studied with platelets from concentrates prepared with PGE_1. They were diluted, to a final concentration of 300,000/mm^3 (about 1:10), in ABO-compatible, citrated, platelet-poor plasma. Aggregation in response to ADP ($2 \times 10^{-6}M$), collagen, and epinephrine ($10^{-5}M$) was normal. The survival times (measured with ^{51}Cr) of platelets that were concentrated and then immediately suspended in PGE_1 were also normal (Fig. 2).

Platelet clumping was inhibited by PGE_1 (12 to 750 ng/ml) in whole blood that was stored at 4°C; 50 to 100 percent of the platelets could be recovered even after the blood was chilled for 24 hours. Suspension of concentrated platelets was almost immediate in samples having the greatest concentrations of PGE_1, and with lesser concentrations, up to 30 minutes was required. Platelets obtained in this way were spherical in shape, but otherwise had normal structure. No additional change in shape occurred after incubation at 37°C for 1 hour. Aggregation in response to ADP, collagen, and epinephrine was the same as that of fresh platelets. Recovery (percent of total platelets remaining in the circulation 1 hour after injection) of such platelets labeled with ^{51}Cr was normal on transfusion, but their survival time was short (half-life, 1.5 days) and comparable to that of platelet concentrates stored for the same period of time at 4°C.

To determine whether PGE_1 might aid in short-term preservation of platelets, we performed 36 studies with platelet concentrates containing 50 to 700 ng of PGE_1 per milliliter. After the addition of PGE_1, the concentrates were stored for 24 to 120 hours, either at room temperature or at 4°C. In the presence of PGE_1, the change of shape of the platelet from a disc to a sphere was delayed, but it was not possible to demonstrate that platelet ultrastructure and function were better preserved with PGE_1 than they were in the absence of the drug. These were also the results of four studies in which 50 μg of PGE_1 was added daily to the concentrates previously mixed with the drug to compensate for inactivation of PGE_1 during storage. Survival studies with ^{51}Cr were performed only on platelets stored at 4°C with PGE_1 (250 ng/ml). Survival times of these platelets were uniformly short and were comparable to those of platelets stored without PGE_1 at 4°C for the same period of time.

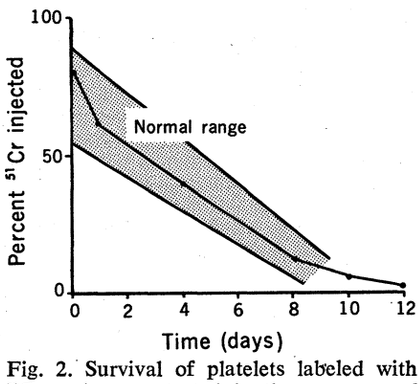

Fig. 2. Survival of platelets labeled with ^{51}Cr and concentrated in the presence of PGE_1 (24 ng/ml).

Platelet concentrates prepared with 250 ng of PGE_1 per milliliter to facilitate suspension do not tolerate freezing (*6*) any better than untreated platelets do.

Concentrations of PGE_1 as low as 24 μg/ml permit platelet pellets to be suspended almost immediately after rapid centrifugation with no adverse effect on their function or survival. Platelets similarly treated, but with no added PGE_1, clump irreversibly. Thus, addition of small amounts of PGE_1 at the time whole blood is collected reduces by 1 to 2 hours the time required for preparation of platelet concentrates. The addition of PGE_1 may also improve the quality of the final product by preventing even microscopic clumping of platelets.

Addition of as little as 4 μg of PGE_1 to a unit of whole blood at the time of collection permits 50 to 100 percent of the platelets initially present to be recovered in platelet concentrates, even after 24 hours of storage at 4°C. Although the survival time of such platelets is short, they appear to function normally in vitro. Clinical studies indicate that they are effective in stopping hemorrhage in thrombocytopenic patients. Although a concentration of PGE_1 of 12 ng/ml is insufficient to inhibit ADP-induced platelet aggregation, it does prevent platelet clumping in stored whole blood.

