Isolated Kidney Perfusion: A Model for Testing Platelet Function[1] (37554)

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(Introduced by Charles A. Hall)

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The early observations of Danielli (1) and the recent studies by Gimbrone *et al.* (2) and ourselves (3) indicate that platelets play an important role in maintaining the integrity of the endothelium and vascular bed during organ perfusion. We therefore developed the isolated, perfused rabbit kidney as a model system for evaluating platelet function. This model provides two important advantages. It permits the simultaneous monitoring of several parameters of renal function, using the contralateral kidney as a control; and it avoids the problems that would arise from interactions with other organ systems or with the physiologic control mechanisms in the intact animal. Essential to the development of this model was selection of the correct perfusion medium into which platelet concentrates or additives could be introduced.

Materials and Methods. Perfusion system. Isolated rabbit kidneys were prepared and perfused for 4 hr as described previously (4, 5) except that the initial hour of "flush" perfusion was omitted. The apparatus used in the current experiments is illustrated in Fig. 1. The organ was suspended in a plastic mesh basket to permit optimal positioning of the ureter and continuous measurement of kidney weight. Renal arterial perfusion pressure was measured with a mercury manometer, and urine was collected via a flexible plastic ureteral catheter, which drained into a calibrated test tube. A duplicate system without a kidney in the circuit served as a control for changes induced by the perfusion apparatus and by the peristaltic action of the pump. With the exception of the siliconized glass oxygenator and reservoir, the apparatus consisted entirely of polyvinyl chloride plastic.

The perfusion fluid consisted of 56 ml of 6% human albumin in Tyrode's solution, 8 ml of 3.8% trisodium citrate, and 16 ml of washed rabbit red blood cells. The red cells were washed 6 times in modified Tyrode's solution (without divalent cations), and careful attention was given to removal of the buffy coat. Platelet counts of this perfusate ranged from 2000 to 8000/mm^3. Two milliliters of 3.8% trisodium citrate were added hourly to maintain the final citrate concentration at 0.38%. Without this additive, platelet–fibrinous masses formed and became trapped in the system. In some experiments, platelet concentrates prepared (as described below) from rabbit, dog or human blood were added to the system.

Samples of perfusate for measurement of platelet count, hematocrit and clot retraction were taken from the renal venous outflow or reservoir at the following times: 0, 10 and 30 min, 1, 2, 3 and 4 hr. Platelets were counted by phase-contrast microscopy; hematocrit was measured by microhematocrit. Clot retraction was determined at regular intervals by a modification of the technique of Fearnley, Balmforth and Fearnley (6): One milliliter of perfusate was drawn from the reservoir through a siliconized 18-gauge needle into a plastic syringe containing an equal volume of citrated, intact, platelet-free rabbit plasma which had been diluted 1:5 with imidazole-buffered saline. This mixture was added to a 10 × 75 mm glass tube containing 0.1 ml of 25 units/ml bovine thrombin (Parke-Davis, Detroit, MI) and incubated in a water bath at 37°. Clot re-

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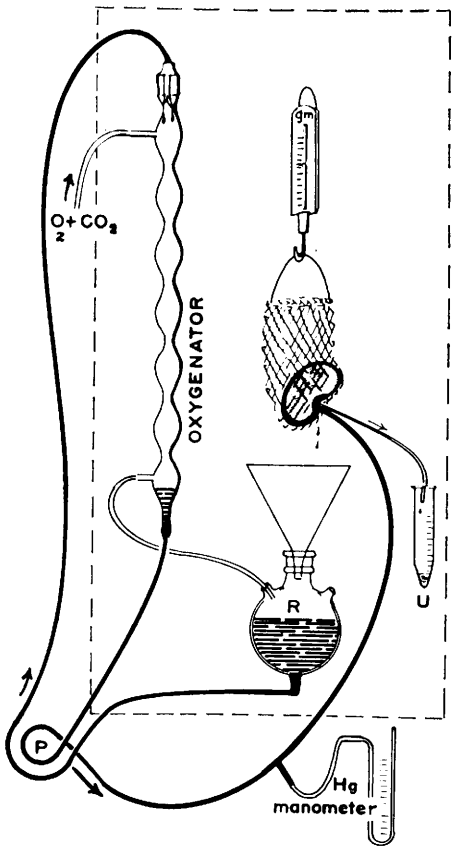

FIG. 1. Renal perfusion apparatus. (P), pump; (U), urine collecting tube; (R), reservoir; (O_2 + CO_2), air inlet.

traction was estimated visually every 30 min and quantitated after 2-hr incubation by measuring the volume of serum expressed from the clot.

