Platelet Survival and Thrombosis

Peter D. Winocour, Marco Cattaneo, Diana Somers, Mary Richardson, Raelene L. Kinlough-Rathbone, and J. Fraser Mustard

This study examined the relation among platelet survival, thrombosis, and repeated vessel injury. With the use of $^{51}$Cr-labeled platelets, indwelling aortic catheters were shown to reduce platelet survival in rabbits and rats. In rabbits, thrombi were observed mainly at the aortic bifurcation and at the tip of the catheter. The amount of thrombus that formed in rabbits with short and long catheters was similar, but platelet survival was shortened only in rabbits with long indwelling aortic catheters. In rats, the aortic catheters did not cause thrombosis, and platelet survival was shortened significantly in rats with both short and long catheters, but was more pronounced in animals with longer catheters. In both rabbits and rats, long aortic catheters caused more extensive vessel injury than the short catheters and this was associated with greater platelet interaction with the vessel wall. Platelet survival cannot be used as an estimate of thrombus formation, but may reflect the extent and frequency of vessel wall injury. Thus, shortened platelet survival may represent increased platelet interaction with the damaged arterial wall and increased platelet consumption.

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Shortened platelet survival occurs in a number of conditions, including vessel wall injury, thrombosis, and the insertion of prosthetic devices into the circulation. The mechanisms responsible for the shortened platelet survival observed in association with vascular disease have not been established. Several possibilities have been considered: 1) platelets are removed from the circulation by incorporation into thrombi to an extent sufficient to reduce platelet survival; 2) since thrombi may undergo episodic dissolution and reformation, it is possible that the platelets that return to the circulation may have been modified in such a way that their rate of removal from the circulation is accelerated; 3) transient platelet interaction with the surface of a damaged or stimulated vessel wall in the absence of thrombosis modifies the platelets so that their survival in the circulation is diminished.

In previous experiments in rabbits and rats, we have found that the removal of the endothelium from aortas with a balloon catheter does not shorten platelet survival (Winocour, unpublished observation). The injury sites rapidly became nonreactive to extensive interaction with circulating platelets. In other experimental circumstances that cause repeated or continuous injury to the vessel wall, such as homocystinemia or hypercholesterolemia, platelet survival is shortened. Meuleman and his colleagues found that indwelling aortic catheters that cause repeated injury shorten platelet survival in rats and rabbits but they did not establish whether thrombus formation or repeated vessel injury was directly responsible for the shortened platelet survival. In our present experiments we examined the relation among platelet survival, repeated vessel injury, and thrombosis in rabbits and rats.

Methods

Platelet Survival Studies in Rabbits

Washed rabbit platelets were prepared from blood pooled from several New Zealand white rabbits (2 to 3 kg). The platelets were labeled with Na$_2^{51}$CrO$_4$ (specific activity 200 to 500 mCi/mg chromium, 100 Ci/mg for the platelets from each rabbit; Amersham Searle, Arlington Heights, Illinois) in the first washing fluid, washed once in calcium-free Tyrode solution, and resuspended in plasma. Platelets ($7 \times 10^9$ re-suspended in 5 ml plasma) were injected into rabbits via a marginal ear vein. For platelet survival studies with autologous platelets, washed platelets were prepared from 30 ml of blood taken from the central
ear artery into acid-citrate-dextrose. The platelets were prepared in the same way as for studies with homologous platelets and were resuspended in each animal's own plasma. The platelets (4–6 × 10⁸ resuspended in 2 ml of plasma) were re-injected via a marginal ear vein. The red cells from the 30 ml of blood were washed twice in Tyrode solution (pH 9.3) containing 0.1% glucose, resuspended to a volume of 10 ml with autologous plasma, and re-injected. Two hours following injection of ⁵¹Cr-platelets, samples of blood (1.5 ml) were collected into 0.5 ml of acid-citrate-dextrose anticoagulant. Platelet recovery was calculated as previously described by expressing the radioactivity of blood samples taken at 2 hours as a percentage of the injected radioactivity. The recovery of labeled platelets ranged from 70% to 80%. The radioactivity in the 2-hour sample was taken as 100% and the radioactivity in subsequent samples was expressed as a percentage of the 2-hour value. Further blood samples were taken 4 hours after injection of the platelets, at the same times twice daily for the next 3 days, and once on Day 4. The mean platelet life span was calculated from the radioactivity in individual samples using the gamma function described by Murphy and Bolling, Murphy et al., and Scheffel et al. The computer program for these calculations was kindly supplied by E.A. Murphy, Johns Hopkins University School of Medicine, Baltimore, Maryland. By ensuring that the samples were always collected at the same times, it was possible to avoid the large variations in the calculated platelet survival values that have been reported by others.