One of the factors limiting the availability of platelets for therapeutic purposes is that much of the blood used for transfusion is collected at mobile sites and must, by regulations of the National Institutes of Health and the American Association of Blood Banks, be "chilled immediately" upon collection. By the time this blood has been transported to the processing center, recovery of platelets in the form of concentrates is virtually impossible. Use of PGE_1 may permit recovery of platelets suitable for transfusion from such blood. The maximum delay permissible before platelets are extracted from chilled blood to which PGE_1 has been added, without shortening their survival time in vivo, remains to be determined.

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17 December 1971

Prostaglandins in the Preparation of Blood Components

Abstract. *Prostaglandin E_1 significantly improved the separation of blood components in blood bags. The recovery in vivo and the life-span values of the platelets were not altered. The hemostatic effectiveness of platelets treated with prostaglandin was shown to be normal in man.*

At the suggestion of Shio and Ramwell (*1*), we carried out experiments in which prostaglandin E_1 (PGE_1) was added to whole blood that was collected in the anticoagulant citrate-phosphate-dextrose (CPD) and to the platelet-rich plasma (PRP) prepared from this blood. We studied the effects of this procedure on (i) recovery in vitro of platelets from whole blood, (ii) the survival in vivo of both liquid-stored and previously frozen platelets, (iii) the survival in vivo of liquid-stored red cells, and (iv) physical, structural, and metabolic parameters of human red cells stored with PGE_1 and PGE_2 (*2*).

Table 1. Relative effectiveness of transfusing fresh and frozen platelets, prepared with and without PGE_1. Platelets originally obtained from 450 ml of whole blood mixed with CPD.

Condition	In vitro recovery of platelets from original unit: Amount (%)	Circulating platelets at 2 hours: ^{51}Cr recovery (%)	Relative effectiveness* (%)
	With PGE_1 (8 ng/ml)		
Fresh	80	65	50
Frozen-thawed-washed	68	45	30
	Without PGE_1		
Fresh	65	65	40
Frozen-thawed-washed	55	45	25

* Relative effectiveness is the product of in vitro recovery × ^{51}Cr in vivo recovery.

Within 4 hours after collection in a CPD double pack unit (JF-25) and after storage at room temperature (22° ± 1°C), PGE_1 was added aseptically to whole blood mixed with CPD (final concentration, 8 ng of PGE_1 per milliliter of whole blood). Other units of the blood mixture, but without added PGE_1, were treated in a similar manner. Each unit of whole blood was centrifuged (4500*g* for 3 minutes at 22°C); the PRP was transferred to the attached plastic bag which was then centrifuged (4500*g* for 5 minutes) and the platelet-poor plasma (PPP) was removed. The platelets sedimented from the PRP containing PGE_1 were easily suspended in 30 ml of PPP, while the platelet concentrates prepared from PRP without PGE_1 had to be stored at room temperature for 1 hour before they could be suspended.

The recovery in vitro of platelets prepared from whole blood, with and without PGE_1, was determined. Platelet counts of whole blood were performed with phase microscopy (*3*), and platelet counts of PRP were determined with the Coulter model B counter. The addition of PGE_1 (8 ng per milliliter of whole blood) produced an increased platelet recovery of statistical significance ($t = 3.49$, $P < .01$, d.f. = 33); the mean recovery of platelets (± standard deviation) from 22 units of whole blood mixed with CPD was 64 ± 14 percent, whereas the recovery of platelets from 17 units of whole blood mixed with CPD but containing PGE_1 was 79.5 ± 11 percent.

Some tests on these platelets were carried out in vitro after storage in the cold (4°C) and at room temperature (22°C). After storage at either temperature for 3 days a mixture of acid-citrate-dextrose (ACD) anticoagulant and whole blood to which PGE_1 or PGE_2 (8 ng/ml) had been added showed decreases in platelet count and in plasma *p*H. These decreases were similar to those in whole blood mixed with ACD, but without the addition of prostaglandins. The response of platelets to hypotonic stress, proposed as an index of viability in vitro (*4*), also indicated no alteration by this concentration of prostaglandin.

