Platelets in blood stored in untreated and siliconed glass bottles and plastic bags

I Studies *in vitro*

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SYNOPSIS The number and function (clot retraction) of platelets in blood stored at 4°C. for three weeks in plastic bags and in untreated and siliconed glass containers were determined using EDTA and acid-citrate dextrose (ACD) as anticoagulants. No essential differences were found.

During recent years platelet transfusions have been used with increasing frequency in the treatment of thrombocytopenic bleeding, but there are still many unsolved problems connected with this treatment which are of both practical and theoretical importance. The present paper is mainly concerned with the containers and anticoagulants used for collecting blood as Cronkite and Jackson (1959) have recently given an excellent survey of the broader aspects of platelet transfusion.

It is generally claimed that platelets are preserved and handled much better in siliconed glass or plastic containers than in untreated glassware. As a matter of fact, it has been claimed that ACD-blood in untreated glass bottles (conventional blood-bank bottles) is without any value as a platelet source, for example, in the treatment of patients with thrombocytopenia (Gardner, 1958; Stefanini and Dameshek, 1955; Tullis, 1953). However, certain investigations have recently shown that the importance of using siliconed equipment in handling platelets seems to have been overemphasized (Lundevall, 1958; Hellem, 1960). McIlvanie (1958) compared plastic, untreated, and siliconed glass containers for platelet transfusions, and he found that untreated glass containers gave results which were only slightly inferior to those obtained with plastic and siliconed glass. This is in accordance with my own experience from many years' work with

blood platelets, including experience in the treatment of thrombocytopenia with concentrated thrombocyte suspensions. I have found that platelets from blood collected in ordinary untreated glass containers are quite valuable, even after storage for about 24 hours.

It is of importance for clinicians, and, especially, for blood bank staffs, to know how to handle blood in order to secure a high recovery of viable platelets when the blood is transfused, and it is also of financial importance. The following questions illustrate some aspects of the problem: Is it necessary to use fresh blood? If not, how long is it safe to store the blood before use? Is it necessary to use siliconed glass bottles? Are plastic bags superior to glass bottles? A study of the literature does not give a satisfactory answer to these questions.

Plastic bags are a modern and very elegant blood-transfusion equipment but the relative importance of the advantages and disadvantages has not yet been solved (Dudley, Richmond, McNair, Paton, and Cumming, 1958; Gibson, 1958; Kissmeyer-Nielsen, 1960). It is certain, however, that plastic equipment, at least for the present, is expensive in comparison with the conventional glass equipment. This paper attempts to show essential quantitative and qualitative differences in platelets stored in various containers. Our results, however, did not give any definite answer, and consequently these studies were followed by investigations *in vivo* in which the survival of platelets stored in various containers was determined. The results are published in Part II.

MATERIAL AND METHODS

PLATELET COUNTS   It was necessary to choose a reliable method which could be used for counting platelets in blood stored for three weeks, taking into consideration that spontaneous sedimentation of erythrocytes in plasma disappears quickly when blood is stored.

For counting platelets, the contents of the container were thoroughly mixed, and 1 ml. of blood was transferred to an untreated test tube containing 4 ml. of 3·8% sodium citrate ($2H_2O$). The contents of the tube were mixed, and the tube was left at room temperature for about 15 minutes for sedimentation of the red cells. From the middle of the supernatant a small volume was transferred to a Thoma counting chamber (height 0·05 mm.), which was left for sedimentation of the platelets in a moist chamber for at least 15 minutes. The platelets in 40 small squares were counted, and this figure multiplied by 1,000 gives the number of platelets in 1 mm.³ of whole blood (ignoring the volume of the red cells in the mixture of 1 ml. whole blood + 4 ml. citrate). The accuracy of the method was calculated from 25 duplicate determinations on blood bottles immediately after bleeding. The difference between the two values varied between 0 and 20,000 (S.D. 6,400, and coefficient of variation 2·7%). The number of platelets in the 25 bottles immediately after bleeding, without correction for the volume of anticoagulant added, varied between 159,000 and 360,000 (mean 255,000). The platelet counts were always performed in duplicate by the same technician.