Three types of experiments were done with the rabbits: Experiment 1: platelet survival was measured in the same animals before and after placement of short aortic catheters (10 cm); Experiment 2: platelet survival was measured in a group of control rabbits and a group with longer aortic catheters (20 cm); and Experiment 3: platelet survival was measured in a group of rabbits that had had a sham operation and a group with the longer aortic catheters (20 cm). The experiments were done in this order because we initially expected the shorter catheters to cause thrombosis and shorten platelet survival (Experiment 1). When we failed to show shortened platelet survival, we carried out Experiment 2 using the longer catheters and we compared platelet survival under these conditions with platelet survival in normal rabbits. Then because of the possibility that the operative procedure itself might influence platelet survival, we carried out Experiment 3 with sham-operated animals as the controls.

**Platelet Survival Studies in Rats**

Suspensions of twice-washed platelets were prepared from blood obtained by cardiac puncture from several Wistar rats anesthetized with ether. The platelets were labeled with Na₂⁵¹CrO₄ (40 μCi/rat) in the first washing solution, washed once in calcium-free Tyrode solution and resuspended at a concentration of 1.5–2.5 × 10⁸ platelets/ml in Tyrode solution (pH 7.35) containing 0.35% bovine albumin. The labeled platelets (1 ml) were injected into a superficial penile vein through a 27 G needle; the rats were anesthetized with ether during the injection. The syringes used were weighed before and after injection to determine the exact volume of platelet suspension injected. Samples (100 μl) of the platelet suspension were weighed and the radioactivity was determined in a gamma counter. From this information, the volume of suspension injected and the total amount of radioactivity injected into each rat was calculated.

Two hours after the injections of ⁵¹Cr-platelets, samples of blood were taken into heparinized microhematocrit tubes (DADE Division American Supply Corporation, Miami, Florida) from freshly cut ends of the tails of rats anesthetized with ether. Blood samples were centrifuged in a microhematocrit centrifuge (International Equipment Company, Needham Heights, Massachusetts) and after the hematocrits and the length of the columns of blood were recorded, the radioactivity of the samples was determined in a gamma counter. Using the weight of known lengths of water in a number of microhematocrit tubes, a standard diameter of the tubes was established. Knowing the length of the column of blood collected and the radioactivity of the sample, we calculated the radioactivity of each sample per cubic millimeter of blood. The samples taken 2 hours after injection of ⁵¹Cr-platelets were considered to represent the maximal circulating radioactivity and were assigned values of 100%. Platelet recovery was calculated in the same way as for rabbit platelets and ranged from 65% to 75%; the blood volume for these calculations was estimated as 4.5% of the body weight of the rats. Blood samples were taken 2 hours after injection of the platelets, and at 4, 17, 25, 41, 65, and 89 hours; the aortic catheter was introduced immediately after the 2-hour sample. Radioactivity in the samples was determined, the values were adjusted for changes in hematocrit due to blood loss, and the mean platelet life span was calculated in the same manner as for rabbit platelets.

The platelet turnover was estimated in the rabbits and rats from the whole blood platelet count (determined microscopically on the second day of the study) and the mean platelet life span.

