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THE EFFECT OF VARYING TEMPERATURES ON THE POST-TRANSFUSION SURVIVAL OF WHOLE BLOOD DURING DEPOT STORAGE AND AFTER TRANSPORTATION BY LAND AND AIR¹

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The successful establishment of a military whole blood transfusion service is dependent upon reducing damage to the cells from mechanical agitation incident to land, sea, and air transportation to a minimum. Robertson (1) successfully transported whole blood in Rous-Turner solution by ambulance for short distances in World War I. Blood from civilian donor centers was shipped to front lines during the Spanish Civil War (2). Maycock has reported on the use of blood transported in refrigerated trucks during the Battle of Flanders (3). During the invasion of Europe blood banks were in operation in many theatres, blood being obtained from military personnel, a necessarily limited source of supply.

The establishment of the American Red Cross Blood Donor Service, taking blood for processing of plasma, afforded a potentially adequate supply source, provided blood could withstand shipment across the Atlantic and far out into the Pacific oceans. The distances involved were so great that air transport was the only feasible means of transportation that would get blood to medical personnel before it had seriously deteriorated.

It was obvious that only the best known whole blood preservatives would suffice, and 2 solutions, Alsever's (4) and acid-citrate-dextrose (5), were under consideration by the Armed Forces at the time this study was undertaken. The comparative value of these 2 solutions for depot storage under constant refrigeration has been reported (6), ACD being superior to Alsever's. The survival of these cells in these preservatives after long-range ship-

ment, with and without controlled refrigeration, was not, however, known.

De Gowin (7) in 1941, transported whole blood in a modified Rous-Turner solution in vacuum collecting bottles for distances up to 720 miles by automobile, and up to 3,500 miles by airplane. The bottles were refrigerated with cracked ice which was replaced, if melted, every 12 hours. These bloods were transfused without reactions, although no post-transfusion survival studies were made. Marked destruction of cells as evidenced by hemolysis did not occur. Kendrick, *et al* (8) concluded that "blood collected in Alsever's solution, after adequate pre-chilling (24 hours), could be flown unrefrigerated for 24 hours from the United States to the United Kingdom and would be safe to use for 21 days after collection, that is, when the blood is refrigerated continuously following its arrival in the United Kingdom."

These experiments were designed to define the optimal temperature of refrigeration and the effect of uncontrolled refrigeration both during depot storage and during land and aerial transportation. The effects of extreme ranges of temperature, from -4° C. to $+40^{\circ}$ C., were studied in bloods in both ACD-1 and Alsever's solutions stored in depots. Since the problem of successful transportation involved the determination of adequate refrigeration range, experiments were conducted in which bloods were transported by air both under controlled and uncontrolled refrigeration.

The method of measuring post-transfusion survival by means of 2 isotopes of radioactive iron has been previously described (9, 10).

DEPOT STORAGE EXPERIMENTS

In all these experiments blood from the same donor was taken in equal amounts into both solutions (except

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.

Experiments 51 through 55), and aliquots of each were transfused after storage for equal periods of time under identical temperature conditions.

Experiments 65 (Alsever) and 66 (ACD)

The aliquots of blood were stored at 4° C. for 4 hours and then removed to a constant temperature apparatus and kept at -4° C. for 24 hours. They were then returned to storage at 4° C. until transfused, the blood in ACD at 8 days and that in Alsever's solution at 9 days after drawing. Each transfusion contained about 50 ml. of cells. The blood did not freeze and there was only slight hemolysis in the supernatant fluid. Survival in both experiments was about 75 per cent. Neither recipient experienced any reaction.

Experiments 57, 59, 61 (Alsever) and 56, 58, 60 (ACD)

The aliquots of this blood were kept at 4° C. throughout the whole storage period and were transfused at 11, 15, and 21 days after drawing, described above in the

section on whole blood preservatives. Survival in both solutions was satisfactory (70 per cent or better) up to 21 days.

Experiments 63 (Alsever) and 62 (ACD)

Bloods were stored 46 hours at 4° C., then allowed to stand at room temperature (20 to 25° C.) for 24 hours, and returned to storage at 4° C. until transfused 18 days after drawing. Each transfusion contained about 100 ml. of cells. The recipient of the ACD blood had a mild transient febrile reaction, and rabbit pyrogen test on the supernatant was positive. The other recipient had no reaction and the pyrogen test was negative. Survival of blood in ACD was better than that in Alsever's, but both were less good than was obtained for bloods in both solutions stored at 4° C. for 18 days.