The effect of PGE_1 on the life-span of stored platelets was studied using ^{51}Cr labeling methods (*5*, *6*). Platelets prepared from PRP treated with 8 ng of PGE_1 per milliliter of PRP, were stored as platelet concentrates at +4°C for 24 hours in TA-2 transfer packs (Fenwal) under continuous agitation. When transfused back into the same healthy donors, these platelets had slightly better recovery in vivo, as measured by ^{51}Cr techniques, than did the platelet concentrates prepared with either 4 or 12 ng of PGE_1 per milliliter or the platelet concentrates prepared without PGE_1 (Fig. 1). In one case, the recovery and life-span of the platelet concentrate prepared with PGE_1 (8 ng per milliliter of PRP) and stored at 22°C for 24 hours were similar to those of fresh platelets. Platelets prepared from PRP containing 8 ng of PGE_1 per milliliter and then frozen with 5 percent dimethyl sulfoxide (DMSO) (*7*) had recovery and life-span values (measured with ^{51}Cr methods) similar to those of platelets not treated with PGE_1.

The influence of PGE_1 treatment on the effectiveness of platelets as hemostatic agents was studied with platelet concentrates (stored at room temperature for less than 4 hours) prepared from PRP obtained from blood mixed with ACD. When transfused into normal volunteers who had been treated with aspirin 24 hours earlier 6 units of fresh platelet concentrates corrected the bleeding time in 2 hours (*6*). The correction of an aspirin-induced thrombocytopathy (correction of bleeding time) in normal volunteers occurred either with platelets treated with 8 ng

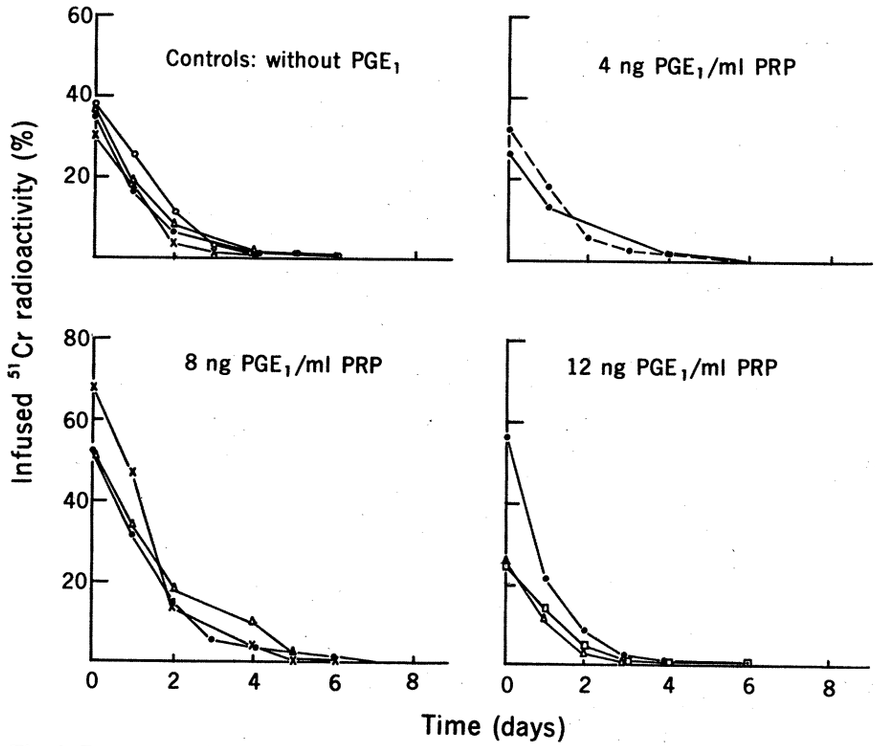

Fig. 1. Recovery and life-span (measured with ^{51}Cr) of platelets prepared from platelet-rich plasma of healthy volunteers and then transfused back into the same donors after storage as platelet concentrates for 24 hours at 4°C and either (i) treatment with PGE_1 (final concentrations of 4 to 12 ng per milliliter of PRP) or (ii) no added PGE_1.

of PGE_1 per milliliter of platelet concentrate, or with untreated platelets; these findings indicate that PGE_1 does not impair the hemostatic effectiveness of fresh platelet concentrates.

The effects of PGE_1 on stored red cells were examined. The PGE_1 was added (8 ng/ml) to whole blood mixed with CPD, the PRP was removed, and the concentrated red cells were stored for about 1 week at +4°C [maximum hematocrit of approximately 90 percent achieved by removing all the visible plasma (approximately 250 ml)].