**Insertion of Indwelling Aortic Catheters**

The right femoral arteries of the rabbits and rats anesthetized with sodium pentobarbital (20–30 mg/kg) were isolated close to the aortic bifurcation. In Experiment 1, the rabbits weighed approximately 4 kg and in Experiment 2, between 2 and 3 kg. The rats weighed between 450 and 500 g. A polyethylene catheter (rabbit: PE 90; rat: PE 10, Clay Adams, Becton, Dickinson and Company, Parsippany, New Jersey) was sealed at one end with wax and intro-
duced into the aorta via the femoral artery. The femoral artery was ligated with the catheter in place and the incision was closed with silk sutures. In some experiments, control animals had their femoral artery exposed and ligated as a "sham operation." Surgery was performed approximately 2 hours after the injection of $^{51}$Cr platelets into the animals.

The aortas were removed 4 days after insertion of the catheters. The rabbits were anesthetized with ketamine (Ketaset; 100 mg intramuscularly; Rogar/STB, Division of BTA products, London, Ontario) and atropine (0.1 mg subcutaneously) 5 to 10 minutes before they were given sodium pentobarbital (20 to 25 mg/kg intravenously). The rats were anesthetized with sodium pentobarbital (30 mg/kg intraperitoneally). The left carotid and left femoral arteries were also cannulated (rabbit: PE 190 for carotid and PE 90 for femoral artery; rat PE 90 for carotid artery and the femoral artery was transected). Immediately before the animals were perfused, they were given an injection of heparin (500 U/kg intravenously; Hepalean, Harris Laboratories, Brantford, Ontario). The animals were perfused via the left carotid artery with Locke's-Ringer solution (37°C) containing heparin (1 U/ml) at a pressure of 70 to 100 mm Hg. When the perfusate draining from the cannulated femoral artery was almost clear of blood, the Locke's-Ringer solution was replaced by 4% paraformaldehyde in phosphate buffer, pH 7.3. When we detected paraformaldehyde draining from the cannulated femoral artery, the vessel was tied off, and the perfusion was continued for approximately 10 minutes, after which the carotid and femoral arteries were tied.

The animals were left at 4°C for at least 18 hours, after which the aortas with the catheters in place were isolated, dissected free of extraneous tissue, removed from the animals, and cut into segments of about 2.5 cm. The radioactivity associated with each segment was determined and calculated as a percentage of the total radioactivity circulating 2 hours after the injection of $^{51}$Cr-labeled platelets. After we determined the radioactivity associated with the aorta and catheter, the thrombus associated with each segment of aorta was dissected free, allowed to dry overnight, and weighed.

In a few experiments with rabbits, $^{111}$In-labeled platelets were used to assess platelet accumulation on the injured aortas and in the thrombi. In six rats and six rabbits the extent to which the catheter had injured the aortas at 4 days was determined by giving them an injection of a solution of Evans blue dye (9 mg/kg; Harvey Laboratories, Incorporated, Philadelphia, Pennsylvania) 60 minutes before they were killed. The extent of Evans blue accumulation in the vessel wall is an estimate of endothelial alteration or injury.

Scanning Electron Microscopy

Segments of aorta (2.5 cm) were glued onto a cover glass with cyanoacrylic glue (Eastman 910; Eastman Chemical Company, Toronto, Ontario) using the method of Richardson and Moore. This adhesive glue forms a strong bond between the wet tissue and the cover glass but does not penetrate the tissue; the bond is formed within 1 to 2 minutes and the contraction on hardening is minimal. The tissue was kept moist with buffer solution (0.2M sodium cacodylate, pH 7.3) during mounting. Mounted samples were postfixed in osmium tetroxide, dehydrated in graded ethanol, critical-point dried from CO$_2$, mounted on a scanning electron microscopy stub, gold-coated, and examined with a Philips 501 SEM.

Light Microscopy

For light microscopy, the paraformaldehyde-fixed specimens were dehydrated through graded ethanol, embedded in Histowax, and cut in 4 μ-thick sections, which were stained with haematoxylin and eosin or martius yellow-scarlet red-celestine blue. Specimens were examined with a Zeiss microscope.

