THE SURVIVAL OF PRESERVED RED CELLS AFTER TRANSFUSION *†

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Although the practice of storing citrated blood for short periods in the cold is not entirely new, the widespread adoption of this procedure in the larger hospitals as a means of making available supplies of blood for immediate use, definitely is a development of the past decade. Stored blood was first used for transfusion by Robertson in France toward the end of the first World War. At that time, the circulatory aspects of shock became apparent to medical scientists and the necessity of replacing lost blood in the prevention and treatment of wound shock was realized. Robertson (1), at one of the British casualty clearing stations, stored blood up to twenty-two days and used it with excellent results. This innovation marks the first effective attempt to combat wound shock on the battlefield.

It is difficult to understand why Robertson's remarkable achievement remained forgotten on this continent for more than twenty years. In Europe, particularly in Russia, interest was revived ten years earlier than in America, by the experiments of Shamov, who, in 1927, demonstrated the capacity of blood, taken from a recently dead dog, to function normally in the circulation of a living animal. The successful performance of a corresponding experiment in man by Yudin in 1930 led to the extensive utilization of cadaveric blood, particularly in the city of Moscow. By 1936, Yudin and his associates had set up an unique and very efficient organization for the expeditious collection of blood from victims of fatal accidents, and for its distribution among the hospitals of the city. The successes of the Russian workers doubtless were responsible for the immediate use of stored blood in the treatment of wound shock during the civil war in Spain in 1937.

With a second world war in the offing, the military significance of blood preservation became apparent to medical scientists in all countries, and particularly in America and Great Britain. On this continent interest in the use of preserved blood in civilian hospitals received a tremendous impetus with the initiation of the idea of the blood-bank by Fantus (2) in 1937. Allied to, and almost coincident with this, was the

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introduction on a commercial scale, in the United States, of the vacuum desiccation process by means of which plasma and serum, indispensable in the treatment of burn shock, can be dried and preserved indefinitely.

Thus we have in brief review the development of a new facility which has enabled medical science for the first time in history to cope with the long-standing problem of shock. The use of preserved blood may well be regarded as one of the most important advances in medicine in the present century.

In the storage of whole blood the primary concern is the preservation of the erythrocyte. This implies not only keeping the cell membrane intact, but also the retarding of autolytic changes within the cell which impair its functional properties when it is again placed in the circulation. While the integrity of the cell membrane may be influenced by many agencies directly, as for example by the action of hemolytic agents either present in, or added to, the blood, it is now realized that hemolysis during storage is brought about largely as a result of autolytic changes within the cell. As soon as blood is removed from the body, the cell membrane undergoes marked changes in permeability especially in the cold. Potassium ions diffuse out of the cell while sodium ions enter from the plasma. Complex organic phosphates in the cell undergo autolytic hydrolysis with the liberation of phosphate; glucose breaks down to lactic acid and other intermediates. Some of the products of autolysis, including phosphate, do not pass through the membrane readily at lower temperatures. Hence as the osmotic pressure gradually increases, water enters the cell, causing it to swell and ultimately to burst. Individual bloods differ in regard to cell stability, i.e. rate of hemolysis during storage, and preservative solutions may differ greatly in their cell-stabilizing qualities. Sodium citrate solution, by itself, although still fairly widely used, is among the poorest of preservatives. In this solution hemolysis usually becomes visible after about the fifth day and the majority of red cells undergo irreversible changes by the tenth day. The addition of dextrose to citrate, on the other hand, greatly improves its preservative qualities. In isotonic citrate-dextrose, the rates of autolytic change and of cell swelling are much reduced. Blood may be kept in this mixture for six weeks and sometimes for two months with negligible cell-breakdown. The stability of the red cells is influenced also by the proportion of diluent used. With isotonic citrate-dextrose the maximum stability appears to be obtained with most bloods in the 1 : ½ dilution, i.e. one volume of blood to half a volume of diluent. In phosphate-buffered citrate-dextrose, blood may be diluted much further and may be stored at higher temperatures, e.g. 8 or 10 C., without impairing cell stability.