Experiments 67 (Alsever) and 64 (ACD)

Bloods were stored at 4° C. for 4 hours, then transferred to an incubator where they remained at +40° C. for 24 hours, and returned to storage at 4° C. until trans-

EFFECT OF VARYING STORAGE TEMPERATURES ON POST-TRANSFUSION SURVIVAL

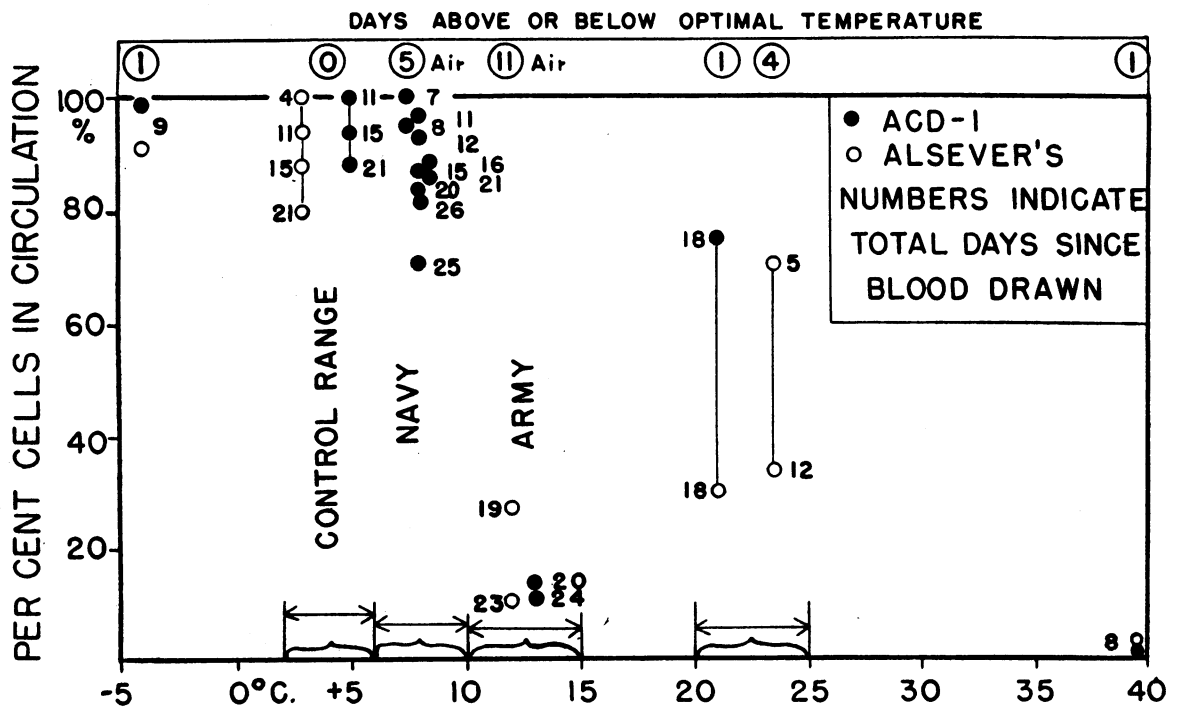


FIG. 1

Figures within circles indicate the days bloods were at, above, or below the optimal (control) range. In all instances the bloods were returned to storage at 4 to 6° C. after exposure to the test temperature. Aerial transportation, with adequate refrigeration, is not deleterious. Lack of adequate refrigeration is deleterious both during aerial transport and subsequent optimal refrigeration. Exposure to room temperature for even short periods is harmful.

