

NEWER KNOWLEDGE OF BLOOD
TRANSFUSIONS*JOHN SCUDDER, CHARLES R. DREW, ELIZABETH TUTHILL, B.Sc.,
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ABEL¹ in 1914 reported on combating experimental hemorrhagic shock in dogs by infusing red cells suspended in Locke's solution after discarding the plasma. Subsequently, Rous and Turner² demonstrated that the longevity of the red blood cells could be enhanced if such erythrocytes were kept in a dextrose-citrate solution in a refrigerator. These two experimental observations led Robertson³ to use preserved red cells in treating the wounded at the casualty clearing stations of the Third Army of the B. E. F. during the first World War. Twenty-two such red cell transfusions, some fortified with gelatin, were given to twenty individuals. Of these, eleven were discharged to the base hospital and nine died. Among the latter, all except one were temporarily benefited by the stimulating effect of the cells.

Perry⁴ extended the preservation of erythrocytes by showing that the oxygen-carrying power of the red blood cells was maintained better in a solution of dextrose and lithium citrate than in one containing sodium citrate. Cells so preserved were transfused to man after removing the supernatant plasma.

Prior to this use of preserved cells, Richard Weil,⁵ in 1915, had transfused citrated human blood which had been kept for several days in cold storage.

The observations of Yudin⁶ on the transfusion of stored cadaver blood and the experimental wide scale use by Durán Jordá⁷⁻¹⁰ of conserved citrated blood during the Spanish Civil War stimulated investigations in many laboratories.^{17, 18, 19}

In the present war, plasma or serum is being used in preference to both red cell and preserved blood transfusions.

What has led to this complete reversal in current medical thought?

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TABLE I
CHANGES IN CELLULAR ELEMENTS OF PRESERVED BLOOD

Over 30 days in refrigerator at 4-6° C.		
<i>Constituent</i>	<i>Heparin</i> ²³	<i>Sodium citrate</i> ²³
Red Blood cells	Cell counts at constant level for a month	Moderate destruction of erythrocytes after 15th day with decrease of 1,000,000 to 1,500,000 at end of 30 days
Mean cell diameter (Halometer method)	20 per cent decrease in 30 days	
Hemoglobin (Hellige)	Total remains constant 15-25% in plasma in 30 days	Total remains constant 15-25% may diffuse out in 30 days
White blood count	50% decrease in 24 hours	Fall 27% in first 5 days
Polymorphonuclear neutrophiles	Show earliest and most rapid degenerative changes with nuclei losing shape. 50% decrease in 48°	Nuclear changes in 24 hours. In 48 hrs. 50% decrease. In 15 days liquefaction and droplet formation becoming subsequent smudges
Eosinophiles	Show least changes in size, shape and staining qualities over 1 month.	Well preserved at end of 30 days
Basophiles		Well preserved at end of 30 days
Lymphocytes	Retain shape, size and staining properties better than neutrophiles. Recognizable at end of 30 days	More resistant than polymorphonuclears Recognizable at end of 30 days
Monocytes	More resistant than neutrophiles	Difficult to trace
Thrombocytes	Rapid decrease during first 3 days	Early decrease

CONDITIONS OF EXPERIMENT

Donor	Type O	Type O
Blood (venous)	5.0 cc.	4.5 cc.
Anticoagulant	5. mg. dry heparin (Connaught)	0.5 cc. 3.5% sodium citrate solution
Test tube	Round bottom—Internal diameter 1.1 cm.	Flat bottom—Internal diameter 1.6 cm.
Mixing of sample	0.5 cc. plasma removed for K analysis after centrifugation 1 hr. 3000 RPM. Blood mixed inverting tube 15 x.	Shaking
Time of experiment	Fall, 1938	Winter, 1939.

