechanism that could be inhibited by aspirin, while ADP and epinephrine caused a smaller but still significant K+ loss.

**MATERIALS AND METHODS**

**Materials**

Bovine thrombin (Topical, Parke Davis and Co., Detroit, Mich.) was purified by ion exchange chromatography to a specific activity of 2300–2900 NIH U/mg protein. ADP (Sigma Chemical Co., St. Louis, Mo.) was dissolved at a concentration of 0.5 mM in 0.1 M sodium phosphate buffer, pH 6.8, and stored frozen until used. A stock solution of 2.5 mM l-epinephrine bitartrate (Sigma) was prepared in 0.01 N-HCl to minimize oxidation and stored in the dark at 4°C. Human fibrinogen (Kabi, Stockholm, Sweden) was dissolved in 0.15 M LiCl at 37°C at a concentration of 30 mg/ml as determined using a ε at 281 nm = 15.1. The solution was dialyzed against 0.15 M LiCl at 23°C for 48 hr with multiple changes of buffer. Acetylsalicylic acid (Fisher Scientific Co., Fairlawn, N.J.) was dissolved in water by adding a chemically equivalent amount of lithium hydroxide, and the stock solution was adjusted to 100 mM. Triton X-100 and crystalline human serum albumin were obtained from Sigma Chemical, lithium orthophosphate and lithium carbonate from K and K Laboratories, Inc., Plainview, N.Y., and lithium chloride and magnesium chloride from Fisher Scientific.

**Preparation of Platelet-rich Plasma (PRP)**

All PRP samples were handled in plastic equipment. Nine volumes of venous blood were collected into a syringe containing 1 volume of 0.13 M trisodium citrate as anticoagulant. The blood was centrifuged at room temperature for 10 min at 120 g and the supernatant PRP removed by aspiration. Platelet count of the PRP varied from 200,000 to 350,000 per μl, while the white blood cell count was 3 and the red blood cell count was 2/106 platelets. Platelet-poor plasma (PPP) was prepared from PRP by centrifugation at 2500 g for 15 min at 23°C.

**Incubation of PRP With 42KCl and 14C-Serotonin**

Stock 42KCl (2 mCi on assay date; New England Nuclear Corp., Boston, Mass.) was added to PRP at 37°C to give a final concentration of 2–8 μCi/ml. Potassium concentration of PRP was increased by this addition to between 5.9 and 9.3 mM. PRP was incubated with 42KCl for 90 min prior to gel filtration. 14C-serotonin, 0.01 volumes of stock solution, (30 μCi/ml in water; New England Nuclear) was added to PRP and incubated at 37°C for at least 20 min. When platelet K+ and serotonin were labeled together, both isotopes were added to PRP at 37°C for 90 min prior to gel filtration.

**Tyrode Media**

A modified Tyrode's buffer, without added Ca2+ ions was used both for equilibration of the gel column and for elution of PRP. The Li+-Tyrode buffer contained 127 mM LiCl, 11.9 mM Li2CO3.
and 0.42 mM Li$_3$PO$_4$ adjusted to pH 7.4 with 1 N HCl (approximately 14.0 ml/liter). The stock solution was prepared and stored at 4°C, and the following additions were made on the morning of each experiment: 10 mM glucose, 0.35 g/dl crystalline human serum albumin, and 1.05 mM MgCl$_2$. The final pH of the complete Tyrode was checked, and when necessary adjusted to pH 7.4 with 1 N LiOH. The complete Li$^+$-Tyrode medium was analyzed for other cations and contained approximately 6 mM K$^+$, 30 mM Na$^+$, and 20 μM Ca$^{2+}$ ions. In several experiments a Na$^+$-Tyrode buffer, pH 7.4, without added K$^+$ or Ca$^{2+}$ ions was used.

Separation of Platelets by Gel Filtration

Platelets were separated from plasma by gel filtration on a Sepharose-2B column modified from Tangen et al. The Sepharose-2B gel was mixed with 0.15 M LiCl or NaCl, and the slurry poured into a plastic syringe barrel and allowed to settle under gravity to form a column. A silk net with rectangular openings was used to retain the gel. The column was washed with at least 3 bed volumes of the Li$^+$ Tyrode or Na$^+$ Tyrode medium. PRP (3.0-4.0 ml) was applied to the top of the column and was eluted with a Li$^+$-Tyrode or Na$^+$-Tyrode medium in 1-ml fractions. Fractions corresponding to the void volume contained most of the applied platelets. These platelet fractions were pooled to give 3-4 ml of platelet suspension containing 150,000-250,000 platelets/μl. Platelet separation from plasma proteins was confirmed by finding an optical density at 280 nm of less than 0.02 of the Tyrode (without albumin) after centrifuging the pooled platelet fraction at 2500 g for 15 min. The suspension of gel-filtered platelets will be referred to as GFP.