WHOLE BLOOD IN ALSEVER'S SOLUTION pH 6.8

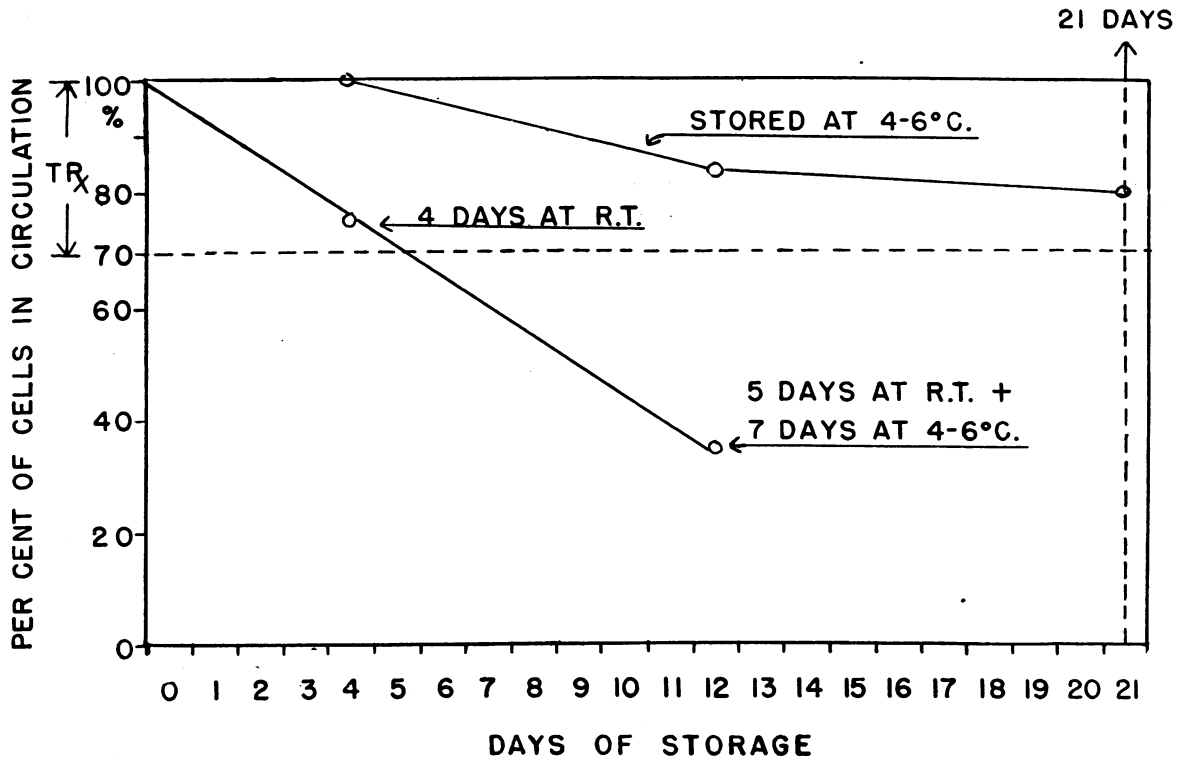


FIG. 2

All aliquots of blood in Alsever's solution were drawn from the same donor. Storage for 4 days at room temperature (20 to 25° C.) initiated deteriorative changes, as evidenced by the superior (25 per cent) survival of the aliquot in depot storage at 4° C. for 4 days, the rate of which was not retarded by subsequent refrigeration at 4° C.

fused 8 days (Alsever's) and 7 days (ACD) after drawing. Each transfusion contained about 80 ml. of cells. The supernatants of both bloods were deeply hemolyzed, but neither recipient had a reaction. Virtually no radioactive cells were found in the blood stream of either recipient 24 hours after transfusion.

The survival data obtained in these depot storage experiments are shown in Figure 1.

Experiments 51 through 55 (Alsever)

These experiments were designed to determine whether deteriorative changes that might occur at room temperature could be arrested by subsequent storage at lower ranges.

Blood from 1 donor was taken into Alsever's solution and divided into 5 equal aliquots. Three of these were transfused after storage at 4° C. for 4, 12, and 21 days after drawing. Another was transfused after storage at room temperature (20 to 25° C.) for 4 days. The other was kept at room temperature for 5 days, then stored at 4° C., and transfused 12 days after drawing. Each transfusion contained about 50 ml. of cells. No reactions occurred.

As shown in Figure 2, survival after 4 days at room temperature was markedly less than after 4 days at 4° C., and even worse after subsequent refrigeration at 4° C. for 7 additional days than was the control stored at depot temperature.

TRANSPORTATION EXPERIMENTS

Two series of experiments were carried out; in one, blood was taken into ACD and transported under controlled refrigeration,² while, in the other, bloods in both ACD and Alsever's solution were transported under uncontrolled refrigeration.³ In both experiments, the bloods were flown about 6,000 miles, in the first overland and in the second overseas.

² This experiment was undertaken at the request of the U. S. Navy, Medical Corps, in cooperation with Capt. Lloyd Newhouser, U.S.N., M.C.

³ This experiment was undertaken at the request of the U. S. Army, Medical Corps, in cooperation with Capt. John Elliott, A.U.S., Sn.C., Capt. John Reichel, A.U.S., M.C., and Capt. Ellis Vaubel, A.U.S., M.C.