Many attempts have been made to devise a practical method for assaying the vital capacity of preserved erythrocytes. Such a method obviously would greatly facilitate the improvement of methods of preservation and would enable the operator of the blood-bank to know when

stored samples have reached the limit of their usefulness. Up until two years ago, when much attention was given to prevention of hemolysis during storage, the ordinary clinical fragility test was used for testing the stability of red cells. Later it was shown by Mollison and Young (3) and has been confirmed by the authors (4) that there is no correlation between the stability or instability of preserved cells to hypotonic or isotonic saline solutions and to plasma, and their capacity to survive in the circulation after transfusion. Since there is no in vitro test for the vital capacity of red cells, the only means of testing the influence of storage and of preservative mixtures is to transfuse the cells and follow their survival in the recipient.

Numerous studies have been reported on the survival of erythrocytes after transfusion. Values given for the life of the average red cell vary from thirty to eighty days, depending on the method employed. According to the differential-agglutination procedures of Ashby (5) and of Wiener (6), the average life span of the red cell is about eighty days but some may remain in the circulation for one hundred thirty days or even longer. In the former of these methods, a blood of group O is introduced into a subject of group A or group B. Periodically thereafter samples are withdrawn from the recipient and the latter's cells are selectively agglutinated by using the appropriate test serum (anti-A) leaving the surviving donor cells to be counted in the hemacytometer. Wiener's method is similar in principle excepting that the M and N blood types are used. Donor cells of group OM are injected into a recipient group ON (or vice versa) and periodically a sample of the recipient's cells is agglutinated with anti-N serum, leaving the surviving donor cells free. The two procedures when used concomitantly, e.g. group OM cells may be transfused into a recipient AN (or BN), give close agreement.

Wiener and Schaefer (7, 8) were the first to apply this method to the study of the survival of preserved red cells. For blood stored in citrate alone, they showed that if the blood were fresh, or if it were stored up to a week, the donor cells could be detected in the circulation for three or four months. If stored for longer periods the cells were eliminated much more rapidly. A twenty-one-day-old specimen, for example, was eliminated completely within twenty-six hours after transfusion. The period of useful storage in sodium citrate solution, according to these investigators, is from seven to ten days. This observation has been widely confirmed by clinicians, although some instances are known in which citrated blood was stored up to twenty days and was used without reactions and with beneficial results.

Cell survival is greatly prolonged when preservative mixtures containing dextrose are employed. Dextrose appears not only to stabilize the cell membrane but also retards autolytic changes during storage, hence may be considered a true preservative. Survival studies by numerous workers (4, 5, 9–16) have shown that cells stored up to four-

teen days in citrate-dextrose mixtures, with or without added saline or phosphate buffers, survive as well as fresh cells after transfusion. Here again individual bloods differ considerably with regard to preservation and survival capacity. Many specimens after storage for eighteen days show survival comparable with that of fresh cells after transfusion. When stored for longer periods, an increasing number of cells suffer irreversible deterioration, hence the rate of elimination from the blood stream is more rapid than the normal.

EXPERIMENTAL

The survival experiments described in the following sections were performed to test the merits of two preservative mixtures which the authors have found to be very satisfactory for red-cell preservation in vitro. The study was carried out at the Verdun Protestant Hospital (for mental patients) at Verdun, Quebec, and cell survival was followed by Wiener's method. Thirty-five transfusions were performed using bloods ranging from strictly fresh to samples stored up to fifty-seven days. All subjects were serologically negative and were in good health from a nutritional point of view.

Methods of Preservation

Blood was preserved in two mixtures, as follows:

1. Modification of DeGowin's citrate-dextrose formula.

Blood 400 cc., citrate (3.2 per cent) 80 cc., dextrose (5.4 per cent) 120 cc. This gives a ratio of blood to isotonic diluent of 1:½, a proportion which we have found to give optimal preservation with most bloods.