Platelet K$^+$ Concentration

GFP suspended in Li$^+$-Tyrode were dissolved by addition of 0.02 volume of 10% Triton X-100 after an aliquot had been taken for platelet counting. The K$^+$ concentration of the solubilized platelets was measured by flame photometry in a Varian Techtron Model 1200 atomic absorption spectrophotometer at 766.5 nm using acetylene fuel plus compressed air. In each experiment the K$^+$ concentration of the Li$^+$-Tyrode buffer was measured (4-8 mM), and this value was subtracted from the values obtained for solubilized platelets. Platelets were counted in a Coulter Model ZB electronic particle counter (Coulter Electronics, Hialeah, Fla.).

Isotopic K$^+$ Uptake

K$^+$ entry was calculated from the uptake of radioactivity by platelets in PRP incubated with $^{42}$KCl. Platelets were separated from plasma by gel filtration and 1-ml aliquots of the pooled platelets were added to small polystyrene tubes and solubilized with 0.02 volume of 10% Triton X-100; the radioactivity was measured by gamma counting. A 1-ml aliquot of diluted plasma was counted together with the platelet samples. The uptake of K$^+$ for each sample was calculated as follows:

\[ \text{cpm/10}^8 \text{ platelets in sample} \times \frac{\text{specific activity of plasma K}^+}{\text{10}^8 \text{ platelets}} \]

and expressed as nEq/10$^8$ platelets. The initial rate of uptake of K$^+$ (i.e., the influx) was calculated from the uptake values after 20 and 40 min of incubation. After the aliquots of solubilized platelets were counted for radioactivity, they were analyzed for K$^+$ concentration by atomic absorption spectrophotometry. The simple ratio of isotopic K$^+$ uptake to the total K$^+$ concentration in the same sample represented the fraction of platelet K$^+$ which had exchanged with plasma.

Isotopic K$^+$ Release

Platelets prelabeled with $^{42}$K were separated from plasma by gel filtration using Li$^+$-Tyrode elution. The effluent platelets were pooled and samples taken for measurement of radioactivity, K$^+$ concentration, and platelet count. Aliquots were then added to tubes and warmed at 37°C, and aggregating agents were added. At least two control tubes containing no aggregating agent were incubated in parallel. Initial experiments showed that maximum K$^+$ release was not reached until 10 min of incubation with thrombin (0.04 U/ml). Thus platelets were incubated for 12 min,
after which the tubes were spun for 4 min at 12,000 g in an Eppendorf Model 3200 centrifuge. The supernatant was platelet free and was removed for radioactivity measurement and subsequent K+ analysis by atomic absorption spectrophotometry. The fractional release of platelet K+ to the supernatant could then be calculated, as well as the specific activity (cpm/nEq K+) of both platelet K+ and K+ released to the supernatant. Loss of K+ in the control tubes averaged 38% of cell K+ (23% at zero time plus another 15% during incubation and sampling).

**14C-Serotonin Release**

Following gel filtration of 14C-serotonin labeled platelets less than 3% of the serotonin was found in the extracellular medium. Platelets were incubated with aggregating agents and the per cent serotonin released was calculated as:

\[
\frac{cpm/ml \text{ in supernate after release} - cpm/ml \text{ in supernate of control}}{cpm/ml \text{ in whole GFP} - cpm/ml \text{ in supernate of control}} \times 100
\]

Control tubes containing no aggregating agent always released less than 4% of their serotonin. In experiments involving the simultaneous use of 42KCl, the samples taken for 14C-serotonin measurement were stored 5 days prior to liquid scintillation counting to allow the complete decay of 42K isotope.

**β-Glucuronidase**

For this assay a kit method (Sigma) was used which measured hydrolysis of phenolphthalein glucuronide substrate.24 Enzyme activity in the supernatant after centrifuging gel-filtered platelets was expressed as a percentage of the total enzyme activity obtained when the platelet suspension was dissolved in 0.02 volume of 10% Triton X-100.11 In agreement with Holmsen and Day,13 it was found that the maximum release of this enzyme which could be induced by thrombin was only about 20% of total platelet activity. Addition of Triton X-100 to the supernatant did not influence the enzyme activity.