PLATELET FUNCTION   It is difficult to assess the function, *i.e.*, the viability, of platelets *in vitro*, but this is supposed to be of major interest, especially in view of the experiments of Hjort, Perman, and Cronkite (1959) who showed that the viability of the platelets was of essential importance for their haemostatic function in dogs.

We made an attempt to assess the viability by determining the ability of the stored, washed platelets to give clot retraction of platelet-free, fresh, citrated plasma. The platelets from the stored blood were isolated by differential centrifugation. Platelet-rich plasma was produced by centrifugation at about 300 *g* for five minutes. (The centrifugal force is calculated for the middle of the test tube.) The platelet-rich plasma was transferred to siliconed test tubes; 1/10 by volume of 2% triton (WR 1339) in 0·9% saline was added, and the mixture was centrifuged at about 600 *g* for 30 minutes. The super-

natant was discarded, and the platelets were then washed twice in saline with 1/10 by volume of triton 2%. After the last washing the platelets were resuspended in 0·9% saline without triton. The amount of saline added was adjusted by platelet counts, so as to give a platelet suspension containing about 10^6 platelets per mm.³. The ability of this platelet suspension to give clot retraction was assessed in the following mixtures:—

One millilitre platelet-free, normal citrate plasma (the platelet-free plasma was prepared from 1 ml. 3·8% citrate + 9 ml. blood centrifuged at 1,300 *g* for 30 minutes); 0·2 ml. of the washed platelet suspension; and 0·1 ml. 0·25 M $CaCl_2$.

The test was performed in round-bottomed test tubes (75 by 10/11 mm.) pre-heated in a Bunsen flame almost to melting point. This pre-heating is, as shown by Lüscher (1956), essential, as it completely abolishes any adhesion between the clot and the glass. After clotting the tubes were left undisturbed at 37°C. for one hour. After this time the serum formed was carefully removed using a fine Pasteur pipette, taking care not to squeeze the clot. The volume of serum was measured, and this volume as a percentage of the total volume (1·3 ml.) represents the clot retraction. Using this method for 32 determinations in 16 normal subjects, values between 85 and 97% were found (mean 92, S.D. 2·9). The accuracy of the method was calculated from 12 duplicate determinations on normal subjects. The standard deviation was found to be 1·9 and the coefficient of variation 2·1%. As in the platelet counts, the clot retraction was determined in duplicate.

It was hoped that it would also have been possible to investigate platelet function during storage using the above-mentioned washed platelet suspension in the classical thromboplastin-generation test (Biggs and Douglas, 1953), mixing the platelet suspension with normal $Al(OH)_3$-adsorbed plasma and normal serum. However, the platelet function remained absolutely unaltered during the three weeks of storage; consequently, the results of this part of the investigation are not again referred to.

BLOOD CONTAINERS, ANTICOAGULANTS, AND DONORS   Unselected donors and unselected blood containers were used for the experiments. All the bleedings

TABLE I
CONTAINERS AND ANTICOAGULANTS USED

Container	Number	Anticoagulant	Amount of Blood (ml.)
Untreated glass	5	ACD (Danish Pharmacopoea) 150 ml. Citric acid, H_2O 4·8 g., Na_3-citrate, $2H_2O$ 13·3 g., dextrose, H_2O 30 g. Distilled water to 1,000 ml.	500
Siliconed glass	5	ACD (Danish Pharmacopoea) 150 ml.	500
Plastic (Fenwal)	5	ACD (Unites States Pharmacopoea) 75 ml. Formula A	500
Untreated glass	5	EDTA 50 ml. 1·5% Na_2EDTA, $2H_2O$ and 0·7% NaCl	500
Siliconed glass	5	EDTA 50 ml.	500
Plastic (Fenwal)	5	EDTA 50 ml.	500

were performed by the head nurse of the blood bank or by myself. The various containers and anti-coagulants used are shown in Table I.