2. Buffered citrate-dextrose mixture.

The phosphate buffer was prepared as follows:

0.3 molar (4.14 per cent) monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$),	1,000 cc.
0.3 molar (1.2 per cent) sodium hydroxide (NaOH),	925 cc.
Water,	480 cc.

This mixture is isotonic with physiologic saline solution and has a pH of 7.4. Blood and diluent were mixed as follows:

Blood 400 cc., citrate 60 cc., buffer 60 cc., dextrose 80 cc.

Fresh twice-distilled water was used in all solutions, and sterilization was carried out without delay. The dextrose solution was sterilized separately and added aseptically to the other ingredients of the diluent after the solutions had cooled. If dextrose is added beforehand it tends to undergo caramelization during sterilization. Subsequent studies in our laboratory and in other centres have shown that the degree of caramelization produced in the usual autoclaving procedure has

no detrimental effect either on the preservation of red cells during storage or on the recipient.

In addition to the above preservative mixtures, DeGowin's formula was used in three stored specimens, the proportion of blood and diluent being as follows:

Blood 200 cc., citrate 40 cc., dextrose 260 cc.

As storage under an atmosphere of carbon dioxide has been advocated by some authorities, some of the bloods were equilibrated aseptically with carbon dioxide before storage to test the effect of this procedure on the preservation of the cells. All blood samples were drawn into chilled diluent without the aid of aspiration and by the method described in a previous report (17). They were then stored in a refrigerator automatically maintained at 4 ± 1 C.

Testing of Samples Prior to Transfusion

In addition to cross-matching the stored specimen with the recipient's blood, the amount of free hemoglobin, i.e., the degree of hemolysis if any, was estimated, and a red-cell stability test was performed. The free hemoglobin was estimated by a modification of the micro-benzidine method of Wu (18), or by the pyridine-hemochromogen method of Flink and Watson (19). The latter analyses were done on the plasma of an auxiliary specimen after resuspending and then centrifuging the cells. A stability test was carried out by mixing 0.2 cc. of the preserved specimen with 2 cc. of compatible plasma. After permitting the mixture to remain for four hours at room temperature, it was centrifuged and the free plasma hemoglobin, i.e. the additional hemolysis, was determined. Subsequently it was found that simply by resuspending the stored sample, permitting it to stand at room temperature for four hours, and then estimating the additional cell breakdown, the results were practically the same as when the sample was mixed with different plasma. The purpose of the test was to ascertain whether the amount of additional hemolysis could be related to the initial rate of destruction of red cells after transfusion. In other words it was desirable to know whether the cells that broke down in their own or in compatible plasma were also disposed to break down in the recipient's circulation shortly after transfusion. With the methods of preservation described, the degree of cell breakdown after resuspension of the stored samples was usually very small even when suspended up to twenty-four hours at room temperature. With some methods of preservation, however, extensive hemolysis may occur within half an hour under these conditions.

Transfusion Procedure

Anticipating that the introduction of 600 cc. of preserved blood into a normal subject might give rise to a slight polycythemia, and hence re-

sult in an increased rate of cell elimination, the practice in this study was, with one exception (a case of pernicious anemia), to remove 400 cc. of blood from the subject prior to transfusing the stored sample. While the blood was being collected, the cells in the stored specimen for transfusion were resuspended by carefully swirling the bottle for from five to ten minutes. From the handling the temperature was raised to about 10 C. The blood was then administered without further warming, being preceded and followed by about 50 cc. of saline solution. About a minute after the transfusion was completed, a 5 cc. sample of blood from the recipient was collected into exactly 1 cc. of 3 per cent citrate in a graduated centrifuge tube. Subsequent samples for cell survival counts were collected in the same manner at the fourth, twelfth, twenty-fourth hour, the third and sixth day, and thereafter at weekly or ten-day intervals. Free hemoglobin and bilirubin in the plasma were estimated by the methods of Evelyn (20, 21) on the four-hour sample and sometimes on the first four samples, especially after transfusion of the older stored specimens. Details of the method used for estimating the survival of donor cells after transfusion are given in a previous paper (4). Cell counts were continued on all subjects until the survival curve reached the recipient's free-cell count.