**Lactic Dehydrogenase (LDH)**

LDH was assayed spectrophotometrically at 340 nm from the rate of oxidation of NADH in the presence of sodium pyruvate at pH 7.5.25

**Platelet Aggregation**

An aggregometer (Chrono-Log Corporation, Havertown, Pa.) was used as described by Born.26 Platelet-rich plasma, 0.5 ml, was stirred at 1200 rpm at 37°C in a glass cuvette, 8 mm in diameter, with use of a Teflon-coated bar, 1 x 0.5 mm. Light transmittance was continuously recorded (Chrono-Log Recorder) for 12 min following the addition of the aggregating agent. The per cent transmittance of PRP was recorded as 0, and that of PPP as 100. Platelet aggregation was unrelated to platelet concentration within the range studied. Prior to aggregating gel-filtered platelets with ADP or epinephrine, human fibrinogen was added to give a final concentration of 1.5 mg/ml. This addition increased the K+ concentration by 12 μM. No fibrinogen was added to gel-filtered platelets when thrombin was employed as the aggregating agent.

**Treatment of Platelets With Aspirin**

Two different protocols were used to pretreat the platelets with aspirin. The first consisted of preincubating PRP with 1 mM aspirin for 20 min at 37°C. In the second method, the donor ingested aspirin by mouth, 0.5 g twice daily for the 3 days immediately before venesection. After separation of PRP it was also preincubated with 1 mM aspirin for 20 min at 37°C. Aspirin-treated PRP was then gel filtered, and aliquots of the pooled platelets were added to the tubes and incubated 12 min at 37°C with thrombin.

**Statistics**

Mean values ± 1 SD are shown with the number of separate experiments in parentheses. The effect of aspirin on the extent of release was measured in experiments performed on different
Table 1. Release of Platelet K⁺ Resulting From Gel Filtration

<table>
<thead>
<tr>
<th>Incubation Time Prior to Gel Filtration (min)</th>
<th>Gel-filtered Platelets</th>
<th>Supernatant After Gel Filtration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isotopic ⁴²K (cpm/10⁸ Platelets)</td>
<td>Total K⁺ by Flame Photometry (nEq/10⁸ Platelets)</td>
</tr>
<tr>
<td>0</td>
<td>2,876</td>
<td>118</td>
</tr>
<tr>
<td>20</td>
<td>17,974</td>
<td>115</td>
</tr>
<tr>
<td>40</td>
<td>22,764</td>
<td>92</td>
</tr>
<tr>
<td>60</td>
<td>23,770</td>
<td>78</td>
</tr>
</tbody>
</table>

PRP was incubated with ⁴²KCl for various times and then gel filtered as described in Materials and Methods. An aliquot of the GFP was analyzed for total K⁺ by flame photometry and for isotopic ⁴²K by gamma counting. The platelet count of GFP varied from 1.36 to 2.18 x 10⁸/ml. Specific activity was calculated as the isotopic ⁴²K divided by total K⁺ (cpm/nEq). The remainder of the GFP was immediately centrifuged at 12,000 g for 4 min. The supernatant was analyzed for total K⁺ isotopic K⁺, and the specific activity (S.A.) was calculated as above. Potassium in PRP had a specific activity of 558 cpm/nEq.

days on the same donor and analyzed by the Student's t test of significance. Spearman's rank correlation, r, was used for correlations.

RESULTS

Characteristics of Lithium-Tyrode Gel-filtered Platelets

Filtration of platelet-rich plasma through a Sepharose 2B column and elution with Li⁺-Tyrode gave a platelet suspension in which Li⁺ replaced Na⁺ and K⁺ as the extracellular cation. The platelets were centrifuged immediately following elution and no LDH was found in the supernate, confirming that cytolysis had not occurred. If the platelets were prelabeled with ¹⁴C-serotonin, less than 3% of total radioactivity was found in the supernatant, which suggested that the release reaction had not occurred during the filtration procedure.

In contrast to these results, there was an immediate appearance of K⁺ in the supernatant following gel filtration which amounted to 23 ± 2 (n = 8) per cent of total platelet K⁺. Two observations indicated that this K⁺ came from within the cell and that it did not represent incomplete separation of platelets from plasma K⁺. First, filtration of PPP through an identical column showed a K⁺ concentration of less than 0.2 μM in the fractions in which platelets normally were eluted. This value was less than 1 per cent of supernatant K⁺ concentrations found when these fractions contained platelets.