The effects on physical structural and biochemical measurements were similar whether the concentrated red cells stored at +4°C were treated with PGE_1, with PGE_2 (8 ng per milliliter of whole blood), or without prostaglandins. There was no apparent difference in the red cell concentrations of 2,3-diphosphoglycerate, adenosine triphosphate, sodium ion, or potassium ion, or in the red cell *p*H, the *p*50 value of the oxyhemoglobin dissociation curve, red cell osmotic fragility, or red cell filterability (*8*) whether PGE_1, PGE_2, or no prostaglandins were used (Fig. 2).

Concentrated red cells prepared from whole blood and CPD, treated with PGE_1 (8 ng per milliliter of whole blood), and stored at +4°C for 1 week, were transfused into healthy recipients. When tested 24 hours later, about 90 percent of these cells were viable, with the range being from 88 to 95 percent. Removal of the nonviable red cells during the 30-minute period after transfusion was not associated with hemoglobinemia (as evaluated by plasma hemoglobin and plasma radioactivity). These results were similar to those of red cells not treated with prostaglandin.

These data demonstrate that when added to whole blood collected in CPD, 8 ng of PGE_1 per milliliter significantly improved platelet recovery in vitro. Improved recovery of platelets from blood treated with PGE_1 suggests that red cell concentrates can be prepared from whole blood with minimal platelet contamination. After washing, these red cells should be relatively free of platelets. This is important because patients requiring homotransplantation, or those with deficient immunological states, or with a history of febrile, urticarial, or anaphylactoid transfusion reactions, should receive red cells free of white cells and platelets (*9*).

Platelets stored at +4°C for 24 hours, both with and without PGE_1, had exponential rates of removal from the circulation, after transfusion. Other investigators have also reported an exponential rate of removal of platelet concentrates stored at +4°C for 24 hours prior to transfusion (*10*). On the

