xanthone ketochloride, respectively (*3*). (II) has also been prepared by the action of copper bronze on 1:2-benzoxanthione (IV), which may be prepared by the action of thioacetic acid on 1:2-benzoxanthone ketochloride; similar reactions are known in the case of xanthione (*4*).

When dixanthylene (I) is heated with sulfur, xanthione is formed (*3*). Similar reactions were carried out with (II) and (III), and thus 1:2-benzoxanthione (IV), mp, 141°, and 3:4-benzoxanthione, mp, 148°, were obtained, respectively. When (II) and (III) are treated with oxalyl chloride, followed by the action of water, 1:2-benzoxanthone and 3:4-benzoxanthone are obtained, respectively (*5*).

A full report describing the previous reactions, the action of lithium aluminum hydride, the Grignard solutions on 1:2-benzoxanthone and 3:4-benzoxanthone, respectively, and the action of ethereal diazomethane on 1:2-benzoxanthione and 3:4-benzoxanthione, together with photochemical behavior of 9-aryl-1:2-benzoxanthen and 9-aryl-3:4-benzoxanthen toward oxygen (*6*) and the photo-action of *p*-benzoquinone (*7*) and oxygen (*6*), respectively, on 1:2-benzoxanthen will be published soon.

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Method for Obtaining Large Yields of Human Platelets as a By-Product of Blood Collection[1]

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Clinical and experimental use of platelets has been limited by the expense and impracticality of obtaining them in quantity. The development of methods for storing blood (*1–4*) and isolating its constituents (*5, 6*) has permitted mass collection of human blood and detailed study of its fractions, including the formed elements (*7*). Among the latter, probably platelets are the least well understood, but the most important in thrombocytopenic conditions accompanied by bleeding. Recently the use of ion-exchange resin for the purpose of rendering blood incoagulable by fixing calcium ions (*8, 9*) proved, fortuitously, to cause partial disappearance of platelets from the blood. This apparent obstacle to the eventual harvesting of platelets by centrifugation for laboratory study and clinical use was turned to advantage when elution of the resin proved to be an efficacious means of salvaging quantities of platelets (*10*).

Blood-collecting and -transfusion sets (*9*) made of translucent plastic material (polyvinyl chloride acetate copolymer) and containing a column of ion-exchange resin (sulfonated polysterene divinyl benzene copolymer[2]) for making the blood incoagulable by decalcification were used. Needles were coated with tris (2-hydroxyethyl) dodecylamine.[3] The column of resin, contained in 28-mm plastic tubing, was suspended from the donor needle on the afferent end by small-caliber tubing and was led to the blood receptacle by similar tubing on the efferent end. The ion-exchanger consisted of 50 g of Dowex-50 beads on the sodium cycle. The resin was washed with saline solution and kept moist. Platelet suspensions were received in clean silicone-lined[4] flasks or bottles. The eluting fluid was unbuffered solution of 0.085% NaCl in distilled water. Ordinary 50-ml glass syringes equipped with metal adapter tips were used to deliver the physiologic saline eluting solution.

The usual blood donation unit of 500 ml was collected by means of the described blood-collecting set (*9*). The resin container was then cut free at both ends, leaving a few centimeters of tubing attached at each end. The contents of the resin container were then washed with the physiologic saline solution, simply by introducing the adapter tip of a syringe filled with saline solution into the open end of the snugly fitting afferent tube, forcing the saline through the resin container, and catching the washings in a silicone-lined bottle. Approximately 10 ml of saline solution was added at a time, and the resin and saline were manipulated by kneading the contents of the container from the outside with the fingers, to aid in freeing whatever blood elements were attached to the resin beads. This process, and straight flushing with portions of saline solution, were alternated as necessary.

In order to obtain platelets in concentrations equivalent to that of whole blood, for purposes of counting, each resin column was washed with 500 ml of saline solution, the original blood volume. Elutions were made, also, with smaller quantities, for purposes of concentrating the platelets. Platelet counts were made by the direct method, using 3.8% sodium citrate solution as a diluent (*11*). Sealing the tubing at both ends of the resin container, dielectrically (*9*), would simplify recovery of platelets under sterile conditions if that were desirable.