Controlled refrigeration. Experiments 68 through 77

Full bleedings (480 ml.) were taken on the same day from each of 10 group O, Rh positive radioactive donors into standard 600 ml. vacuum bottles containing 120 ml. of ACD and were immediately refrigerated at 4° C. The

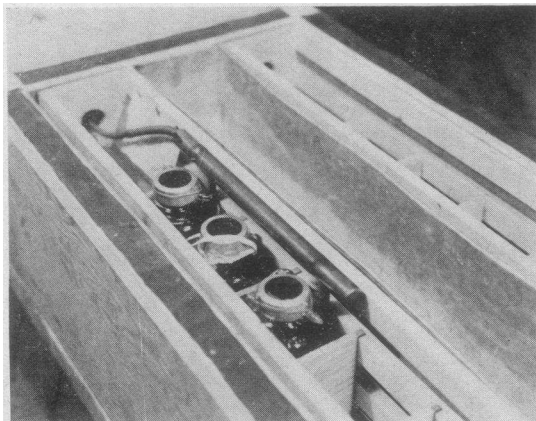


FIG. 3A. REFRIGERATOR USED FOR AERIAL TRANSPORT OF WHOLE BLOOD IN ACD-1

The temperature-sensitive element of the gas-filled recording thermometer was fixed in position in close proximity to the bottles of blood. Note also the dioxane indicators fastened to the bottle necks.

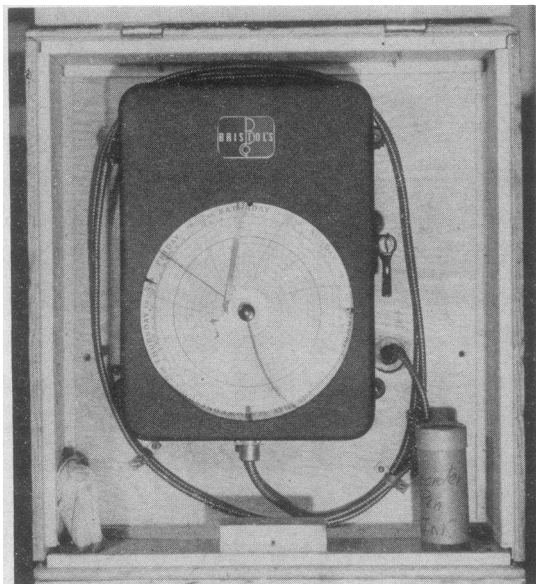


FIG. 3B. REFRIGERATOR USED FOR AERIAL TRANSPORT OF WHOLE BLOOD ACD-1

Bristol recording thermometer, mounted on icebox. A continuous recording was obtained during the 5 days en route.

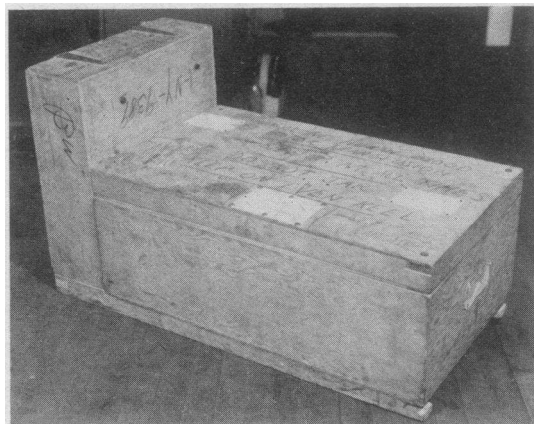


FIG. 3C. REFRIGERATOR USED FOR AERIAL TRANSPORT OF WHOLE BLOOD ACD-1

Refrigerator on return from Oakland, California.

refrigerator (Figure 3) in which these bloods were transported was made for this experiment at the Naval Medical Center, Bethesda, Maryland, and consisted of an insulated wooden chest, with 12 compartments for blood bottles and a single large central compartment for water ice and a tightly fitting insulated cover. A Bristol gas-filled recording thermometer was attached to the refrigerator in such a way that the thermal element lay between the 2 rows of bottles. A thermal indicator was attached to the neck of each bottle. These were "L" shaped glass bulbs containing dioxane, the melting point of which is +10° C. They were frozen with the dioxane all in 1 arm of the tube and so mounted on the bottle that the frozen arm was uppermost. Exposure to temperatures above 10° C. melts the dioxane which then flows into the lower arm, where it remains even though re-frozen.