CLEANING OF GLASSWARE AND BOTTLES The cleaning procedure used may be of some importance in the experiments under consideration. The glassware used was of our routine pattern and no special precautions were taken. Test tubes were treated as follows:—

Immediately after use they were immersed in a detergent, which is a 0·5 solution of equal parts of technical Na_2CO_3, sodium metasilicate, and trisodium phosphate. The test tubes were boiled for 10 to 15 minutes in this solution, rinsed three times in running tap water, immersed for at least half an hour in 1% HCl, rinsed three times in running tap water, immersed in technical demineralized water, and finally rinsed once in running demineralized water and dried in a hot oven at 80°C.

The bottles were cleaned in a semi-automatic washing machine at the hospital pharmacy. The cleaning procedure included 2% NaOH + 1% trisodium phosphate, 80°C., 2% trisodium phosphate, 60°C., tap water, 60°C., cold tap water, decalcified tap water, and drying in a hot oven.

COATING OF GLASSWARE WITH SILICONE The bottles were siliconed in the hospital pharmacy, after cleaning with sulphuric chromic acid, using silicone M.S. 1107 dissolved in neutral, water-free trichloroethylene followed by rinsing in distilled water. The bottles were baked at 180°C. for three hours. The test tubes were siliconed, using a 10% solution of silicone M 441 (I.C.I.) in petroleum ether, followed by rinsing in several changes of distilled water and air-drying (no baking).

RESULTS

In a preliminary experiment, the number and function of blood platelets in five untreated and five siliconed glass bottles were followed for three weeks.

All the bottles contained ACD, 150 ml. and 500 ml. of blood. (ACD, according to the Danish Pharmacopoea, is composed of citric acid 4·8 g., Na_3 citrate, 2 H_2O 13·3 g., dextrose 30 g. in 1,000 ml. distilled water.)

Containers were examined immediately after the bleeding, and after three, seven, 14, and 21 days of

TABLE II
PLATELETS AND ABILITY FOR CLOT RETRACTION AFTER STORAGE IN VARIOUS CONTAINERS

Time of storage in days		0		3		7		14		21		Total Percentage Decrease in Platelet Count
Untreated glass	ACD	283	81·5[1]	276	89·8	211	64·8	187	24·6	122	23·7	54
	EDTA	248	88·8	244	94·4	237	86·3	169	72·7	118	49·2	52
Siliconed glass	ACD	199	93·6	226	89·0	207	66·0	178	33·1	144	29·2	36
	EDTA	279	93·5	266	91·3	210	87·9	186	63·1	155	35·5	44
Plastic (Fenwal)	ACD	229	91·2	213	81·2	144	47·8	99	51·8	87	25·3	62
	EDTA	239	91·6	252	89·1	182	83·9	146	80·7	118	27·6	51

[1]The first figure in each column is the number of platelets (× 1,000), and the second is the clot retraction. Each figure is the average of the results obtained for the five containers included in each group.
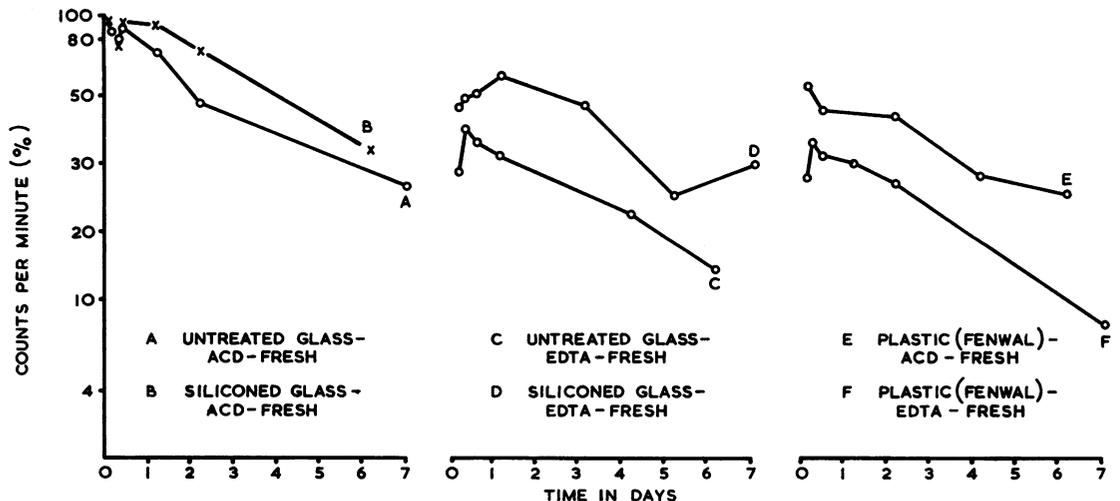