Washing of the resin with sufficient saline solution to make up the original blood volume resulted in high yields of platelets that had been filtered out by the resin. A series of resin columns, through each of which 500 ml of whole blood had been passed once, were eluted with 500 ml of saline solution. The average concentration of recovered platelets was approximately 119,000/mm^3, or about 40% of the total normal

[1] Work done in the laboratories of the Harvard Medical School in cooperation with the Blood Characterization and Preservation Laboratories of the Bussey Institution of Harvard University, University Laboratory of Physical Chemistry, and the American National Red Cross.

[2] Dowex-50, Dow Chemical.
[3] A-15, Armour and Company.
[4] Dri-Film 9987, General Electric.

TABLE 1

PLATELETS RECOVERED FROM ION-EXCHANGE COLUMNS AFTER PASSAGE OF 500-ML UNITS OF WHOLE BLOOD

Specimen	Platelets/mm³ whole blood	
	Loss*	Recovery†
1	138,000	130,500
2	102,000	78,000
3	128,000	106,000
4	178,000	162,000
Av	136,500	119,125
Percentage of filtered platelets recovered	88	

* Calculated as difference between counts on whole blood before and after passage through the resin column.
† Direct count on suspension in 500 ml of saline solution.

platelet complement (Table 1). Platelet counts made on the blood from the tubing below the needle immediately after cessation of flow from the donor and again after passage of the blood through the resin indicated losses of a similar magnitude; therefore, approximately 88% of the platelets filtered from the blood were recovered (Table 1). By passing the same unit of blood through a series of four identical resin columns, it was possible to recover an average of 89% of the platelets filtered from the blood (Table 2). Between 90 and 95% of the total platelet complement from 500 ml of blood is generally filtered out with four columns, the residual portion tending to remain with the blood even after further resin treatment.

Elution was carried out also with smaller volumes of saline solution. Approximately 70% of a possible total yield could be recovered with a volume of saline solution equivalent to 25% of the original blood volume. By manipulating the contents of the resin container, as many as 600,000 platelets/mm³ were obtained in a volume of 50 ml of saline solution, although usually only about half that concentration was seen. Although normal donors were used almost exclusively, considerable variability in platelet loss and recovery could be expected in any individual test. The platelet counts made on blood within the plastic tubing were erratic because of clumping, but served adequately as a check on recovery counts. There have been periods during which lower yields than are reported here were obtained. The circumstances with respect to the resin and the preparation of columns are being investigated.

TABLE 2

PLATELETS RECOVERED FROM SERIES OF FOUR ION-EXCHANGE COLUMNS AFTER PASSAGE OF INDIVIDUAL 500-ML UNITS OF WHOLE BLOOD

Specimen	Platelets/mm³ whole blood	
	Loss*	Recovery†
1	246,000	234,000
2	188,000	174,000
3‡	756,000	651,000
Total	1,190,000	1,059,000
Percentage of filtered platelets recovered	89	

* Calculated as difference between counts on whole blood before and after passage through four columns of resin.
† Sum of direct counts on four suspensions in 500 ml of saline solution each.
‡ Donor with polycythemia vera.

Platelets eluted from resin by the method described appeared entirely normal by visualization in the phase microscope.[5] Also, the prolonged clotting time of oxalated, platelet-poor, normal plasma was returned to normal by addition of such platelets in saline suspension.

When desirable, elimination of most of the red cells caught with the platelets by the ion-exchange resin could be carried out by allowing the suspension to settle in the cold for several hours, or by light centrifugation at 1,000 rpm[6] for 5–8 min at 4° C. Further differential centrifugation could be carried out to obtain purified platelet material if platelet morphology or individuality did not need to be preserved. Purification and concentration were always performed at the sacrifice of absolute quantity.

The accumulation of large quantities of human platelets as a by-product of blood collection during which ion-exchange resin is used to prevent coagulation provides a practical and substantial source of human platelets for use in the laboratory or in the clinic. The elimination of high-speed centrifugation, washing, and resuspension, which have been in general use, favors the preservation of the natural state of these elements. Also, the ease with which elution takes place and the physiological simplicity of the eluting fluid tend to maintain morphological and physiological integrity. Methods for improving the concentration and preservation of such platelet suspensions are being studied.

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[5] James L. Tullis was kind enough to make this observation.
[6] International Centrifuge, No. 1.