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Research Article

THE RATE OF POST-TRANSFUSION LOSS OF NON-VIABLE STORED HUMAN ERYTHROCYTES AND THE RE-UTILIZATION OF HEMOGLOBIN-DERIVED RADIOACTIVE IRON [1]

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When human erythrocytes are transfused within a few hours of taking in citrate solution, practically all of them remain intact in the recipient's blood stream (1). Thereafter, they disappear from the circulation at a rate of about one per cent per day (2, 3), and it is generally believed that this is the normal death rate of homologous erythrocytes.

Chemico-physical changes occur in red cells during storage *in vitro*. Depending upon the preservative value of the anticoagulant used, deteriorative changes may occur gradually and proceed at relatively constant rates for considerable periods, or they may be initiated early in storage and proceed at accelerated rates. Post-transfusion viability of preserved cells is a function of the length of storage (1).

Thus, the transfusion of stored blood, which always contains a certain quantity of cells in which irreversible changes have occurred, necessitates the removal of those dead or dying red cells from the circulation. These cells may rupture in the blood stream and, through release of hemoglobin, give rise to hemoglobinemia. It is also possible that they may be removed, with membranes relatively intact, by phagocytosis, or segregation in the spleen (4), and subsequently destroyed. In either event a load is imposed upon the mechanism for scavenging the blood stream and for the handling of hemoglobin-derived pigments: the reticuloendothelial and the erythropoietic systems. Hemoglobinemia also involves renal function since there is a plasma level for hemoglobin above which hemoglobinuria occurs. The degree of this burden will be determined by the quantity of mortally damaged cells transfused (which will in turn depend upon the size of the transfusion, and, for any given preservative solution, the length and conditions of storage prior to transfusion), and the rate at which the blood is transfused.

In previous communications (5) it was concluded that the greater part of the non-viable cells are completely removed from active circulation, usually within 24 hours after transfusion, and that the remainder of the transfused cells resume normal functional capacity and enjoy a sojourn in the body equivalent to the remainder of their normal life expectancy. It is therefore only the non-viable portion of the transfused red cells that need be considered in the study of the effects of the transfusion of stored blood.

In a number of experiments in which blood, the cells of which were tagged with radioactive iron, was transfused, the initial recipient blood sample was taken within 60 minutes, and several subsequent samples within the first 14 hours of the beginning of the transfusion. Since little if any reutilization of hemoglobin-derived radio-iron can be expected to occur in this period, the radioactivity of those blood samples accurately measured the quantity of transfused cells removed from the blood stream. These values may be expressed in terms of the percentage of total cells transfused or of the contained quantities of hemoglobin or of iron.

Such data were obtained in 9 transfusions of stored whole blood and in 15 transfusions of stored resuspended cells. The whole bloods were drawn into ACD-1 and Alsever's solution; they were transfused from 8 to 26 days after collecting.

Five of the bloods in ACD-1 had been transported, by

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Massachusetts Institute of Technology, in collaboration with the Peter Bent Brigham Hospital, and the Massachusetts General Hospital.

TABLE I

Quantities of whole blood (or resuspensions) and red cells transfused, storage periods, and percentages of non-viable red cells removed from circulation at successive intervals from beginning of transfusion