FIG. 1. Number and function of platelets stored in untreated and siliconed glass and plastic bags when ACD was used as anticoagulant.

E PLASTIC (FENWAL)–ACD–72 HOURS
F. PLASTIC (FENWAL)–ACD–FRESH

A UNTREATED GLASS – ACD–72 HOURS
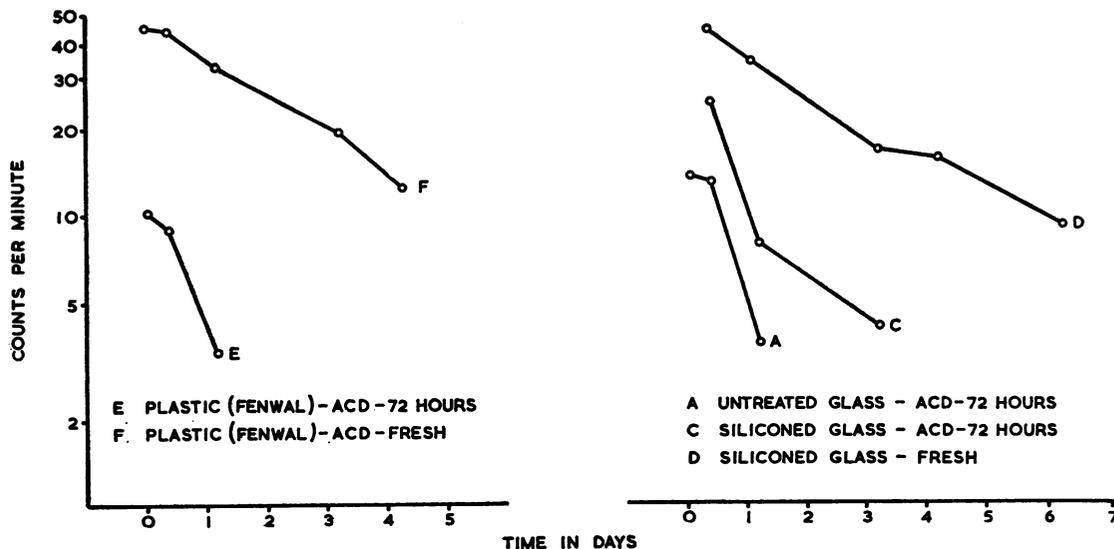C SILICONED GLASS – ACD–72 HOURS
D SILICONED GLASS – FRESH

FIG. 2. *Number and function of platelets stored in untreated and siliconed glass and plastic bags when EDTA was used as anticoagulant.*

storage at 4°C. Contrary to expectation, it was found that the number of platelets and their clot-retraction ability decreased identically in the two groups of containers. During the three weeks the average number of platelets in both groups decreased by 61% of the original value.

Immediately after bleeding, isolated platelets from both groups gave a clot retraction of 90%; after three weeks the results were 28 and 27% respectively.

The number of platelets and the clot retraction remained unaltered during the first three days of storage.

The investigations were repeated on six more representative groups of containers and anticoagulants (Table I). Each group contained five specimens, and consequently the whole material included 30 'pints' of blood.

The EDTA solution used was the Fenwal formula, although it was realized that this solution, because of lack of dextrose, was supposed to give haemolysis during storage.