This shipment of blood went by National Air Transport Service from the East Boston Airport to the National Airport at Washington; from there to Oakland, California, and was returned by National Air Transport Service from Oakland directly to Boston. The package was placed in a cold room at the Naval Medical Center in Bethesda, Maryland, and also at Oakland while awaiting air transportation. The time elapsed between leaving from and returning to Boston was 122 hours. The package was in flight 22 hours, and in mechanical refrigerators for 34 hours. During the remaining 66 hours, the only refrigerant was the ice in the box itself. The box was re-iced once in Oakland during the 5-day trip. The distance covered by air was about 6,000 statute miles.

The recorded temperatures (Figure 4), when corrected for variations in altitude during flight, did not exceed 15° C. at any time during the trip, and 6 of the dioxane indicators were melted and 4 still frozen, with the dioxane in the upper arm of the tube, on arrival. They were promptly returned to depot storage at 4° C.

The condition of these bloods, as determined by *in*

TABLE I

Condition of human erythrocytes transported in ACD and Alsever's solution under continuous and intermittent refrigeration, as determined by *in vitro* tests

Exp. no.	Days since drawn	Transfused		Total Hb	Supernatant			Cells hem. in 0.6 per cent NaCl	Survival by radio-iron
		Whole blood	Red cells		Hb*	Cells hemolyzed**	pH		
		ml.	ml.	grams	mgm. per cent	per cent		per cent	per cent
Continuous refrigeration 4 to 10° C.—ACD									
68	7	563	220	65.3			7.04	18	94
69	8	542	247	70.2	36	0.15	7.17	10	89
70	11	552	243	65.8	196	0.92	6.97	12	90
71	12	556	233	66.3	19	0.07	6.94	12	87
72	15	538	230	67.0	51	0.24	6.97	15	81
73	16	565	253	72.5	58	0.25	7.20	24	83
74	20	558	234	56.6	74	0.42	7.02	26	81
75	21	568	246	68.8	112	0.52	7.01	18	83
76	25	326	150	38.7	115	0.52	6.95	22	72
77	26	284	125	27.6	159	0.44	7.12	28	78

Intermittent refrigeration 4 to 15° C.—Experiments 78, 80 in Alsever's; 79, 81 in ACD

78	19	575	161	44.3	79	0.75	6.70	50	27
79	20	366	156	41.3	166	0.85	6.70	63	13
80	23	515	151	40.8	109	1.09	6.65	83	10
81	24	285	122	31.6			6.80	70	9

* Determined by hemochromogen technic.

** Percentage of total cells corresponding to quantity of hemoglobin in supernatant by the equation

$$\text{per cent cells hemolyzed} = \frac{\text{ml. supernatant} \times \text{Hb. in mgm. per cent}}{\text{Total Hb. in mgm.}}$$

in vitro studies carried out just prior to transfusion, is shown in Table I. Very little hemolysis had occurred, amounts present in the supernatant of a centrifuged sample representing hemolysis of less than 1 per cent of the total cells drawn. The pH of the supernatants ranged from 7.2 to 6.94. Fragility tests showed from 10 to 28 per cent hemolysis in 0.6 per cent NaCl.

These 10 bloods were transfused into group A, Rh positive individual recipients, each of whom had previously been bled 500 ml., at intervals of from 7 to 26 days after drawing. All the blood in the bottle was given except in the cases of the 25- and 26-day-old bloods in which about ½ of the contents was given. Two recipients, with allergic histories, experienced anaphylactoid reactions, and 3 exhibited symptoms which could be attributed to rapid breakdown of red cells. Subsequent analysis of data in one of these receiving a high titre blood, indicated that his own cells had been destroyed (10).

As shown in Table I and Figure 1, the post-transfusion survival of these bloods was not less than 80 per cent up

to 21 days after drawing, and not less than 70 per cent in the two oldest bloods.

Uncontrolled refrigeration. Experiments 78, 80 (Alsever); 79, 81 (ACD)

Five hundred ml. of whole blood from each of 5 donors were taken into 500 ml. of pre-chilled Alsever's solution in standard 1,000-ml. vacuum bottles, and 480 ml. of whole blood from each of 5 donors into 120 ml. of pre-chilled ACD in standard 600-ml. vacuum bottles. All donors were group O, Rh positive, and were bled the same day. The bloods were refrigerated immediately after drawing. The bottles were prepared for shipment in a manner simulating that in routine use at the time by the Army Medical Corps for overseas aerial transport. They were replaced in the manufacturer's cartons which were then wrapped in heavy brown paper and sealed with gummed tape. Just prior to this, a dioxane (M.P. 10° C.), caprylic acid (M.P. 15° C.) and a water (F.P. -2° C.) thermal indicator was attached to the neck of each bottle. The 2 cartons, one containing blood in Alsever's and the other blood in ACD, were fastened together and placed in a light wooden box attached to the same recording thermometer used in the previously described experiments, the thermal element being enclosed within the cartons.