Kidney surface bleeding times were monitored at hourly intervals by puncturing the uppermost kidney surface with a 25-gauge needle to a depth of approximately 1 mm. Clearance of urea (20 mg) and creatinine (4 mg) by perfused kidneys was measured as another index of renal function. Urine "hematocrit" was determined whenever red cells leaked into the urine. Gross appearance of the kidney was monitored throughout the perfusion period, and sections of tissues were taken for light microscopy. Histologic sections were prepared from formalin-fixed specimens and from frozen tissue slices stained with either hematoxylin–eosin or by the Picro-Mallory method, which identifies endothelium, fibrin and platelets (7).

Preparation of platelet concentrates. All procedures were performed at room temperature. Platelet-rich plasma (PRP) was prepared from 60 ml of citrated whole rabbit, dog or human blood by centrifugation in polycarbonate test tubes at 1500 rpm for 2–4 min. PRP was drawn off and the blood was respun as before. Both samples of PRP were pooled into polycarbonate test tubes, and the pH was adjusted to 6.5 with 0.25 *M* citric acid. This acidified PRP was centrifuged at 1500 rpm for 10–15 min. The resulting platelet buttons were left undisturbed for 1 hr before the platelet-poor supernatant plasma was removed (8, 9). The platelet buttons were then resuspended in 4 ml of 4% human albumin in modified Tyrode's solution containing 0.4% potassium chloride. In some studies, the solution used to resuspend the platelets contained 10^{-4} *M* adenosine, which inhibited platelet aggregation as measured with 1×10^{-4} and 1×10^{-6} *M* adenosine diphosphate (ADP).

Results. Figure 2 compares several functional parameters of kidneys perfused without additional platelets; with fresh, acidified rabbit platelet concentrates; and with fresh, acidified, adenosine-treated rabbit platelet concentrates. Organs perfused without extra platelets were significantly heavier than those perfused with platelets, and there was a noticeable increase in kidney size. Perfusion pressure increased gradually during perfusion with or without additional platelets in the medium, but adenosine-treated platelets produced a marked elevation to an average of 150 mm of mercury at 4 hr. The rapid increase in perfusion pressure with adenosine-treated platelets was accompanied by a drop in perfusate hematocrit from 2–4 hr, a decrease in circulated platelet count, and the appearance of many red blood cells in the urine. Similar results were obtained when 1–2-day-old or clumped platelets were perfused through kidneys.

The hematocrit of perfusates without additional platelets decreased steadily throughout perfusion, and there was moderate leakage of red blood cells into the urine. By