Exp. no.	Solution	Days stored	Transfused		Time intervals in minutes from start of transfusion				
			Total	Cells	Per cent cells removed in interval				
			ml.	*ml.*					
69	ACD-1	8	543	247	35 5	88 6	233 6	428 7	1,320 9
72	ACD-1	15	536	230	95 4	147 5	322 7	1,320 14	
73	ACD-1	16	565	253	58 2	114 3	354 7	1,470 8	
76	ACD-1	25	325	150	36 0	99 7	249 22	449 26	1,290 30
77	ACD-1	26	285	121	36 1	112 2	232 6	1,350 17	
78	Alsever's	19	575	161	50 16	117 33	242 55	482 67	1,260 76
79	ACD-1	20	366	156	54 28	91 44	236 72	1,260 83	
80	Alsever's	23	515	151	38 35	95 57	476 67	1,260 87	
81	ACD-1	24	285	122	34 22	89 55	239 82	479 82	1,290 92
88	Citrate-buffered citrate-dextrose with added globulin	1	505	212	57 0	127 0	337 0	1,320 5	
90		18	420	191	52 0	117 1	363 3	1,260 6	
92		17	525	215	55 0	106 5	246 7	1,260 10	
133	Citrate-buffered citrate-dextrose pH 5.0	13	467	217	65 6	155 12	355 28	1,440 40	
134		15	520	235	153 5	372 28	1,500 29		
135		20	315	147	43 11	123 32	273 36	1,615 47	
136		11	510	256	55 11	135 32	335 36	1,500 47	
137		14	500	234	46 13	130 28	290 45	1,440 52	
138		15	497	245	55 22	124 34	392 58	1,500 68	
139		18	357	175	76 16	176 26	326 42	1,410 45	
140	10 per cent corn syrup	13	502	227	61 —	164 3	349 6	1,500 8	
141		9	487	169	45 2	140 4	300 6	1,530 11	
142		15	475	178	44 14	74 21	184 43	1,440 46	
143		20	470	193	61 28	115 —	275 33	1,440 59	
146		14	520	220	75 32	155 45	350 48	1,360 49	