The second line of evidence was derived by incubating PRP with ⁴²KCl for various times, gel filtering the platelets, and immediately centrifuging an aliquot of the filtered platelets. The specific activity of supernatant (extracellular) K⁺ and the total platelet K⁺ was then measured at each time (Table 1). Over 1 hr the platelets progressively gained isotopic K⁺. On the other hand, the total K⁺ content measured by atomic absorption decreased in this experiment. After each gel filtration approximately 20% of the isotopic K⁺ taken up by platelets appeared in the supernatant, while a similar fraction of total platelet K⁺ was also found in the supernatant (extracellular) fluid. Table 1 also shows that the specific activities of platelet and supernatant K⁺ both rose with time and reached 305 and 228 cpm/nEq, respectively, at 60 min. Since the specific activity of plasma K⁺ was constant at 558 cpm/nEq, it was clear that the super-
natant K⁺ came from the platelets as a result of gel filtration. Thus, in subsequent studies both the intra- and extracellular K⁺ of the effluent platelets were analyzed together as an estimate of total platelet K⁺. The immediate loss of cell K⁺ was the same whether platelets were eluted with Na⁺-Tyrode or Li⁺-Tyrode medium and was observed even in aspirin-pretreated cells. Moreover, loss of cell K⁺ continued as the gel-filtered platelets stood in Tyrode media. This further loss amounted to 30%, 18%, and 5% of cell K⁺ over 30 min incubation at 37°C, 22°C, and 4°C, respectively.

**K⁺ Permeability of Platelets**

The uptake of ⁴²K by platelets suspended in citrated plasma at 37°C was studied. At various times up to 4 hr of incubation an aliquot of PRP was removed from the incubation tube and the platelets separated from plasma by gel filtration with Li⁺-Tyrode elution. Platelet count, isotopic ⁴²K, and total K⁺ were measured on each sample. Initial platelet K⁺ for 12 normal donors was 116 ± 16 nEq/10⁸ platelets, while the initial rate of K⁺ uptake by platelets (i.e., influx) was 65 nEq/10⁸ platelets/hr. Half the K⁺ of platelets in citrated plasma (pH 7.5–7.6) exchanged in approximately 50 min, and when the logarithm of (1 – (fraction of platelet K⁺ exchanged with plasma)) was plotted versus time, a straight line was obtained for the first hour, indicating a first-order kinetic relationship (Fig. 1). K⁺ influx was accelerated if the citrated plasma was adjusted to pH 7.9 prior to isotope addition, while in contrast the addition of 20 mM imidazole CI, pH 7.5, slowed K⁺ influx. An incubation period of 90 min was routinely chosen for labeling platelets with ⁴²K in citrated plasma since 60%–70% of platelet K⁺ exchanged with plasma in this time.
K⁺ Release by Aggregating Agents

ADP and epinephrine initiated both aggregation and ¹⁴C-serotonin release (release I) from gel-filtered platelets suspended in Li⁺-Tyrode. The magnitude of both responses was similar to that of platelets in plasma (Table 2). ADP and epinephrine also caused a release of 8%–10% of potassium to the extracellular fluid. Thrombin (0.1 U/ml), caused a release of 23%–26% platelet K⁺ to the extracellular fluid. When platelets were prelabeled with ⁴²K and then gel-filtered with Li⁺-Tyrode, it was possible to measure the specific activity of the K⁺ released. This specific activity was between 110 and 133 cpm/nEq K⁺ for each of the three aggregating agents, and these values did not differ from the specific activity of the total platelet K⁺ (136 cpm/nEq K⁺). The greater release of K⁺ by thrombin did not arise from cytolysis of platelets by this enzyme, since LDH was not detected in supernatants after incubation with thrombin. It was confirmed that thrombin caused significant release of β-glucuronidase (an α-granular enzyme) (26% ± 5%), whereas ADP and epinephrine gave values (5% ± 3%, 12% ± 5%) which were only moderately increased above the no-agent control. Although stirring is a necessary cofactor for aggregation, it was not required for the release reactions of thrombin since that enzyme produced an unimpaired release of K⁺, serotonin, and β-glucuronidase in an un-stirred suspension of platelets (Table 2).