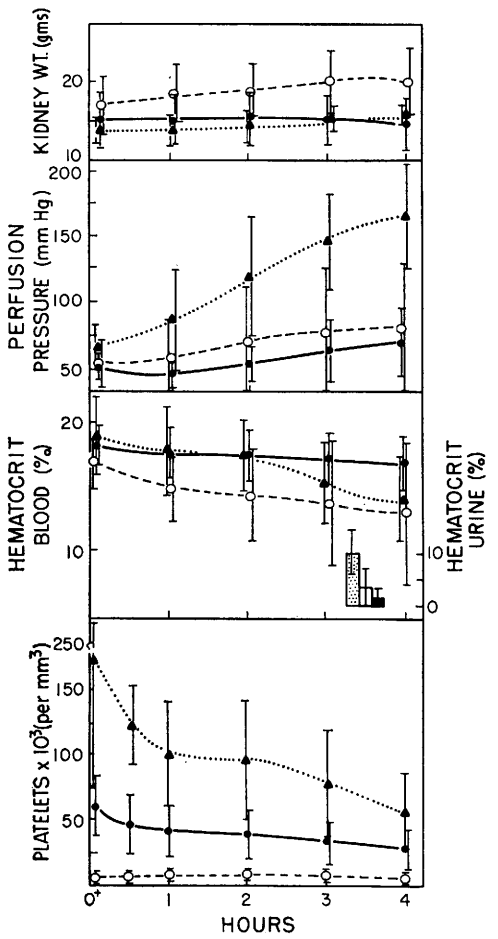

FIG. 2. Perfusion of rabbit kidneys without additional platelets (○- -) (11 experiments); with fresh, acidified rabbit platelets (●—) (15 experiments); and with fresh, acidified, adenosine-treated rabbit platelets (▲···) (13 experiments). The mean data ± SD at each sample point are shown. The data at 0⁺ represent samples obtained after 10 min to allow for complete mixing of the system. Curves were obtained by computer after fifth order regression analysis. The "hematocrit" of the urine (mean ± SD) after 3.5 hr of perfusion is shown by the bar graphs, as follows: fresh, acidified, adenosine-treated platelets, dotted bar; no platelets, open bar; fresh, acidified platelets, solid bar.

contrast, kidneys perfused with fresh, acidified rabbit platelets maintained a fairly stable hematocrit for 4 hr, and red cell leakage into the urine was minimal or absent. Continual oozing of blood from the kidney surface was observed in most kidneys perfused without additional platelets but did not occur with fresh, acidified platelets.

The platelet count during perfusion remained less than 15,000/mm³ if no additional platelets were provided. Following addition of fresh, acidified platelet concentrates, the count was increased to 30,000 to 80,000/mm³, with relatively little decrease during the 4-hr perfusion period. Addition of adenosine-treated platelet concentrates resulted in variable initial platelet counts ranging from 75,000 to 275,000/mm³. Most of these platelets were rapidly removed from the circulation.

Figure 3 compares histologic sections from kidneys perfused without additional platelets, with fresh, acidified rabbit platelets, and with adenosine-treated platelets. As mentioned above, interstitial hemorrhage and edema were a feature of perfusions without additional platelets (Fig. 3A), whereas vascular integrity was maintained in the presence of fresh, acidified platelets (Fig. 3B). Many blood vessels in kidneys perfused with adenosine-treated platelets were partially occluded by platelet–fibrinous deposits (Fig. 3C), and large platelet aggregates could be demonstrated in the lumen of some vessels (Fig. 3D).

Experiments similar to those described above with rabbit platelets were also performed with fresh dog and human platelet concentrates. Data from these studies indicated that the isolated rabbit kidney functions efficiently with platelet concentrates of different species. It was therefore feasible to conduct multiple parallel experiments with aliquots of fresh human platelets. Subsequent experiments determined that renal vascular integrity and hemostatic function are maintained by circulating levels of rabbit or human functional platelets between 20,000 and 100,000/mm³; these findings agree with those of Roy and Djerassi (10). Higher levels did not improve organ function and were often associated with rapid, early reduction in platelet counts (*e.g.*, adenosine-treated platelets, Fig. 2) and the appearance of small platelet clumps. These findings suggest that large numbers of platelets produce mechanical damage in the microcirculation.