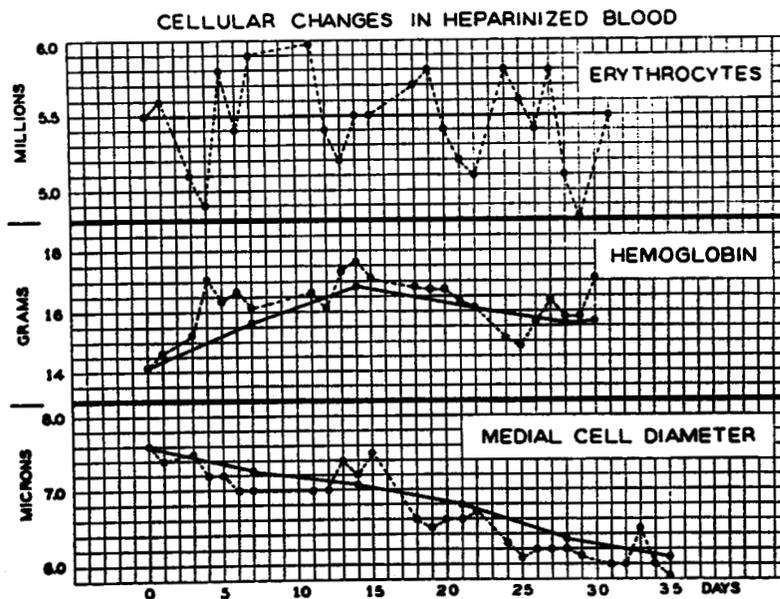


Figure 1

The answer lies in the fact that blood on leaving the vascular system starts to undergo degeneration immediately.²⁰ To appreciate what some of these changes are, will enable one to use those measures which will hinder or retard them, thereby prolonging the usefulness of preserved blood.

The following report deals only with the work done in our laboratory. It will be treated under three separate heads:

1. Changes which occur in the cellular elements of the blood.
2. Changes which occur in the electrolyte distribution.
3. Changes which appear in the protein patterns.*

CHANGES IN THE CELLULAR ELEMENTS

Methods. Two sets of experiments were carried out. Equal amounts of freely flowing venous blood were collected in each of thirty-five sterile test tubes. The first used dried heparin (Connaught); the second used sodium citrate solution as anticoagulant. The results are tabulated in Table I and a few of the changes are illustrated in Figs. 1-5.^{22, 23}

It is apparent that changes take place with both anticoagulants. The neutrophilic leukocytes show the earliest alterations.

* These were carried out under D. A. MacInnes of the Rockefeller Institute for Medical Research.²¹