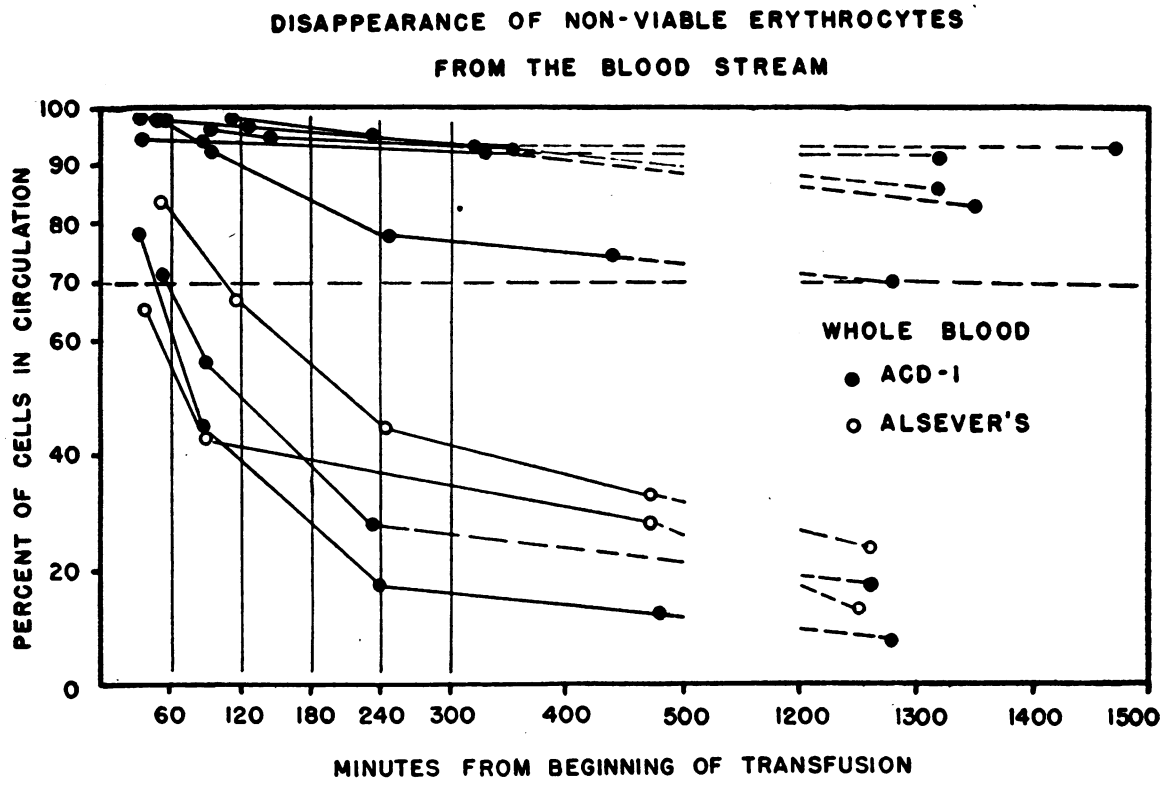

FIG. 1. DISAPPEARANCE OF NON-VIABLE ERYTHROCYTES FROM THE BLOOD STREAM

The percentage of total transfused tagged cells stored as whole blood in ACD–1 and Alsever's solution remaining in circulation during the first 1,500 minutes after beginning transfusion.

air, under constant refrigeration, from Boston, Massachusetts to Oakland, California, and back (5 days elapsed), and subsequently were stored at 4° C. Two bloods in ACD–1 and 3 in Alsever's solution had been transported by air, without constant refrigeration, from Boston to Paris, France (11 days elapsed), and subsequently were stored at 4° C.

The packed cells were resuspended in: a 10 per cent corn syrup; a citrate-buffered citrate-dextrose solution of pH 5.0; and a citrate-buffered citrate-dextrose solution containing 3 per cent Fraction IV–3, 4, Lot 301 [2] (α and β globulin). The cells in the globulin-fortified solution had been in depot storage at 4° C. until transfused. The cells in corn syrup and in the acidified-citrate-dextrose solution were transported by truck, under controlled refrigeration (4 to 10° C.), from Boston to New York, and transfused into patients on the wards of the New York Hospital.[3]

[2] Prepared in the Pilot Plant of the Department of Physical Chemistry, Harvard Medical School, under the direction of Dr. E. J. Cohn.

[3] These experiments were carried out, under request from medical authorities of the U. S. Navy, represented by Lt. (s.g.) Henry Blake, U.S.N.R., in collaboration with Dr. Ralph G. Stillman, of the New York Hospital, and Dr. William Thalhimer.

The transfused quantities of whole blood in ACD and Alsever's solution ranged from 315 to 525 ml. (121 to 253 ml. of cells); cell resuspensions in corn syrup and in the citrate-buffered citrate-dextrose ranged from 315 to 520 ml. (147 to 256 ml. of cells); and cell resuspensions in the globulin-fortified resuspension solution, from 420 to 505 ml. (171 to 215 ml. of cells). The average of the 24 transfusions was 194 ml. Time required for transfusion varied from 13 to 65 minutes, and the rate of flow from 8 ml. to 36 ml. per minute, averaging 18 ml. per minute. The hematocrits of whole blood were from 28 to 46; and of cell resuspensions from 34.7 to 50.5; the average hematocrit of all the transfusions was 42.7. Survival during the first 24 post-transfusion hours ranged from 92 to 8 per cent for whole bloods and from 95 to 32 per cent for the cell resuspensions.

These quantities of whole blood and cells are well within the ranges encountered in single clinical transfusions; hence the data may be treated as a whole in applying the findings to transfusion practice. The average rate of inflow, 18 ml. per minute, is perhaps more rapid than that of the usual routine transfusion.