In six experiments in which kidneys were

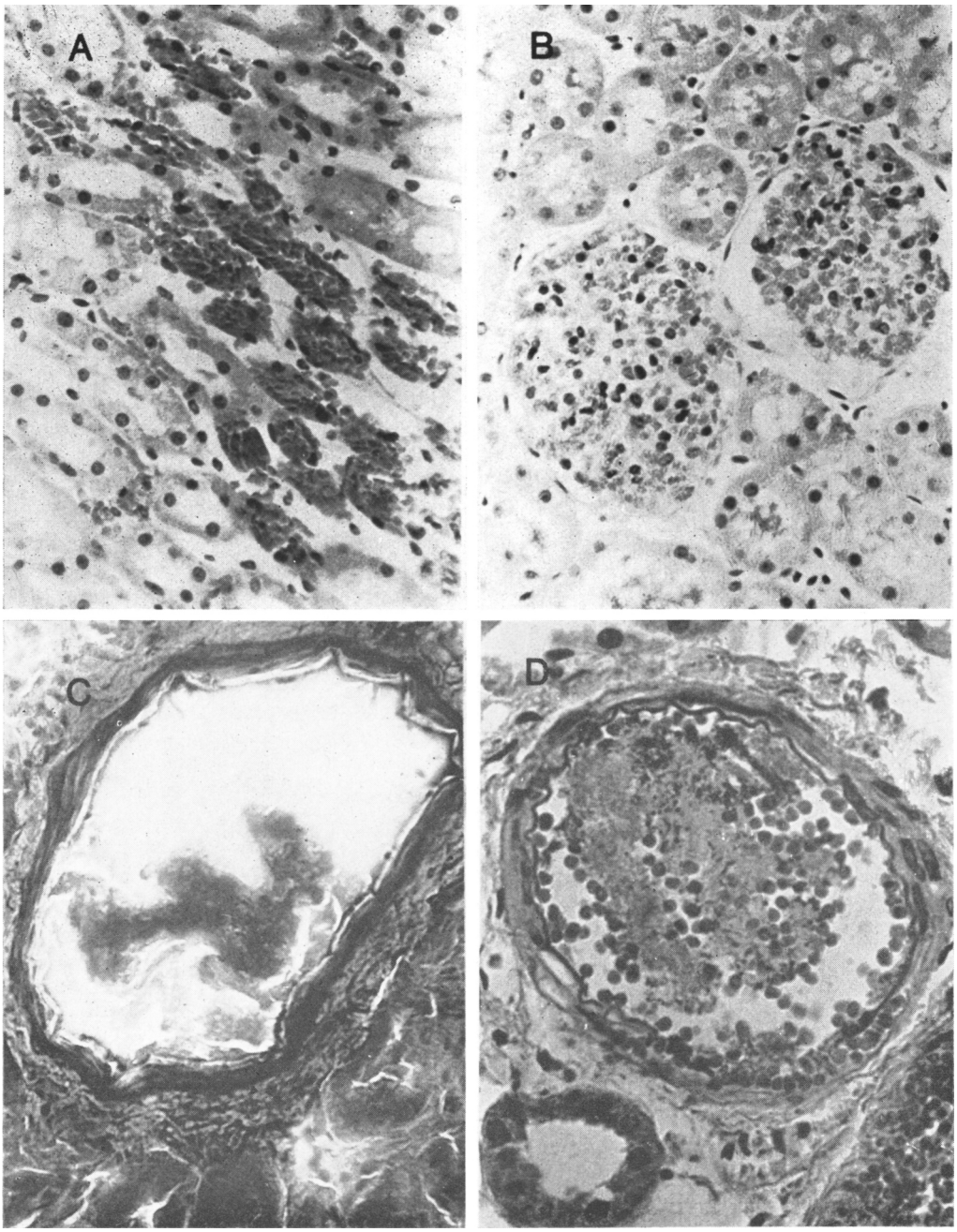

FIG. 3. Photomicrographs of histologic sections from perfused rabbit kidneys (81×). (A) Perfusion without additional platelets showing diffuse hemorrhage and edema of renal tubules (H and E stain). (B) Perfusion with fresh, acidified rabbit platelet concentrates. The renal glomerular and tubular architecture is intact with little evidence of interstitial hemorrhage or edema (H and E stain). (C) Perfusion with fresh, acidified, adenosine-treated rabbit platelet concentrates. The vessel is partially occluded by a large mass containing platelets and fibrin as identified by specific differential staining (Picro-Mallory stain). (D) Perfusion experiments as described in (C), showing a large platelet aggregate filling the vessel lumen. Some endothelial cell nuclei, cellular debris and bacteria can be seen trapped within the mass of degranulated platelets (H and E stain).

perfused with fresh acidified platelets, clearance of urea and creatinine was 0.4–1.1 and 0.3–1.0 ml/min, respectively. By contrast, kidneys perfused without platelets or with adenosine-treated platelets demonstrated impaired clearance.

There was significant prolongation of the surface bleeding time (> 30 min) and impairment of clot retraction (46 ± 16%) in kidneys perfused without additional platelets. Organs perfused with fresh, acidified rabbit or human platelets maintained their surface hemostatic capacity for the entire perfusion period. In addition, clot retraction of perfusates was normal (97 ± 7%) throughout the experiment.