This shipment was flown from Boston to La Guardia Field, N. Y., and thence by Air Transport Service to Paris, where it arrived 6 days later. The recorded temperature was 15.6° C. and the package was refrigerated at 3.3° C. in Paris, for 31 hours. It was then flown back to La Guardia Field where it was again refrigerated until returned to Boston. It was en route 11 days. No refrigeration was used during air passage.

Immediately on arrival the bottles were refrigerated at 4° C. All of the dioxane, 5 of the caprylic acid, and all of the water indicators were melted. The exact number of hours the package had been in flight, in refrigerators, or unrefrigerated is not known. The graph of the recording thermometer was blurred so that no readings could be obtained.

The conditions of these bloods, as determined by *in vitro* tests carried out just prior to transfusion, is shown in Table I. Very little spontaneous hemolysis occurred. The pH of the supernatants ranged from 6.65 to 6.80, and hemolysis in 0.6 per cent NaCl from 50 to 83 per cent.

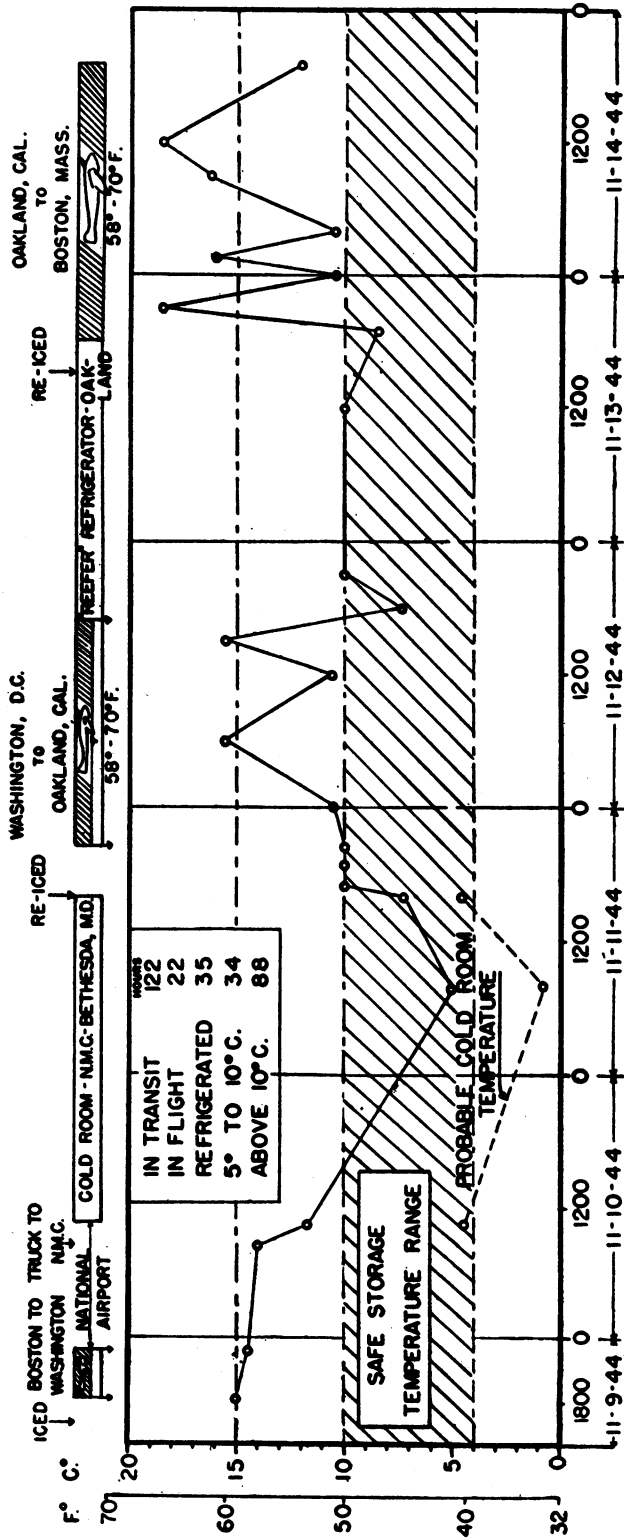
Two bloods in half amounts in Alsever's were transfused at 19 and 23 days, and 2 in ACD at 20 and 24 days after drawing. Each transfusion contained about 125 ml. of cells. The remaining bloods were not transfused. One recipient experienced moderately severe symptoms of rapid breakdown of cells but without lasting sequelae. Post-transfusion survival ranged from 9 to 27 per cent, as shown in Figure 1.