The quantity of whole blood or resuspension, cells transfused, rate of inflow, and percentage of total transfused cells lost from circulation at suc-

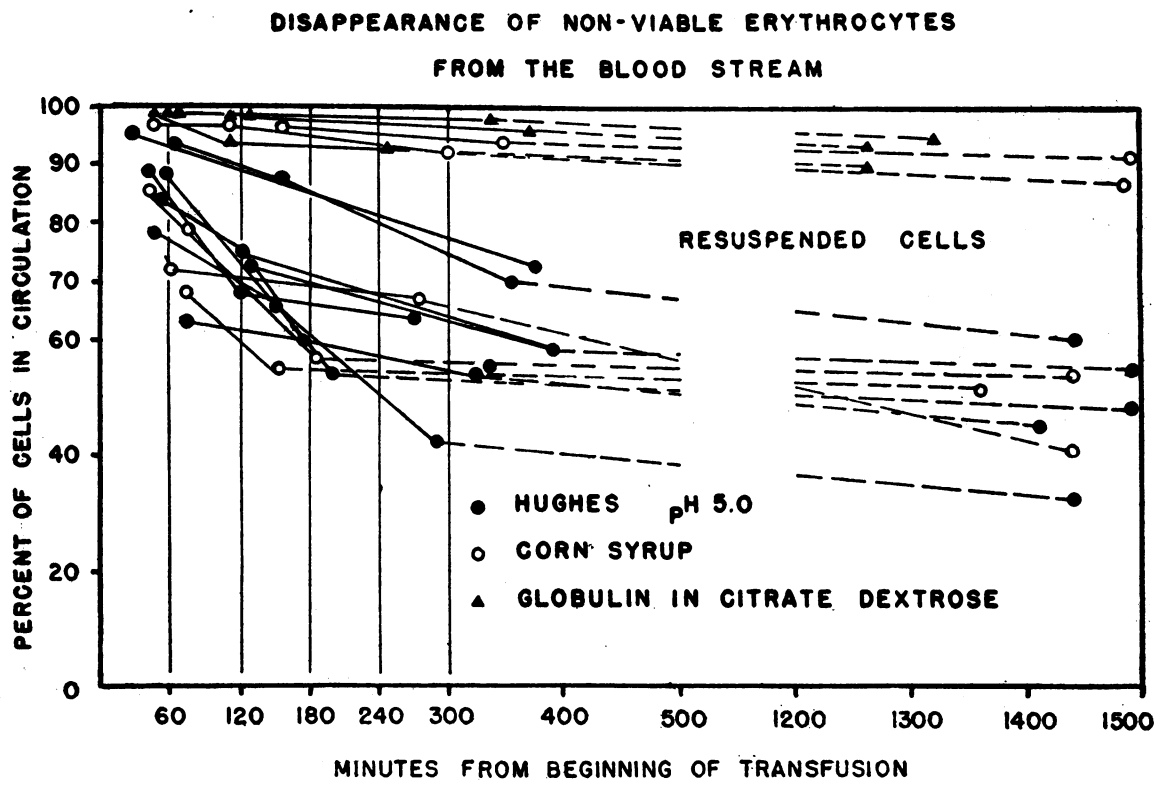

FIG. 2. DISAPPEARANCE OF NON-VIABLE ERYTHROCYTES FROM THE BLOOD STREAM

The percentage of total transfused tagged cells drawn as whole blood in 4 per cent sodium citrate and resuspended in 3 preservative solutions, remaining in circulation during the first 1,500 minutes after beginning transfusion.

cessive intervals from the beginning of the transfusion are shown in Table I. The percentages of cells remaining in circulation at intervals up to 1,500 minutes after transfusion are shown in Figures 1 and 2.

The post-transfusion behavior of both the cells stored as whole blood and the cells in resuspension was similar. The rate of loss of non-viable cells from transfusions in which the eventual survival was better than 80 per cent was slow and relatively constant. At 80 per cent survival, or better, the greater part of the loss occurred during the first 300 to 500 minutes from the start of the transfusion, there being, in general, little further loss during the remainder of the first 24 hours.