Results

Rabbit Experiments

Table 1 shows that the survival of autologous platelets in rabbits weighing approximately 4 kg was not reduced with a short aortic catheter (Experiment 1), but that platelet survival was shortened in experiments with a longer catheter (Experiment 2) in rabbits weighing 2 to 3 kg. In the first experiment, platelet survival was studied before and after insertion of the catheters. The interval between the two platelet survival studies in each animal was 6 weeks. In this experiment, the 10 cm aortic catheters extended approximately 5 cm into the abdominal aorta. The platelet counts, platelet survival, and turnover in each animal was similar before and after the introduction of the catheter (table 1). In the second experiment, the catheters extended 15 cm into the aorta and reached the thoracic region. Under these circumstances, the survival of autologous platelets was significantly shorter in the animals with the indwelling catheters than in the controls studied at the same time. The platelet counts in the rabbits with aortic catheters were significantly reduced, but platelet turnover was similar in both groups of rabbits. The radioactivity associated with the thrombi and aortas in animals with the longer catheters (Experiment 2) was approximately twice that observed in the animals with the shorter catheters. In both Experiments 1 and 2, thrombi were present on and around the aortic catheters principally near the catheter tips and at the bifurcation of the aortas. The weights of the thrombi were similar in both experiments (table 1).

To determine whether the operative procedure without insertion of the catheter affected platelet survival, we performed a third experiment in which one group of rabbits had a sham operation and the other
Table 1. Effect of Different Lengths of Indwelling Aortic Catheters In Rabbits on Platelet Survival, Platelet Turnover, Thrombus Weight and $^{51}$Cr Associated with the Aorta

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Before aortic catheter</th>
<th>After aortic catheter (10 cm)</th>
<th>No. of animals</th>
<th>Platelet survival (hrs)</th>
<th>Platelet count (no./mm$^3$)</th>
<th>Platelet turnover (no./mm$^3$/hr)</th>
<th>Thrombus weight (geometric means) mg</th>
<th>$^{51}$Cr-associated with aorta and thrombi (% total)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>6</td>
<td>55.4 ± 6.8</td>
<td>368,000 ± 20,600</td>
<td>7,010 ± 730</td>
<td>63,000 ± 870</td>
<td>1.38 ± 0.14</td>
<td>0.25 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td>4</td>
<td>62.4 ± 8.8</td>
<td>537,000 ± 36,600</td>
<td>9,040 ± 1,100</td>
<td>46,000 ± 1,020</td>
<td>1.37 ± 0.18</td>
<td>0.53 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Experiment 3</td>
<td>6</td>
<td>37.0 ± 5.6†</td>
<td>292,000 ± 37,000</td>
<td>8,350 ± 1,080</td>
<td>38,000 ± 37,000†</td>
<td>1.45 ± 0.11</td>
<td>0.53 ± 0.11</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE.
$^{51}$Cr was associated with the aorta 4 days after insertion of the catheters or sham operation and expressed as % total circulating prior to surgery.

Significance of the difference from the value before introduction of catheters or from sham-operated animals:

$^*p < 0.05.$
$^tp < 0.02.$
$^§p < 0.01.$
$^tp < 0.001.$

Table 2. Platelet Accumulation on Indwelling Aortic Catheters and Damaged Aortas In Rabbits

<table>
<thead>
<tr>
<th>Time after insertion of catheter when $^{51}$Cr-labeled platelets were injected</th>
<th>No. of animals</th>
<th>$^{51}$Cr associated with thrombus and aorta at 24 hrs* (% $^{51}$Cr platelets injected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days</td>
<td>15</td>
<td>1.6 ± 0.24</td>
</tr>
<tr>
<td>3 days</td>
<td>11</td>
<td>1.3 ± 0.64</td>
</tr>
<tr>
<td>6 days</td>
<td>16</td>
<td>0.96 ± 0.10†</td>
</tr>
</tbody>
</table>

Values are means ± SE.

*In experiments with $^{111}$indium-labeled platelets, more than 90% of the radioactivity was associated with the vessel wall in regions without thrombi.