DISCUSSION

It is evident from Figure 1 that the optimal survival of cells in both solutions, in depot storage,

TRANSPORTATION OF REFRIGERATED WHOLE BLOOD IN ACD-1 REFRIGERATOR TEMPERATURE DURING TRANSIT

OEM Cur-131
IN COLLABORATION
WITH
U.S. NAVY M.C.



DAYS IN TRANSIT

FIG. 4. BLOODS IN BOTH ACD-1 AND ALSEVER'S SOLUTION WERE TRANSPORTED BY AIRPLANE ABOUT 6,000 MILES AS DESCRIBED IN THE TEXT. The cross-hatched bars indicate the time elapsed from leaving and returning to our laboratory; the open bars, the period in depot storage at 4° C. after return. The survival of the bloods transported under controlled refrigeration was equal, at any given observation period, to that of whole blood in ACD-1 in depot storage at 4° C. None of the bloods transported under uncontrolled refrigeration were fit for transfusion, in spite of a period of depot storage at 4° C.

was obtained in the series stored at 4° C. Slight deterioration was evident in the cells stored at -4° C., much more at room temperature, while complete deterioration occurred at +40° C. The results obtained in Experiments 51 through 55 indicate that once deterioration is initiated at room temperature, it is not retarded by subsequent storage at lower temperatures, as shown in Figure 2. The data in Figure 2 also indicate a slightly superior ability of cells in ACD to stand up under adverse thermal conditions than in Alsever's solution.

It is apparent that controlled refrigeration at not over 10° C. is an essential for the satisfactory preservation of cells during transportation.⁴ No significant deterioration of cells in ACD-1 resulted from the mechanical agitation incident to land and air travel, in the experiment in which temperature was maintained with a fair degree of constancy at about 10° C., since the survival after 5 days of travel and 16 days of subsequent refrigeration was as good as that obtained for blood in the same solution after depot storage for a similar period.

It is also evident that lack of constant refrigeration had extremely injurious effects on red blood cells taken in either ACD or Alsever's solution. The results that would have been obtained had the transportation period been 11 days instead of 5 in the first experiment cannot be known, and unfortunately none of the bloods shipped without controlled refrigeration could be transfused until 19 days after drawing. It was stated above that deterioration is accelerated at temperatures in the region above 15° C. and that this rapid deterioration is not slowed by subsequent cooling. The excellent preservation of the ACD bloods would therefore suggest that little damage was done during transport, since the bloods were in depot storage for from 2 to 21 days after their return, ample time for progressive changes to have continued, had they been initiated during flight.

Evidence was obtained that, in the second experiment, the temperature around the bottles had exceeded 15° C. (melted caprylic acid indicators),

⁴ The authors wish to state clearly that no adverse criticism of the performance of the U. S. Army M.C. in flying thousands of bloods in Alsever's solution to E.T.O. is implied. The experiments cited do, however, illustrate the effects of 2 different ranges of temperature control during transportation.

and itinerary records obtained make it clear that the package was exposed to temperatures as low as 4° C. at various times. Hence, the bloods were subjected to a fluctuating rather than evenly maintained temperature. It seems probable that marked damage was initiated during transportation and continued during subsequent depot storage.

Because of the extreme degeneration, no comparison between the value of the 2 preservatives during transportation at high temperatures can be made.

In a previous paper (11), we stated that no serious danger to a recipient need result from a full transfusion of stored blood of which at least 70 per cent of the cells remained viable, since the rate of destruction of the non-viable cells was slow, but that hemolysis of cells might occur at a rate and in quantities sufficient to raise the plasma hemoglobin level well above the renal threshold if less than 70 per cent of the transfused cells were