In transfusions, the survival of which was less than 80 per cent, the rate of loss of non-viable cells was rapid and tended to vary directly with the eventual survival. The rate of loss was greatest during the first 60-minute period, becoming progressively less during the next 24 hours. The

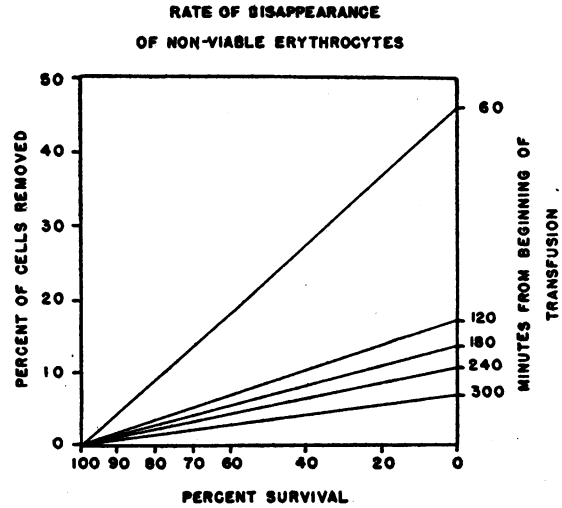

FIG. 3. RATE OF DISAPPEARANCE OF NON-VIABLE ERYTHROCYTES

The average percentages of total transfused tagged red cells removed from circulation during successive 60-minute intervals from beginning of transfusion, in relation to the percentage of survival.

maximum loss was not reached during the first 5 hours, but the rate of loss during the ensuing 19 hours was considerably less than during the first 5-hour period. It should be emphasized that even in the poorest transfusions the 24-hour data still measure disappearance uncomplicated by cell regeneration.

It is therefore apparent that the rate of disappearance of non-viable cells is a function of percentage of survival, which in turn is a function of time of storage.

It is possible, from the time survival curves presented in Figures 1 and 2, to determine, for the entire series of experiments, the average rate of disappearance of cells at given intervals after the beginning of transfusion for any percentage of eventual survival. The family of curves representing these rates at 60-minute intervals up to 5 hours after transfusion are shown, in relation to survival, in Figure 3.

It is evident that the loss of non-viable cells begins very soon after the administration of blood and, in badly deteriorated bloods, may begin during administration. Regardless of the survival, the rate of loss is most rapid during the first 60 minutes' post-transfusion period and decreases considerably during the ensuing four 60-minute periods.

This fact is more clearly evident in Figure 4, in which the average rates of loss of non-viable cells during the first 5 hours after the beginning of transfusion, expressed as per cent of cells lost per minute, are shown for transfusions ranging from 100 to 50 per cent survival.

In the same figure, the total quantity of hemoglobin that would be obtained from *immediate*