The number and function of the platelets were investigated immediately after the withdrawal of the blood and after three, seven, 14, and 21 days of storage at 4°C.

The results are set out in Table II and Figs. 1 and 2.

DISCUSSION

The results obtained with the different containers and the two different anticoagulants were remark-ably similar. The only times during storage at which any differences were noticed were after seven and 14 days. At these times, the EDTA-stabilized blood showed a less marked decrease in clot retraction than the ACD-stablized specimens. However, this difference was not apparent until after one week, and it was not uniform.

These studies *in vitro* do not allow any conclusions as to the superiority or inferiority of any of the three containers and the two anticoagulants included in the investigation. The results are thus of limited value, except for the fact that they give the impression that the claimed superiority of siliconed glass and plastic equipment in relation to number and viability of platelets may be wrong. However, the studies performed were only *in vitro*, and determinations of platelet survival might give quite a different result.

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II   Survival studies

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SYNOPSIS   Platelet survival was determined using untreated and siliconed glass bottles and plastic bags (Fenwal) for collecting and storing blood.   The platelets were tagged *in vivo* with P³² in six polycythaemic patients undergoing treatment with P³². The results showed that fresh ACD blood collected in untreated glass, siliconed glass, and plastic gave the same recovery of platelets in the recipients. The use of EDTA (Fenwal formula) as anticoagulant gave results inferior to those obtained with blood using ACD as anticoagulant. Even after storage up to 24 hours in untreated glass bottles (ordinary bank blood) a satisfactory recovery of platelets was observed. After storage for 72 hours the recovery was less but not negligible.

We have tried to solve part of the problem of preserving platelets for subsequent transfusion and for use in extracorporeal circulation by determining the survival of platelets in blood delivered in different containers, using ACD and EDTA as anticoagulant and storing the blood for periods of varying length before transfusion. It is difficult to determine the survival of thrombocytes but it is possible by using platelets tagged with various isotopes. A survey of the methods used was recently published by Aas and Gardner (1958). Tagging is usually performed *in vitro*, and the rather rough treatment to which the platelets are exposed during tagging has some influence on the results obtained, consequently labelling *in vitro* is not ideal for an investigation in which a relatively small difference in survival time might indicate superiority or inferiority of one container compared with another. We therefore used platelets tagged *in vivo* with P³². We used blood from patients with polycythaemia who were treated with P³². The technique employed is very similar to that used by Adelson, Rheingold, and Crosby (1957) in dogs and human beings. It is well known that tagging is maximal in about a week after the administration of the drug (Adelson *et al.*, 1957). Using blood from patients with polycythaemia gives another advantage, namely, the possibility of drawing several pints of blood from the same donor at very short intervals. The various 'pints' drawn from

the same donor contain the 'same' platelets marked in exactly the same way *in vivo*, thus affording an ideal opportunity for comparing the effect of storage on platelets in various containers before transfusion.

MATERIALS AND METHODS

DONORS   Patients with polycythaemia undergoing treatment with P³² were used as donors. They were given about 5 μ. P³² by mouth and after an interval of five to seven days bleedings were started, 500 ml. of blood being drawn on each occasion, and the bleedings were continued daily for three to six days, depending on the clinical and haematological condition of the patients. Six patients were included in the study, and delivered a total of 24 'pints' of blood. Two patients delivered six bottles each, two four bottles each, one three bottles, and the last donor only one. All the bleedings were performed either by the head nurse of the blood bank or by one of the authors (F. K-N). Automatic shaking was used during bleeding into glass bottles.

RECIPIENTS   It was felt that it was undesirable and unnecessary to use 'normal' recipients, so recipients were patients from the Department of Radiotherapy undergoing heavy *x*-ray therapy for advanced malignant tumours, mostly carcinoma of the cervix uteri. The patients were all in a good clinical condition, but some of them had previously been transfused several times (Table I). The transfusions were preceded by screening for irregular blood-group antibodies (papainized cells and indirect Coombs technique) and by cross-matching