Thrombin Concentration Response Curves

It was clear from the above results that thrombin could induce release of serotonin, β-glucuronidase, and K⁺ in an unstirred suspension of platelets which were not aggregating. Thus, gel-filtered platelets were mixed with thrombin at a final concentration ranging from 0.01 to 0.2 U/ml and incubated without stirring for 12 min at 37°C. The release of serotonin was readily induced by low concentrations of thrombin and 0.04 U/ml caused a maximal effect (Fig. 2). In contrast, the release of β-glucuronidase was less sensitive to thrombin and only reached maximal values at thrombin concentrations of 0.05–0.08 U/ml. A maximum of 15%–20% of the total platelet β-glucuronidase could
be released by thrombin, which agreed with previous reports.\textsuperscript{9,13} Figure 2 shows that the loss of cell K$^+$ paralleled the release of $\beta$-glucuronidase and reached values of 20\% - 25\% of total cell K$^+$ at 0.1 U/ml thrombin, although when thrombin was increased to 0.2 U/ml (not shown), a K$^+$ loss of 25\% - 30\% was observed. There was no detectable loss of LDH, a cytoplasmic enzyme, from platelets incubated with any concentration of thrombin (0.01 - 0.2 U/ml).

\textbf{Correlation of K$^+$ and $\beta$-Glucuronidase Release}

The release of K$^+$, $\beta$-glucuronidase, and serotonin by thrombin in all experiments was compared and analyzed. Results obtained with other aggregating agents, ADP and epinephrine, were not included in the analysis since these agents released less than 10\% of the K$^+$ and $\beta$-glucuronidase. Potassium release correlated extremely well with $\beta$-glucuronidase release ($r = 0.88$, $p < 0.00001$) and analysis showed that approximately 25\% - 30\% of total K$^+$ was released under conditions in which maximal $\beta$-glucuronidase release occurred. There was also a correlation between release of K$^+$ and serotonin ($r = 0.55$, $p < 0.05$).

\textbf{Effect of Aspirin on K$^+$ and $\beta$-Glucuronidase Release}

Aspirin either taken orally in vivo or incubated with platelets in vitro is a potent inhibitor of the platelet release reaction.\textsuperscript{28-30} The effect of aspirin on the release of K$^+$ was therefore studied in gel-filtered platelets incubated with various concentrations of thrombin at 37\degree C. When Figs. 3 and 4 are compared to Fig. 2, it is clear that aspirin inhibited the release of $^{14}$C-serotonin by thrombin at concentrations of 0.01, 0.02, and 0.04 U/ml ($p < 0.01$ at each concentra-
Fig. 3. Effect of aspirin on the thrombin concentration response curves of gel-filtered platelets. Aspirin was taken by mouth for 3 days before the experiment and platelets were also exposed to 1 mM aspirin in vitro. Release of serotonin, β-glucuronidase, and K⁺ were all significantly impaired.

Fig. 4. Effect of aspirin added in vitro on the thrombin concentration response curve. Only the release of serotonin was significantly impaired.
on β-glucuronidase release was not observed when platelets were pretreated in vitro with aspirin, but did occur when aspirin was taken by mouth in addition to the in vitro pretreatment. Under the latter conditions there was a marked inhibition of β-glucuronidase release at concentrations of 0.06, 0.08, and 0.10 U/ml of thrombin \((p < 0.01; \text{Fig. 3})\). Higher concentrations of thrombin overcame the inhibitory effect of aspirin since 0.2 U/ml thrombin (not shown) gave the same release of β-glucuronidase from normal and aspirin-pretreated platelets.

Under all conditions the loss of K⁺ from platelets paralleled the release of β-glucuronidase. A full release of K⁺ amounting to about 25% of total platelet K⁺ could be produced by thrombin from platelets pretreated with aspirin in vitro. In contrast, only about 10% of cell K⁺ could be released from platelets pretreated both in vivo and in vitro with aspirin. Only these latter conditions inhibited the release of β-glucuronidase so that the loss of 10% of cell K⁺ was clearly related to the release of serotonin. A more important result was that aspirin inhibited loss of some 15% of cell K⁺, as well as inhibiting the release of an enzyme of the α-granule.