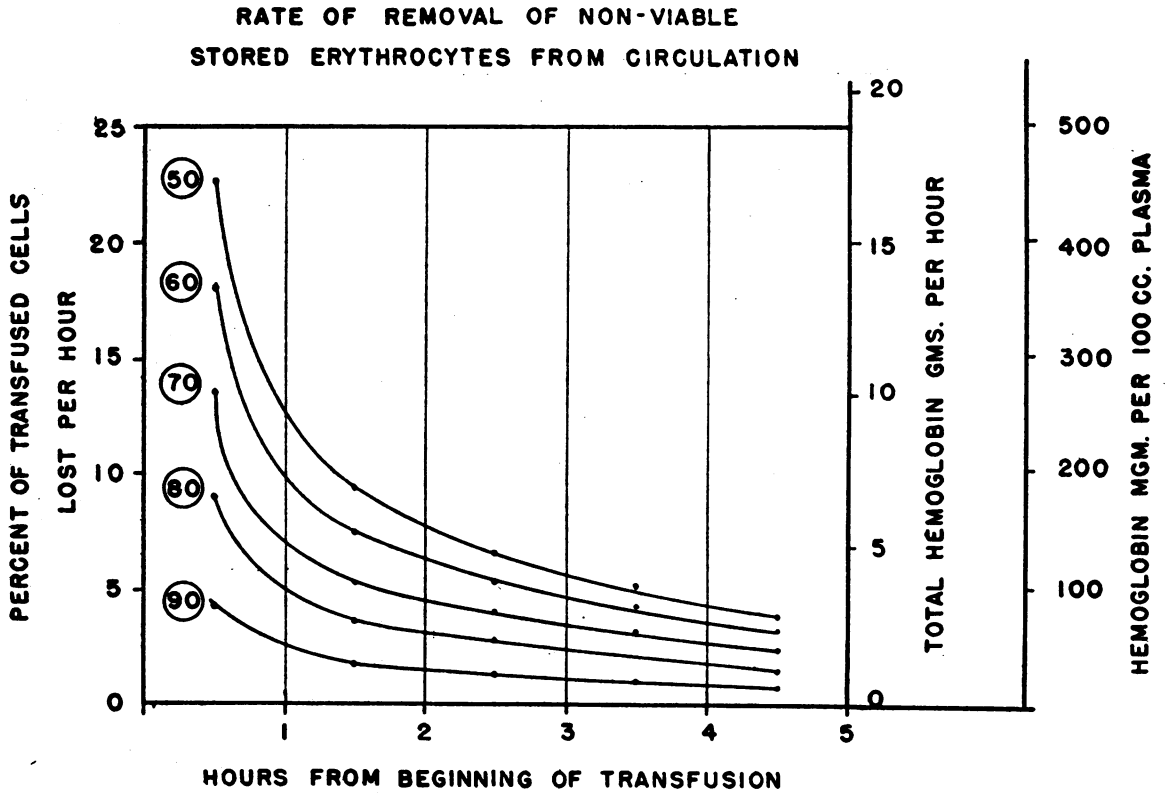

FIG. 4. RATE OF REMOVAL OF NON-VIABLE STORED ERYTHROCYTES FROM CIRCULATION

The average percentages of loss of the total transfused tagged red cells per hour during the first 5 hours from beginning transfusion, in bloods ranging from 50 to 100 per cent survival (indicated by figures in circles). The total amount of hemoglobin, in grams per hour (assuming immediate and complete intravascular hemolysis occurs), liberated from non-viable red cells following transfusion of blood surviving from 50 to 100 per cent, is shown in the first column on the right. The theoretical plasma hemoglobin level, in an individual with a plasma volume of 3,500 ml. resulting from complete retention of the liberated hemoglobin, is shown in the column on the extreme right.

intravascular hemolysis of non-viable cells is shown in relation to the per cent of transfused cells destroyed. These quantities are also plotted as mgm. of hemoglobin liberated per hour per 100 ml. of plasma, in an individual with a plasma volume of 3,500 ml.

Hemoglobinuria was observed only in those of our subjects who received full-sized transfusions of blood the survival of which was less than 50 per cent, and then was a transient affair, the urine being clear within a few hours after transfusion. Even in these subjects the rise in serum total bilirubin did not exceed 2 mgm. per 100 ml. of plasma. It is clear that many dead cells must be removed relatively intact from the blood stream. The role of the spleen in this regard is too well known to elaborate on.

The greatest burden of disposing of cell breakdown products is imposed within the first 2 hours after transfusion has begun. The extent of this load will vary inversely with the size of the transfusion, percentage of non-viable cells, and speed of inflow.

It would appear to be safe practice to set the lower limit of acceptable stored blood at 70 per cent post-transfusion survival. This certainly provides a margin of safety which might not be present were a less good survival accepted.

This concept affords a basis for the assignment of upper dating limit of bloods in the several solutions at present in use in certain blood banks.