In all, 35 transfusions were performed. Three subjects, who eliminated donor cells at an unusually rapid rate, received a second transfusion after the first donor's cells had completely disappeared.

RESULTS

Method of Presenting Results

Not knowing accurately the blood volume of the subjects, it is impossible to calculate the absolute donor cell count anticipated immediately after transfusion. If, however, the plasma volume be approximated from the weight of the recipient, and the number of cells transfused be estimated, an initial count of about 400,000 cells per mm³ would be expected in the majority of the subjects. One method of expressing the results, therefore, is to take 400,000 as the basic value and calculate subsequent survival counts as percentages of the base.

Another method is to take the highest count after transfusion, usually that of the four-hour sample, as the basic value, and to calculate subsequent values as percentages. This method obviously disregards the possibility of the removal of some cells in the interval up to the fourth hour, whereas the former method makes allowance for it.

Table 1 contains information regarding the donors, recipients and transfused samples, and table 2, the experimental data obtained. In figure 1 the hard curves represent the cell survival calculated as percentage of the highest count, and the broken curves, the percentage survival based on an anticipated maximum count of 400,000 per mm.³ The

numbers accompanying each curve correspond with those in the first column in tables 1 and 2.

Table 3 contains the data on the degree of hemolysis in the donor samples which had been stored for thirty-five days or more before transfusion. The plasma bilirubin values, which usually reach a maximum at the third or fourth hour after transfusion, also are included.

TABLE 1

Recipient					Donor		Transfused sample		
No.	Age	Weight	Group	Condition*	Age	Number†	Days duration of storage	Gm. % Hb. in sample	Preservative‡
1	38	159	ON	E	36	2	Fresh		C.D.
2	36	152	OM	S	38	1	Fresh		C.D.
3	32	245 (obese)	ON	E.D.	30	A	1	Fresh	C.D.P.CO$_2$
4	59	130	ON	S.s.	41	12	14		C.D.P.
5	23	145	OM	E	34	B	15	10.0	C.D.
6	50	143	ON	M.d.	35	C	19	7.2	C.D.I.
7	36	150	OMN	S.D.c.	25	D	20	11.2	C.D.P.CO$_2$
8	52	175	OM	P.S.	19	25	21	12.4	C.D.P.CO$_2$
9	20	139	ON	S.s.	56	20	22	11.6	C.D.P.CO$_2$
10	41	161	OMN	S.D.	50	29	23	11.7	C.D.P.CO$_2$
11	20	139	ON	P.s.	38	28	23	10.6	C.D.P.CO$_2$
12	53	135	OMN	S.s.	25	E	24		C.D.I.
13	26	163	OM	S.s.	25	F	24	11.6	C.D.
14	41	130	OM	S.	32	3	24		C.D.
15	48	143	ON	S.D.	30	A	24	10.9	C.D.
16	45	149	OMN	F	20	11	24		C.D.
17	45	140	OM	S.	52	8	25	9.1	C.D.P.CO$_2$
18	43	146	OMN	S.	32	31	26	11.8	C.D.P.CO$_2$
19	26	145	ON	S.	26	G	27	11.3	C.D.
20	56	159	ON	M.d.	48	15	27	10.0	C.D.P.
21	50	132	OMN	S.	20	H	30	8.0	C.D.I.
22	40	145	OMN	S.D.	20	11	32	10.0	C.D.CO$_2$
23	38	160	ON	S.s.	20	9	32	10.6	C.D.P.CO$_2$
24	41	165	ON	S.	25	I	35	10.3	C.D.P.
25	19	139	OM	I	25	J	35		C.D.P.
26	49	133	ON	S.s.	25	K	39	10.6	C.D.P.
27	50	130	ON	P.s.	25	L	41	9.5	C.D.
28	38	157	OM	P.e.l.	21	M	42	11.6	C.D.P.
29	50	126	OMN	S.D.	25	N	57	9.6	C.D.P.
30(a)	57	166	OMN	M.d.A.	45	17	3	Fresh	C.D.P.CO$_2$
30(b)				AT	30	A	16		C.D.P.CO$_2$
31(a)	32	135	OM	S.s.	49	26	19	12.4	C.D.P.
31(b)					32	O	21	8.8	C.D.P.CO$_2$
32(a)	34	168	OM	S.s.	56	27	22	9.5	C.D.P.CO$_2$
32(b)					34	B	22		C.D.P.CO$_2$