**DISCUSSION**

In this study the mean K⁺ concentration of GFP was 116 nEq/10⁸ platelets, which is similar to the value of 127 nEq/10⁸ platelets reported recently by Lages et al.³¹ Both these values were 30%-40% higher than earlier estimates, which measured the K⁺ concentration of a centrifuged pellet of platelets.¹² Gel filtration may modify platelets since there was an immediate loss of 23% of their K⁺ to the extracellular fluid. The nature of the platelet alteration on gel filtration is obscure, since our data showed no loss of serotonin or LDH, and GFP retained their reactivity to ADP, epinephrine, and thrombin. Part of the K⁺ loss may relate to the absence of K⁺ ions in the Tyrode media, which inhibited the pump and caused net K⁺ loss from the cell. However, gel filtration took only 8–10 min, in which time platelets should only lose 6% of their K⁺. Approximately 20% of platelet K⁺ was lost coincident with release II. Thrombin, 0.1–0.2 U/ml, released 25%–30% of platelet K⁺ without producing lysis of platelets. In contrast, epinephrine and ADP released only 5%–10% of platelet K⁺. Since all three agents caused release I while thrombin also elicited release II, the difference between the above figures (20%) must represent the K⁺ released in association with release II. Aspirin inhibited the release of both β-glucuronidase and that fraction of K⁺ which is associated with α-granules. Lages et al.,³¹ on the basis of finding normal platelet K⁺ in patients with storage pool disease, predicted that K⁺ would not be located in the dense granules. They suggested in a recent abstract that K⁺ may be released with acid hydrolases in release II.³³

Aspirin inhibits both the aggregation and the release of serotonin from platelets incubated with low concentrations of thrombin.²⁹ This effect is only observed at concentrations of thrombin just sufficient to produce platelet aggregation, and no inhibitory effect of aspirin is apparent at higher concentrations of thrombin.²⁹,³⁴,³⁵ The results shown in Figs. 3 and 4 confirm the inhibitory effect of aspirin on ¹⁴C-serotonin release from GFP incubated with thrombin.
over a narrow range of concentrations (0.01-0.04 U/ml). Concentrations of thrombin greater than 0.06 U/ml produced full release of serotonin regardless of the type of aspirin pretreatment. The present results also show that aspirin can inhibit the thrombin-induced release of β-glucuronidase, but this inhibition is overcome when thrombin is raised to 0.2 U/ml. Only aspirin taken by mouth as well as added in vitro exerted an inhibitory effect. Simple incubation of platelets with aspirin in vitro does not affect the thrombin-induced release of acid hydrolases, which agrees with a recent report. Similar experiments with aspirin support the concept that a fraction of cell K\(^+\) is associated with the lysosomal contents since inhibition of β-glucuronidase release by aspirin reduces the loss of K\(^+\) from 25% to 10%.

Specific activity (S.A.) measurements excluded the possibility that the K\(^+\) released from α-granules was present in a sequestered or nonexchangeable form since the cell K\(^+\) which was released by thrombin always had the same S.A. as total platelet K\(^+\). Similar results were found with the small amount of K\(^+\) (5%–10%) released in association with serotonin. K\(^+\) release in association with the discharge of α-granules or dense granules probably merely reflected the transfer of aqueous contents with their dissolved cation from within these granules to the surrounding medium. The release of platelet K\(^+\) caused by thrombin could not be due simply to accelerated K\(^+\)-K\(^+\) exchange across the platelet membrane since the loss of cell K\(^+\) was documented both by isotopic and flame-photometric measurements of that cation. Evidence for a “nonexchangeable” pool of K\(^+\) rests largely on the observation that K\(^+\) in plasma equilibrated with platelet K\(^+\) with a biphasic time course. In the present experiments the time course of K\(^+\) exchange between plasma and platelets confirmed the existence of a slowly exchanging fraction of cell K\(^+\), although this fraction did not seem to constitute more than 30% of the total exchangeable K\(^+\). The exchange of K\(^+\) isotope across the cell membrane of other tissues may also deviate from first-order kinetics, but such a deviation may be equally ascribed to a heterogeneity among the individual cells as to the presence of a nonexchangeable pool of K\(^+\) within each cell.

The increased K\(^+\) permeability of the platelet membrane at pH 7.9 correlates with the greater reactivity of platelets to aggregating agents at this pH. Indeed, the optimum pH for platelet aggregation in vitro is around 7.8, while platelets become unreactive at a pH of 6.5. Imidazole Cl, at a concentration of 20 mM and pH 7.5, decreases the K\(^+\) permeability of platelets. As above, this effect is on the transmembrane fluxes of K\(^+\), while the intracellular K\(^+\) concentration is unaltered. Imidazole is known to inhibit the second phase of platelet aggregation induced by ADP and norepinephrine, and it is interesting that a decreased cation permeability correlates with this reduced reactivity to aggregating agents. The parallel changes found between K\(^+\) influx and aggregability suggest that a general connection may be found between membrane permeability and the reactivity of platelets to aggregating agents.

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Potassium uptake and release by human blood platelets

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