Reference has been made (5) to the re-utilization of iron derived from broken down cells in the synthesis of new hemoglobin. It is of interest to know to what extent this blood iron is economized. Twenty-six experiments were completed in which recipient's blood samples were taken at frequent intervals, up to 21 days after transfusion, thus per-

TABLE II

Re-utilization of radio-iron derived from non-viable stored erythrocytes

Solution	Exp. no.	Days stored	Transfused			Per cent of radioactive data		
			Whole blood	Hct.	Cells	Retained	Available	Regen.
			ml.		*ml.*			
McGill II	1	0	157	32	50.3	94	6	4
	2	10	142	29.8	42.3	92	8	6
	3	14	117	31.0	36.0	76	24	11
	4	21	137	31.2	42.6	48	52	30
	5	29	143	31.9	45.6	22	78	52
ACD-1	10	2	84	44.5	37.4	100	0	0
	12	10	77	45.0	34.6	100		
	13	39	82	45.5	36.8	70	30	20
Cells resuspended in saline	6	0	175	40.0	68.0	87	13	5
	7	3	160	42.0	67.2	84	16	6
	8	10	140	46.1	63.7	10	90	32
Cells resuspended in 10 per cent corn syrup	14	1	59	52.0	30.7	100	0	0
	15	5	57	33.5	19.1	60	40	28
	16	12	62	32.7	20.3	43	57	32
	17	21	97	34.2	32.2	17	83	73
DeGowin	18	0	157	25.6	40.4	88	12	5
	19	13	166	24.8	41.2	85	15	3
	20	23	141	27.8	39.2	80	20	14
	21	36	153	19.5	29.8	22	78	71
Parpart's	25	0	120	28.5	34.2	96	4	4
	26	14	118	28.0	33.0	95	5	3
	27	27	126	31.0	39.1	88	12	12
ACD-1	32	15	132	38.7	51.1	85	15	5
	33	29	128	42.6	54.5	51	49	24
	34	41	128	40.1	51.3	37	63	33
		15	132	38.7	51.1	90	10	5
		29	128	42.6	54.5	60	40	18
		41	128	40.1	51.3	42	58	24