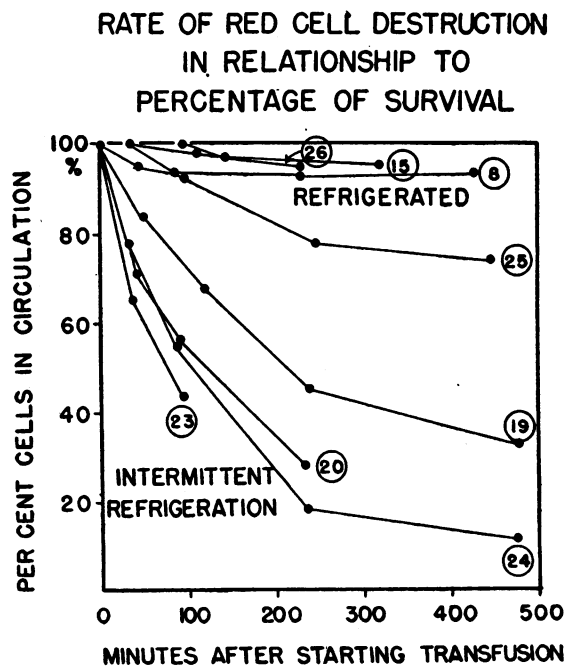


FIG. 5. RATE OF RED CELL DESTRUCTION IN RELATIONSHIP TO PERCENTAGE OF SURVIVAL

The rate of removal of non-viable tagged cells from the blood stream was far less in the refrigerated bloods, the preservation of which was good, than in the intermittently refrigerated bloods, the preservation of which was poor. The figures in the circles refer to the number of days elapsed from drawing to transfusion of blood.

viable. Figure 5 shows the rate of disappearance of cells from the recipient's circulation in both ACD-1 and Alsever's solution, transported under adequate and intermittent (inadequate) refrigeration. It is evident that the greater part of the non-viable cells are eliminated during the first 4 hours after transfusion and that a heavy hemoglobin load may have been placed on those subjects who received the inadequately refrigerated bloods.

The *in vitro* tests were of some value. The relationship of survival as measured by radio-iron and as predicted from changes in osmotic fragility (100 per cent - per cent hemolysis in 0.6 per cent NaCl) was quite good. The supernatant of the refrigerated bloods was slightly alkaline or acid, that of the intermittently refrigerated bloods definitely acid, reflecting a greater production of organic acids in the latter.

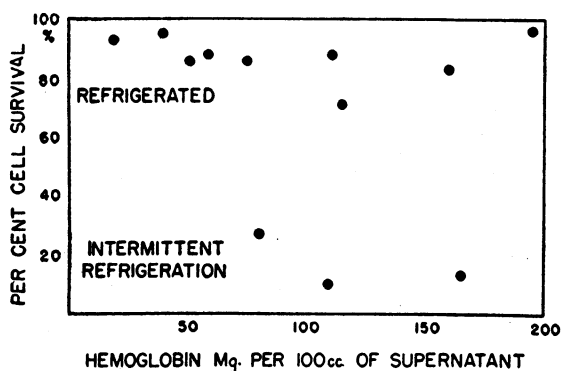


FIG. 6

No correlation between the degree of spontaneous hemolysis, as evidenced by the concentration of hemoglobin in the supernatant plasmas, and the post-transfusion viability of the non-hemolyzed cells was observed in these experiments. The absence of supernatant hemolysis is no criterion of transfusibility. It does not follow that badly hemolyzed bloods can be safely transfused.

A striking fact is the complete lack of correlation between the degree of hemolysis and the measured survival. This is clearly shown in Figure 6. Supernatant hemoglobin ranged from 30 to 196 mgm. per cent in the refrigerated bloods, and from 79 to 166 in the intermittently refrigerated bloods, and yet survival was excellent in the former and extremely poor in the latter series. This does not imply that markedly hemolyzed bloods will have good survival but does demonstrate that absence of spontaneous hemolysis is no

indication that stored blood in either ACD-1 or Alsever's solution is fit for transfusion.

CONCLUSIONS

(1) Refrigeration is an absolute essential for preservation of human erythrocytes drawn as whole blood in either ACD-1 or Alsever's solution.

(2) The optimal range for depot storage lies between 4° C. and 10° C.

(3) Exposure to temperatures below - 4° C. or above 10° C., even for a 24-hour period, increases the rate of *in vitro* deterioration; and this rate is markedly accelerated at temperatures above 15° C.

(4) The rate of deterioration resulting from storage at temperatures outside of the optimal range is not retarded by subsequent storage at 4 to 6° C.

(5) Whole blood in ACD-1 was transported under refrigeration within the optimal temperature range for 6,000 miles by air; and post-transfusion survival was as good up to 26 days as whole blood in ACD-1 maintained in depot storage at 4° C. for a similar period.

(6) Transportation of blood in either ACD-1 or Alsever's under variable temperatures 4 to 15° C. may lead to rapid deterioration of cells in spite of subsequent storage at the point of arrival under optimal refrigeration.

(7) The absence of spontaneous hemolysis in bloods transported under controlled or intermittent refrigeration should not be regarded as evidence that such bloods can be safely transfused.

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