* A—pernicious anemia; c—catonic; D—deteriorated; d—depressive; E—epileptic; F—feeble-minded; I—imbecile; M—maniac; P—paranoid; P.e.l.—postencephalitis lethargica; Ps—psychopath; s—simple; S—schizophrenic; T—after a course of liver treatment.

† Letters denote normal donors, all of whom were male except donor M. Numerals refer to the subjects enumerated in the first column.

‡ C—citrate; D—dextrose; P—phosphate buffer; I—DeGowin's mixture; CO_2—under carbon dioxide.

TABLE 2

Recipient number	Recipient's hemoglobin Gm. % *	Highest free cell count after transfusion	Duration of storage of specimen. days	Percentage cell survival at the end of following periods (days)								Number days donor cells detectable
				3	7	10	15	20	25	30	50	
1	15.4	240,000	Fresh	57	36	34	27	15	27	45	46	120
2	13.7	267,000	"	62	59	45	27	64	70	70	12	120
3	16.2	420,000	1	90	77	75	74	72	73	75	68	130
4	15.5	420,000	14	85	80	82	86	80	75	65	50	110
5	16.3	345,000	15	70	85	77	66	63	77	87	85	130
6	16.5	253,000	19	85	95	95	82	80	75	60	41	95
7	14.8	282,000	20	72	69	67	63	62	62	45	20	100
8	14.2	465,000	21	99	83	81	77	82	81	75	43	130
9	13.9	457,000	22	89	85	86	87	92	95	90	70	140
10	12.2	278,000	23	60	75	90	96	75	70	45	40	115
11	16.5	355,000	23	90	80	87	96	93	90	86	48	105
12	15.1	192,000	24	44	47	40	21	9	5	2	0	35
13	15.7	393,000	24	70	85	90	87	90	85	65	51	130
14	16.0	297,000	24	65	35	22	87	70	50	30	8	105
15	15.3	253,000	24	50	50	52	56	59	46	57	39	125
16	16.8	411,000	24	65	42	42	42	37	39	41	32	115
17	16.2	366,000	25	44	43	35	30	15	10	7	10	105
18	15.1	371,000	26	95	90	86	79	70	62	55	47	105
19	16.7	365,000	27	70	67	60	60	46	22	17	8	74
20	15.3	239,000	27	61	66	68	74	78	83	64	10	75
21	15.0	184,000	30	55	45	41	34	24	25	28	17	75
22	15.0	192,000	32	27	25	20	12	15	27	17	4	65
23	15.2	258,000	32	30	27	26	25	7	7	11	6	65
24	15.6	271,000	35	99	69	71	77	92	80	65	40	90
25	15.1	236,000	35	37	30	31	34	35	25	22	50	90
26	16.4	455,000	39	70	65	67	68	62	57	50	20	75
27	13.5	245,000	41	32	32	29	22	20	22	22	20	70
28	15.5	196,000	42	45	42	37	29	26	24	21	12	70
29	14.8	270,000	57	38	17	21	22	19	15	12	0	45
30(a)	6.8	550,000	3	82	70	62	47	32	22	16	6	60
30(b)	14.5	384,000	16	65	55	30	14	10	5	4	0	30
31(a)	17.1	384,000	19	97	85	75	61	42	25	17	0	45
31(b)	16.4	509,000	21	71	84	80	72	66	55	48	17	90
32(a)	16.7	325,000	22	100	76	65	45	30	20	12	0	35
32(b)	16.0	372,000	22	85	66	62	55	45	30	25	0	50

* Normal taken as 15.6 Gm. per hundred cc. of blood.