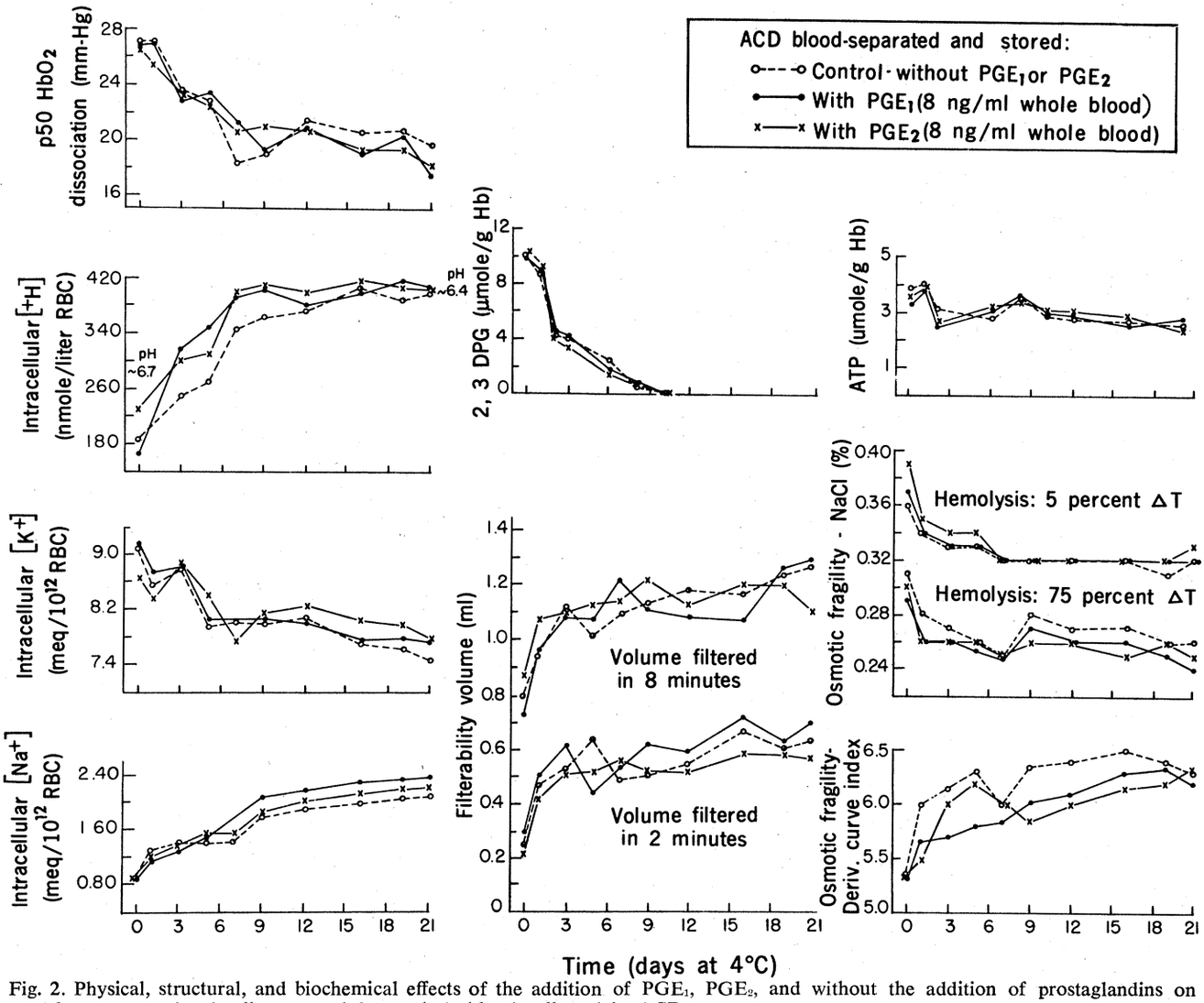

Fig. 2. Physical, structural, and biochemical effects of the addition of PGE_1, PGE_2, and without the addition of prostaglandins on stored concentrated red cells prepared from whole blood collected in ACD.

other hand, a linear rate of removal was observed when ^{51}Cr-labeled platelets, both with and without PGE_1, were stored at 22°C for 24 hours, and when they were frozen with a combination of DMSO and liquid nitrogen. Storage at +4°C for 24 hours produced preservation injury manifested by an exponential loss of platelets from the circulation; PGE_1 did not affect this removal pattern.

The addition of 8 ng of PGE_1 per milliliter of whole blood produced an increase in the number of circulating platelets when transfused from fresh platelet concentrates or from previously frozen platelets (Table 1). This increase reflected only improved recovery of platelets in vitro from whole blood; neither platelet recovery in vivo nor platelet life-span was improved by PGE_1. This treatment of whole blood did not adversely affect the survival of concentrated red cells stored at +4°C for about 1 week. The percent of cells viable after 24 hours was similar to that of concentrated red cells stored for the same period without PGE_1 (*11*). The functional state of the red cells stored with and stored without PGE_1 was similar.

No adverse clinical effects were observed after the administration of either liquid-stored platelet concentrates, previously frozen platelet concentrates, or the small portions of stored, concentrated red cells which had been treated with PGE_1. There were no changes in hematology or chemistry of the recipients during the week after transfusion of these cellular elements.

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Detection of High-Molecular-Weight RNA in Particles from Human Milk

Abstract. *Particles from human milk contain a reverse transcriptase and a high-molecular-weight (60S to 70S) RNA that serves as a template. These particles have two features diagnostic of known RNA tumor viruses.*

Particles morphologically similar to the type-B mouse mammary tumor virus (MMTV), Mason-Pfizer monkey virus, and type-C murine leukemia–sarcoma viruses have been observed in samples of human milk (*1*). Since the causative agents of both murine mammary tumors (*2*) and murine leukemia (*3*) appear in and are transmitted via mothers' milk, the question arose immediately as to the relatedness of the human particles to known RNA tumor viruses.

The oncogenic RNA viruses, or oncornaviruses (*4*), exhibit two biochemical properties unique to them as a group. They possess (*5*) a large single-stranded RNA molecule with a sedimentation coefficient of 60*S* to 70*S*, often referred to as high-molecular-weight RNA. They also contain a reverse transcriptase, an enzyme capable of using the viral RNA as a template to generate a complementary DNA copy (*6*, *7*).