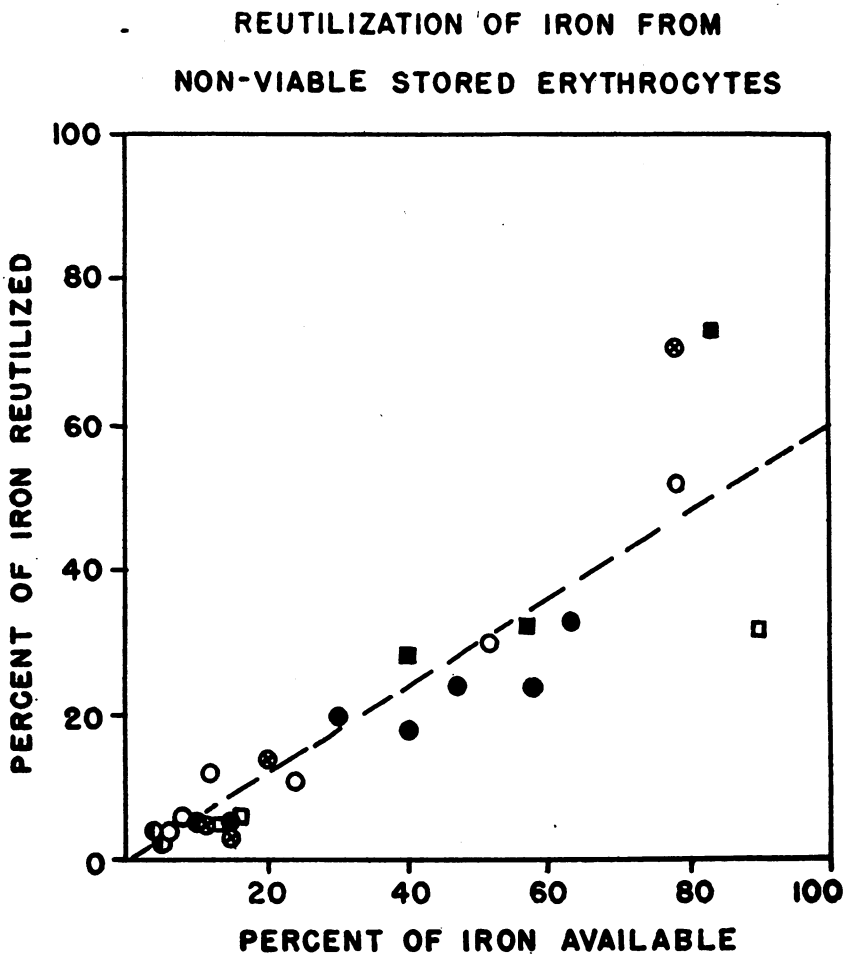

FIG. 5. RE-UTILIZATION OF IRON FROM NON-VIABLE STORED ERYTHROCYTES

The percentage of radio-iron derived from hemoglobin from liberated non-viable tagged transfused erythrocytes found in intact circulating red cells (re-utilized) in relation to the percentage of total transfused cells removed from circulation (available). About 60 per cent of the available iron is economized.

Symbols refer to the following blood preservatives:

● = ACD-1
○ = McGill solution
◐ = Parpart's solution
⊗ = De Gowin's solution
■ ± Cells in 10 per cent corn syrup
□ = Cells in 0.85 per cent saline.

mitting measurement of maximum re-utilization. Significant data from these experiments are shown in Table II. In 5 of the series the cells were transfused as whole blood; in 3 as resuspended cells. The quantity of cells given was small, ranging from 19 to 68 ml., averaging 41 ml., the contained hemoglobin, averaging about 15 grams. Individual circulating red cell volumes were from 1,780 ml. to 2,520 ml., averaging 2,214 ml. Survival was from 100 to 17 per cent.

The method of computing the percentage of transfused cells retained, in circulation and regenerated, has been described elsewhere (5). The percentage of iron available for resynthesis is taken as the arithmetic difference between 100 per cent and the percentage retained. These values are listed in Table II, together with the percentage of available iron actually re-utilized.

The percentage of iron re-utilized is plotted against the per cent available, in Figure 5. It is apparent that about ⅔ of the iron does eventually return to the blood stream in the hemoglobin of

new circulating red cells and that this percentage is relatively constant within the range of the dosage given. Forty ml. of cells contain about 50 mgm. of iron. Hence the iron administered as defunct cells ranged from 0 to 40 mgm.

Hahn, *et al* (6) have demonstrated that the absorption of radioactive iron from the normal gastrointestinal tract, as measured by the amounts detectable in circulating red cells, is extremely small. Cruz (7) also found that radio-iron liberated from hemoglobin from destroyed red cells was utilized nearly quantitatively even in the presence of normal iron reserves. It is of considerable interest that iron given intravenously, as hemoglobin in non-viable cells, is utilized approximately 20 times as efficiently as when iron is given orally.

Sufficient data are not available at present to determine the efficiency of utilization when larger amounts of hemoglobin iron are made available. In many experiments, however, in which full transfusions of very much deteriorated cells were given, the recipient's circulating red cell radioactivities have shown utilization of from 10 to 30 per cent of the available iron on the fifth post-transfusion day. Since ½ of the per cent available utilized is usually present on the fourth to seventh post-transfusion day, this would suggest excellent economy of quantities at least 10 times those dealt with in the above experiments.

CONCLUSIONS

(*1*) Non-viable stored human erythrocytes are rapidly removed from the blood stream after transfusion.

(*2*) The rate of removal of non-viable cells varies inversely with the percentage survival of the transfused tagged cells.

(*3*) At or above 80 per cent survival, non-viable cells are completely removed in 24 hours; below this survival level, loss of non-viable cells may continue into the second post-transfusion day.

(*4*) At any survival level, the majority of non-viable cells are removed from the blood stream during the first 2 hours after transfusion.

(*5*) On theoretical and practical grounds 70 per cent retention of all transfused cells may be considered the lowest safe survival level.

(*6*) The bodily economy of iron derived from the hemoglobin of non-viable cells is such that the utilization is about 20 times that of equivalent amounts of iron given orally.

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