Condition of the Preserved Samples

All stored specimens were in excellent condition judged from the appearance, the degree of hemolysis, and cell stability after resuspension. As is indicated in table 3, hemolysis, even in the oldest samples, was negligible. Cell packing was considerably less dense in the buffered samples, and was greatest in the most dilute specimens. Fibrin precipitation was variable and was usually, though not always, greatest in the older specimens. As a rule the precipitation of fibrin increases more rapidly after the third week of storage and slows down again by the fifth or sixth week. In the resuspended samples, the fibrin usually was in very finely divided form, and except in one sample (donor sample 15,

column 7, table 1), no difficulty was encountered in the transfusions using Baxter equipment with the usual 200-mesh-per-inch stainless steel filter. Storage of blood under carbon dioxide apparently did not have any consistently beneficial influence in reducing fibrin precipitation or on cell preservation or survival.

DISCUSSION

As in a community of people, all the erythrocytes in the blood stream do not survive to a ripe old age. According to the method of Wiener, and also that of Ashby, the age limit for the corpuscle is about 125 days. Some red cells doubtless perish early in life but the majority are believed to remain in the circulation about 80 days. It is usually assumed that only the functional cells remain in the circulation and that as soon as the function of the cell fails or perhaps is impaired beyond a certain degree, the cell is destroyed. The validity of the survival method as a criterion of preservation depends on these assumptions.

TABLE 3

Recipient number	Storage period of sample, days	Total free hemoglobin in sample, Gm.	Per cent of cells hemolyzed	Bilirubin in patient's circulation 4 hours after transfusion, mg. % (in plasma)
24	35	0.23	0.38	Normal
25	35	0.40	0.6	4.1
26	39	0.32	0.5	Normal
27	41	1.86	3.2	3.3
28	42	1.08	1.5	2.8
29	57	0.42	0.7	4.7

In many of the curves in figure 1, and especially in curves 3, 4, 7, 8, 9, 11, 13, and 18, it will be observed that the donor cells disappeared at a steady and almost linear rate. In other instances, notably in 1, 2, 5, 14, and 16 in which the curves extend to one hundred twenty days, the rate of cell removal is less regular. The reason for the unexpected irregularity in curves 1 and 2, which represent the behavior of absolutely fresh transfused specimens, is not known. A repetition of these experiments has been carried out with the same erratic results. Bloods stored for longer than twenty-four days generally show poorer survival after transfusion, as is evidenced by the curves from No. 17 upward. It is noteworthy, however, that occasional donor specimens, for example, cases 18, 20, and 24, in which the samples had been stored for twenty-six, twenty-seven and thirty-five days respectively, show much better survival than the others of comparable age.

Curve 14 presents two peaks of considerable significance. The first maximum occurs about the twentieth day, and the second, between the sixtieth and the nintieth days. That these discontinuities are not merely errors in cell counts was confirmed by repeating some of the counts as

FIG. 1.

Plate 1—Continued.

many as eight times. Furthermore, the same results are obtained when two testing sera are used simultaneously. Similar increases, though less pronounced, may be detected in the majority of the curves in figure 1. Thus a definite increase in the free-cell count is seen at about the twentieth day in curves 1, 4, 8, 9, 10, 11, 13, 19, 20, 24, and 26, and the tendency is detectable in curves 3, 7, 21, and 25. The second peak is less regular in occurrence but it is noticeable in many curves, including numbers 1, 2, 6, 7, 13, 15, and 16. The likelihood of the increases being due to a periodic influx of new immature red cells has been ruled out by Maizels (12).

After considering the phenomenon in question from many angles, the authors have been obliged to conclude that the peaks represent true increases in the number of donor cells in circulation. If this interpretation is correct, it would seem that all the cells that disappear from the circulation are not necessarily destroyed, but that some are stored and may be released again. The process of release is not sudden but gradual, usually taking place over a period of ten days or more. Administration of epinephrine to some patients, and subjecting others to exercise have failed to produce an increase in circulating donor cells, although definite increases in the number of reticulocytes in circulation have been observed. The rather gradual release of donor cells observed does not suggest the action of epinephrine or other rapidly acting agent or mechanism. The increase in count is not accompanied by any change in hemoglobin or in total cell count.