The particles from human milk have a density of 1.16 to 1.19 g/ml and contain a ribonuclease-sensitive DNA polymerase analogous to the reverse transcriptase (*8*). Ribonuclease sensitivity of a DNA polymerase, however, does not per se establish that a reverse

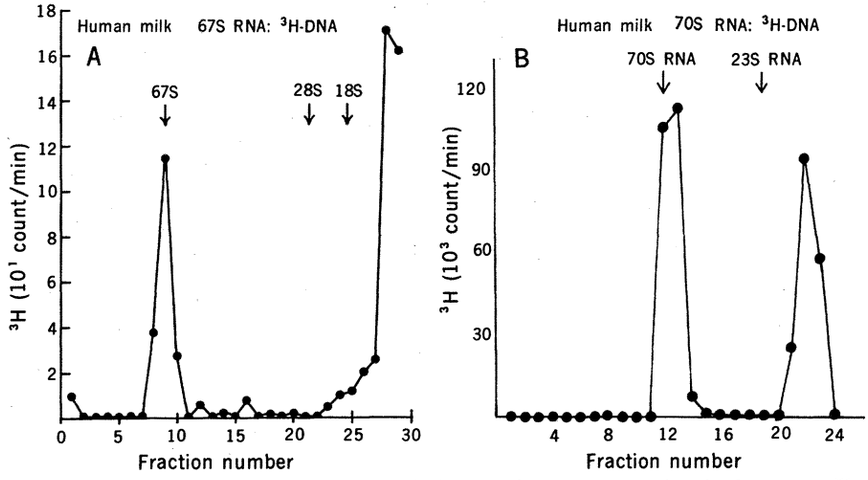

Fig. 1. Detection of high-molecular-weight-RNA : [^{3}H]DNA complex in human milk. (A) Seventy-five milliliters of human milk and 75 ml of 0.15*M* EDTA (*p*H 7.5) were mixed and centrifuged at 3000*g* for 10 minutes. The clear zone between the lipid and precipitated casein layers was removed and incubated at 37°C for 30 minutes in the presence of trypsin (Worthington) at a final concentration of 1 mg/ml. Lima-bean-trypsin inhibitor (0.5 mg/ml) was then added, and the sample was layered over an 8-ml column of 20 percent glycerol in a SW-27 (Spinco) centrifuge tube, and centrifuged at 98,000*g* for 60 minutes at 4°C; the resulting pellet was resuspended in 45 μl of 0.01*M* tris(hydroxymethyl)aminomethane (*p*H 8.3) containing 0.33 percent NP-40 detergent and 0.1*M* dithiothreitol and kept at 4°C for 10 minutes. This suspension was then added to a standard reverse transcriptase reaction mixture (125 μl final volume) containing 6.25 μmole of tris-HCl (*p*H 8.3), 1 μmole of $MgCl_2$, 1.25 μmole of NaCl (instead of KCl to avoid precipitation when sodium dodecyl sulfate is added in a subsequent step), 0.2 μmole each of unlabeled deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, and [^{3}H]deoxythymidine triphosphate to a final specific activity of 8900 count min^{-1} $pmole^{-1}$. After a 60-minute incubation at 37°C, the reaction was terminated by the addition of NaCl and sodium dodecyl sulfate to final concentrations of 0.2*M* and 1 percent, respectively. After addition of an equal volume of a phenol-cresol (7 : 1) mixture containing 8-hydroxyquinoline (3.7 g per 100 ml of mixture), the final mixture was shaken at 25°C for 5 minutes and centrifuged at 5000*g* for 5 minutes at 25°C. The aqueous phase was then layered over a linear glycerol gradient (10 to 30 percent) and centrifuged at 40,000 rev/min for 3 hours at 4°C (Spinco SW-41 rotor). External markers were 28*S* and 18*S* [^{3}H]RNA from NC-37 cells. Fractions were collected from below, and portions were assayed for acid-precipitable radioactivity (*7*). (B) Nineteen milliliters of milk was processed as described in (A). Avian myeloblastosis virus 70*S* [^{3}H]RNA and *Escherichia coli* 23*S* [^{3}H]RNA were used as external markers.