$^tp < 0.02.$
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Table 3. Effect of Different Lengths of Indwelling Aortic Catheters in Rats on Platelet Survival, Platelet Turnover and $^{51}$Cr Associated with the Aorta

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Platelet survival (hrs)</th>
<th>Platelet count (no./mm$^3$)</th>
<th>Platelet turnover (no./mm$^3$/hr)</th>
<th>$^{51}$Cr associated with aorta (% total)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>103.1 ± 0.9</td>
<td>782,000 ± 25,200</td>
<td>7,600 ± 270</td>
</tr>
<tr>
<td>Sham operation</td>
<td>6</td>
<td>97.7 ± 2.4</td>
<td>813,000 ± 51,900</td>
<td>8,400 ± 720</td>
</tr>
<tr>
<td>Aortic catheters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7.5 cm)</td>
<td>4</td>
<td>88.5 ± 2.6†</td>
<td>786,000 ± 64,100</td>
<td>9,390 ± 870</td>
</tr>
<tr>
<td>(12.5 cm)</td>
<td>5</td>
<td>63.7 ± 5.1‡</td>
<td>684,000 ± 42,800</td>
<td>6,900 ± 1,150</td>
</tr>
</tbody>
</table>

Values are means ± SE.

$^{51}$Cr associated with the aorta 4 days after introduction of the catheter or sham operation expressed as % total circulating prior to surgery.

Significance of difference from sham-operated animals:

†p < 0.05.
‡p < 0.001.

Table 4. Platelet Survival, Platelet Turnover and $^{51}$Cr Associated with Aortas in Rats with Indwelling Aortic Catheters for Different Lengths of Time

<table>
<thead>
<tr>
<th>Time of $^{51}$Cr-platelet injection for platelet survival studies*</th>
<th>No. of animals</th>
<th>Platelet survival (hrs)</th>
<th>Platelet turnover (no./mm$^3$/hr)</th>
<th>$^{51}$Cr associated with aorta†(% total circulating at time 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 sham</td>
<td>6</td>
<td>74.0 ± 6.1</td>
<td>12,518 ± 1,366</td>
<td>0.004 ± 0.002</td>
</tr>
<tr>
<td>0 catheter</td>
<td>6</td>
<td>46.8 ± 4.0§</td>
<td>17,122 ± 2,033</td>
<td>0.011 ± 0.003</td>
</tr>
<tr>
<td>6-day catheter</td>
<td>6</td>
<td>46.4 ± 4.1§</td>
<td>16,607 ± 1,122†</td>
<td>0.018 ± 0.004§</td>
</tr>
</tbody>
</table>

Values are means ± SE. Platelet survival was calculated from Murphy’s gamma function.

*In the 0 time experiment, the $^{51}$Cr-platelets were given before insertion of the catheter. In the 6-day study, the labeled platelets were injected 6 days after insertion of the catheter.

†Measured 4 or 10 days after insertion of the catheter.

Significance of difference from 0 sham:

‡p < 0.05.
§p < 0.01.

To ascertain whether the platelet survival and turnover values measured during the initial period following insertion of the catheter would be similar to those measured when the animals had reached a steady state in relation to the chronic vessel injury, we did another set of experiments 6 days after insertion of the catheter (table 4). These studies showed that the effect of the catheter on platelet survival and turnover was similar to that observed in the period immediately after insertion of the catheter.

In the rat experiments, platelet accumulation on the damaged vessel wall was assessed immediately or 4 days after insertion of the catheters (table 5). More than 90% of the radioactivity was associated with the damaged vessel wall and less than 10% with the catheter at 4 days. Platelet accumulation over a 30-minute period on the vessel wall and on the catheters at 4 days was about one-half of the accumulation immediately following the acute injury after insertion of the catheters. However, platelet accumulation 4 days or 10 days after insertion of the catheters was similar (table 4), although much less than at 30 minutes (table 5).

Morphologic Studies

The extent to which the catheters damaged the vessel walls was assessed in rabbits and rats using Evans blue and scanning electron microscopy. Evans blue accumulation was most evident around the

Table 5. Radioactivity Associated with Aortas and Indwelling Aortic Catheters in Rats

<table>
<thead>
<tr>
<th>Time of infusion of $^{51}$Cr-labeled platelets after insertion of catheter</th>
<th>No. of animals</th>
<th>$^{51}$Cr associated with catheter and vessel wall at 30 min (% of injected $^{51}$Cr-labeled platelets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7</td>
<td>0.21 ± 0.061</td>
</tr>
<tr>
<td>4 days</td>
<td>6</td>
<td>0.093 ± 0.014*</td>
</tr>
</tbody>
</table>

Values are means ± SE.