If it is true that some cells are stored and released again, the actual survival is better than indicated by the curves in figure 1. One is forced to conclude that the number of surviving donor cells prior to each peak on the curve may possibly be greater, but is never lower than the peak value. Thus, in curve 16, if the survival at the seventieth day is 80 per cent, the survival prior to this peak must be at least as great.

The period during which donor cells may be detected in the circulation obviously is not the most important index of the usefulness of the specimen. In curves 17 and 18, the transfused bloods stored approximately for the same length of time show equal survival, viz. 100 days, but the proportion of cells surviving at any one time during that period was very much greater in case 18. The important consideration in evaluating the usefulness of stored blood is the proportion of cells in circulation during the critical period of need, i.e., usually a week or even less. It will be observed that in thirty-day-old blood, cell survival after transfusion is usually considerably less than with fresh blood even as early as the tenth day, but in subjects 24 and 26, survival is very good.

In three recipients, namely numbers 30, 31 and 32, and possibly also in number 12, cell elimination was unusually rapid considering the age of the donor sample. A second transfusion from a different donor, therefore, was given after the first cells had disappeared, to ascertain whether the phenomenon was due to the quality of the donor blood, or

to a propensity of the recipient to destroy cells at an abnormal rate. It will be observed that the second donation in each instance also was rapidly disposed of, usually within sixty days.

Subject number 30 had pernicious anemia, and at the time of the first transfusion had a hemoglobin value about 45 per cent of normal. Coincident with the transfusion, liver therapy was started and within a few months the hemoglobin value was restored to normal. Since it is known that the rate of red cell destruction is not abnormal in pernicious anemia, it is almost certain that the rapid elimination of cells was not connected with the anemia. This is indicated, furthermore, by the repeated behavior after the second transfusion (b). Since the second donation also was eliminated rapidly, it seems logical to conclude that the rate of cell elimination depends not only on the quality of the transfused cells, but also on the propensity of the recipient. This is somewhat disconcerting since, if true, the cell-survival method is not a test of the effects of storage alone. In this type of study, therefore, one is dealing with three variables, namely, the quality of the donor cells, the effects of storage, and the properties of the recipient.

If some recipients tend to eliminate transfused cells at an unusually rapid rate, it is logical to suppose that there are others also who tend to destroy them at a rate slower than normal. Subjects number 5 and 15 may belong to such a group. This tendency to destroy cells or to spare them is probably not a constant characteristic of the recipient but the property of elimination may be subject to variation.

In none of the recipients who received blood over 35 days old, was there any sign of icterus. The low bilirubin values at the fourth hour after transfusion indicates that there was a gradual destruction of cells. On the other hand, curve 29 indicates that there was a fairly rapid removal of cells during the first day or so after transfusion but not sufficiently rapid to cause icterus. All the older bloods represented in table 3 were quite stable in their own plasma and also in fresh compatible plasma.

An effort was made in the present study to use the area under the cell survival curves, up to the thirtieth day, in figure 1, as an index of the performance of the respective donor bloods after transfusion. By this procedure there is no correlation between the areas and the respective periods of storage.

Since preserved erythrocytes undergo considerable change in composition by the fifteenth day, and yet are capable of normal survival when placed in the circulation, it would appear that either the transfused cells undergo a reconditioning process, as suggested by Maizels (12), or that the alteration during storage is not sufficient to impair their functional properties. Maizels has submitted evidence that at least the disturbed electrolyte distribution between red cells and plasma is corrected within twenty-four hours after transfusion. It is not unreasonable to suppose that, within limits, autolytic changes also may be

reversible when cells are placed again in the circulation. After fifteen to eighteen days' storage the alteration in an increasing number of cells becomes irreversible, resulting in impaired function and more rapid destruction.