Discussion. Preparation of a kidney for transplant usually involves perfusing the organ with fluids such as dextran–electrolyte solutions under conditions of hypothermia and hyperbaric oxygen. This procedure has been adopted because perfusion of kidneys with whole blood at normothermic temperatures results in increased vascular resistance and multiple thrombi (11). Platelets have been mentioned as one of the major causes of this problem (12–15). However, the routine use of heparin to anticoagulate the perfusion medium may also be responsible. Heparin is known to induce spontaneous platelet aggregation and increase platelet adhesiveness (16, 17); it functions as an anticoagulant primarily by inhibiting activated factor IX (18) and secondarily by preventing the action of thrombin on fibrinogen.

These observations prompted us to avoid heparin and select for the current experiments the albumin–Tyrode's–citrate perfusion medium used in our previous studies (4, 5). Occasional replacement of the citrate with heparin at a final concentration of 10 units/ml resulted in significant increases in renal perfusion pressure within 30 min, often accompanied by leakage of red blood cells into the urine. Perfusions using citrate consistently produced low perfusion pressures throughout the study, with improved organ preservation. Chelation of divalent cations in the perfusion medium by citrate apparently did not interfere with normal organ function. This unexpected finding may be explained by the low divalent cation binding coefficient of citrate, which makes it a relatively inefficient chelator; the available divalent cation concentration in our perfusion fluid is calculated to be 6.3×10^{-4} *M*. In addition, the use of a perfusion fluid devoid of coagulation activity has the distinct advantage of minimizing the chances for fibrin formation, which is unavoidable in perfusion systems that utilize anticoagulated whole blood (4, 11–15). This problem is especially relevant to studies with platelets because of their extreme sensitivity to doses of thrombin much smaller than those required to clot fibrinogen.

The results of our study confirm earlier reports (1–3) indicating that platelets are essential for the maintenance of vascular integrity. In the absence of functional platelets, increased peripheral vascular resistance and kidney weight, hematuria, impaired hemostasis, and hemorrhage and edema into the renal parenchyma were observed (Figs. 2 and 3A). Pretreatment of platelet concentrates with adenosine rendered them insensitive to ADP and resulted in impairment of vascular integrity and renal function, with partial or complete occlusion of vascular lumina (Figs. 2, 3C and D). Although these kidneys showed a marked increase in perfusion pressure, there was no weight gain, which may be explained by inadequate perfusion of renal tissue distal to occluded vessels. The formation of platelet aggregates in vessels perfused with adenosine-treated platelets was unexpected but may be related to the observation of Kinlough-Rathbone and Mustard (19) that platelets incubated with adenosine, washed and resuspended, were more sensitive to aggregation with ADP than similarly handled controls. Also, platelet uptake of adenosine during the incubation period may replenish the metabolic adenine nucleotide pool and render the platelets more sensitive to stimuli encountered during perfusion (19). Effective hemostasis, normal renal function, and preservation of renal architecture were achieved only when fresh, acidified platelet concentrates were incorporated into the perfusate (Figs. 2 and 3B). However, the perfused kidney model used in our experiments

functioned efficiently with platelet concentrates of several species.

The therapeutic value of platelet concentrates is now clearly recognized (20), but their short shelf life presents a major problem to blood banks, and a variety of methods has been developed to prolong platelet preservation. Evaluation of new methods has been severely limited because available platelet function tests measure only specific parameters (such as serotonin uptake and clot retraction) or platelet injury (such as release of platelet factor 3 and platelet enzymes). Unfortunately, none of these tests measure the effectiveness of infused platelet concentrates in maintaining hemostasis. It has been necessary, therefore, to undertake clinical studies in which survival of infused platelets is measured by radioactive labeling or by monitoring platelet counts in thrombocytopenic patients (20). Our studies indicate that the isolated, perfused rabbit kidney is a useful *ex vivo* model for measuring the hemostatic capacity of various platelet preparations. An extension of this study, dealing with the development and evaluation of new methods for platelet cryopreservation, will be reported in a subsequent publication.

Summary. The isolated, perfused rabbit kidney was developed as a model system for evaluating platelet function. Kidneys perfused without platelets or with stored or clumped platelets showed an increase in weight and perfusion pressure, could not maintain surface hemostasis, and produced bloody urine. Perfusion with nonfunctional adenosine-treated platelets demonstrated similar changes without an increase in kidney weight. By contrast, organs perfused with fresh, acidified platelet concentrates from several species functioned well for the duration of perfusion. Pathologic changes in kidneys perfused under these various conditions were consistent with the functional changes observed.

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