*At 4 days, 91% of this radioactivity was with the vessel wall and less than 9% with the catheter.
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Figure 1. Inner surfaces of rat aortas stained in vivo with Evans blue dye injected 1 hour before the aortas were fixed in situ. A. A 7.5 cm catheter had been in place for 4 days. The arrow indicates the position of the tip of the catheter. B. A 12.5 cm catheter extending to the aortic arch had been in place for 4 days. The areas of Evans blue accumulation in the wall corresponded to the regions where the catheters were located and had damaged the wall.

In all animals the increased Evans blue accumulation was associated with the areas in which the catheters came into contact with the vessel wall.

Scanning electron microscopy of the vessel walls from sham-operated rats showed the endothelium to be intact (figure 2 A). In rats with indwelling aortic catheters, there were areas from which the endothelium was missing and platelets and white cells were adherent to the surface (figure 2 B). In contrast to sham-operated animals, the endothelial cells of the aortas adjacent to the injury site were contracted and had prominent nuclei (figure 2 B).

In addition to the platelets and white cells on the exposed subendothelium of rabbits, macroscopic thrombi composed of platelets, fibrin, and red blood cells were observed (figure 3).

Discussion

These experiments show that in rats and rabbits, platelet survival is shortened by indwelling aortic catheters. These observations agree with the results reported by others for the effects of indwelling catheters on platelet survival 10, 11, 20 These findings with indwelling aortic catheters contrast with other experiments in which we have found that the removal of most of the aortic endothelium from rabbit or rat aortas by passage of a balloon catheter does not shorten platelet survival 6 (Winocour, unpublished observations). Following deendothelialization, injured aortas rapidly became nonreactive to further accumulation of circulating platelets, 6 and 7 days after deendothelialization with a balloon catheter, the accumulation of radioactive platelets on the injured surface during a 30-minute period was less than 5% of that observed immediately after injury. Since in the present experiments there was still substantial plate-
Figure 2. A. Scanning electron micrograph of the endothelial surface of the aorta from a rat that had a sham operation. B. Scanning electron micrograph of a rat aorta in which a 12.5 cm catheter had been in place for 4 days. The area shown is from the aortic arch. plt = platelets, wbc = white blood cells, end = endothelium. X 1,500.

Platelet accumulation on the vessel walls 4 or 6 days after insertion of the aortic catheters, it seems reasonable to conclude that the catheters repeatedly injured the wall, resulting in continuous platelet interaction with the wall. It is possible that platelet interaction with the catheter itself contributed to the shortened platelet survival. However, since the indwelling catheters were of polyethylene, which has been reported to have little effect on platelet survival\(^{10, 21}\) and since our studies indicate that most of the platelets accumulated on the vessel wall rather than on the catheter, it seems reasonable to conclude that continuing platelet interaction with repeatedly injured vessel walls is the major factor responsible for shortened platelet survival. This conclusion is compatible with the results of other studies in which continuous or repeated injury to the vessel wall induced by intravenous infusions of homocystine in baboons or by feeding animals diets enriched in cholesterol\(^ {7-8}\) is associated with shortened platelet survival.

In the rabbit experiments, extensive thrombus formation could occur without an effect on platelet survival. In rats, where macroscopic thrombi did not form under the conditions of the experiments, the shortening of platelet survival was related to the length of catheters used.

The results of these experiments indicate that, although platelet survival does not appear to reflect thrombus formation directly, it can reflect the extent and frequency of vessel wall injury.

Figure 3. Light micrograph of thrombus obtained from the aorta of a rabbit with an indwelling aortic catheter. The thrombus is composed largely of platelets (plt), fibrin (f), and red blood cells (rbc). Martius yellow-scarlet red-celestine blue stain. X 150.
References


Index Terms: platelet survival • thrombosis • aortic catheters • vessel wall injury
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