In the operation of a blood-bank it is necessary to maintain a stock of plasma as well as blood. The practice, in the past, has been to discard the cells after removing the plasma. During the past year or so, however, there has been increasing interest in the utilization of the cells, particularly in the treatment of secondary anemias. Usually the cells are suspended in physiologic saline solution and administered immediately. If stored in saline, they begin to hemolize after three or four days, largely because of the low protein concentration of the external medium. This difficulty may be overcome simply by storing the sample after removal of the plasma, and by resuspending the cells in saline dextrose, containing a very small amount of citrate, when they are to be used. If this practice is adopted it is preferable not to use centrifugation, but to permit spontaneous sedimentation of cells for a week before removing the plasma. Red cells stored in the residual plasma keep practically as well as in whole blood. Specimens may be reconstituted after storage for a month or six weeks without hemolysis although they cease to be comparable to fresh cells after about fifteen days.

Conclusions

From the foregoing discussion it is apparent that the conditions of storage are not the only factors influencing cell survival after transfusion. Differences in the stability of individual bloods, in the cell destroying propensities in recipients, and the inconstancy of both these factors in any individual, make difficult the interpretation of results. Whether this variability is accentuated in mental subjects is not known. The difficulty of interpretation in survival experiments may be reduced by dividing each preserved specimen among two or more recipients.

Both the buffered and the unbuffered preservative mixtures used in this study give very satisfactory preservation of the erythrocytes. The buffered solution tends to reduce the packing of cells on sedimentation and hence facilitates resuspension. It also is the more effective in retarding autolytic changes during storage, notably the breakdown of labile organic phosphate compounds. Despite these merits, however, the actual red-cell survival after transfusion was not noticeably superior with the buffered solutions. While some bloods keep better than others, as a rule cells stored up to 18 days may be considered equivalent to fresh cells. No toxicity develops in blood stored up to two months, i.e. long after the majority of the cells have lost their functional qualities.

The relatively rapid disappearance of other elements, such as white cells, platelets, prothrombin, complement, and hemopoietic properties

from blood during storage presents no serious problem since when fresh blood may be required it is usually obtainable from the blood-bank. For secondary anemias, and especially for treating acute hemorrhage, blood stored for three weeks and even longer, is efficacious. Under war conditions, and particularly in areas within bombing range, it is necessary to maintain large supplies of blood and plasma. While it is desirable to keep within the three-week storage limit, there should be no hesitation in using specimens up to a month old in emergencies. Numerous instances have been reported in the present war when month-old blood has been used with good results.

SUMMARY

The merits of two preservative mixtures were compared by storing blood at 4 C. for various periods up to fifty-seven days, and by following red-cell survival after transfusion. One preservative solution consisted of citrate-dextrose and the other, a similar mixture buffered with phosphate at pH 7.4. Both solutions were isotonic with normal plasma and were used with blood in the proportion of one volume of the latter to one half volume of diluent. Under these conditions red cells can be stored for six weeks, and in some instances two months, with less than 1 per cent hemolysis. The buffered solution tends to prevent dense packing and cohesion of cells during sedimentation, and is more effective than the unbuffered mixture in retarding autolytic changes, particularly the breakdown of organic phosphates, during storage.

Thirty-five transfusions were performed and cell survival was followed by the method of Wiener. Despite the merits of the buffered mixture, red-cell survival was not noticeably superior to that obtained with the unbuffered solution. With either mixture, red cells stored up to eighteen days survive as well as fresh cells after transfusion and may be detected in the circulation for about one hundred twenty-five days. Samples stored for twenty-four days undergo slightly more rapid elimination, but in several instances the cells did not disappear completely until about the one hundred twenty-fifth day. Beyond twenty-four days' storage, cell destruction after transfusion is still more rapid and the maximal survival time is decreased. There is evidence that cells are not necessarily destroyed as soon as they disappear from the circulation, but that a considerable number may reappear later, usually between the 15th and the 25th days, and sometimes about the 60th day after transfusion. Even with thirty-day-old blood, cell survival is sufficiently good during the first week after transfusion to warrant using such samples in emergencies.

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