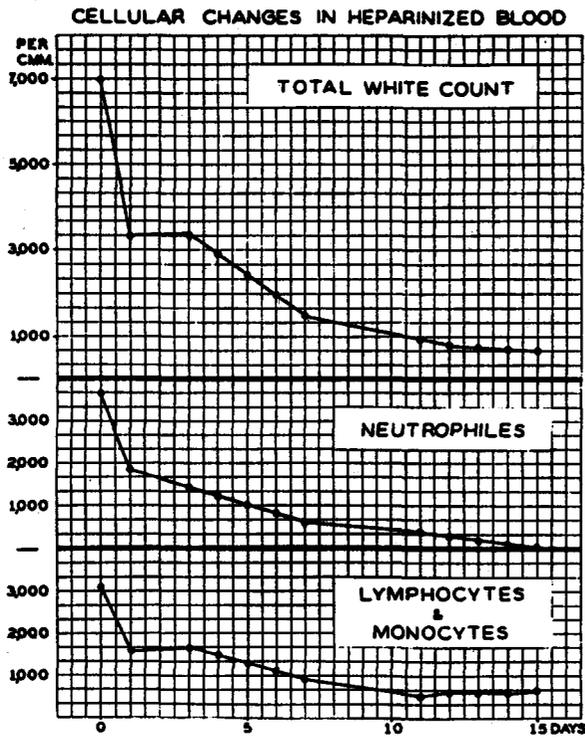


Figure 2

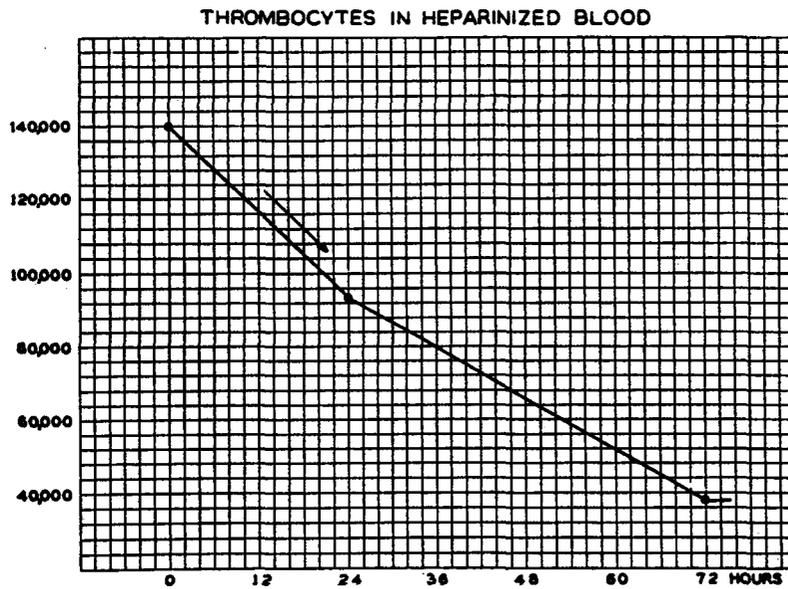


Figure 3

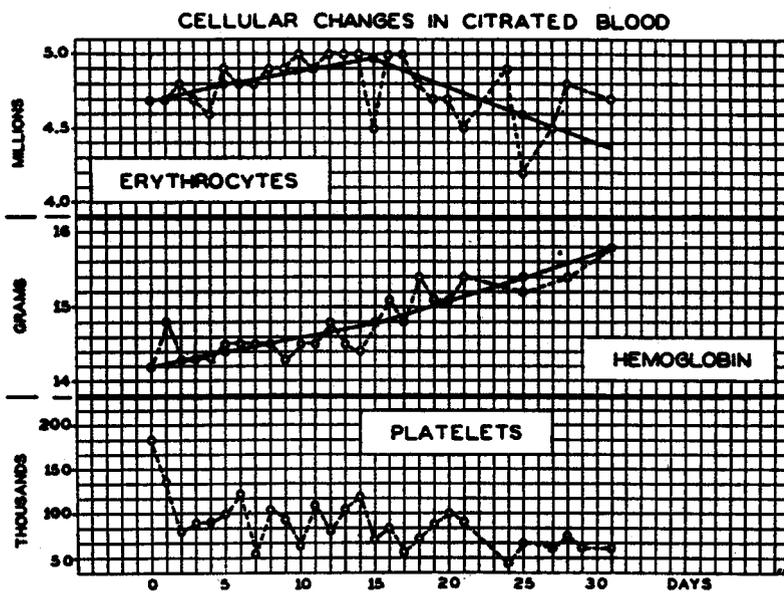


Figure 4

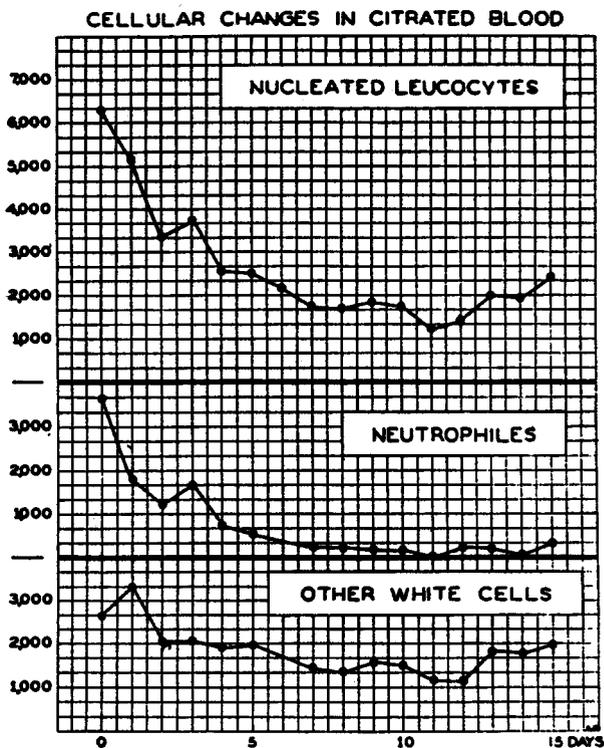


Figure 5

CHANGES IN ELECTROLYTE DISTRIBUTION

The Permeability of Erythrocytes to Sodium and Potassium. The exit of the potassium ion from certain plant cells is one criterion of the permeability, for the integrity of the cell is unlikely to be maintained under adverse conditions on account of the steep concentration gradient which exists between the intracellular potassium and that of the external solution.²⁴⁻²⁷

Gürber²⁸ reported the impermeability of erythrocytes to sodium and potassium, but Hamburger and Bubanovic²⁹ pointed out in 1910 that if the salt concentration of the serum is changed, or the carbon dioxide tension altered, both cations will readily cross the cell membranes.

In vivo, disturbances in both the plasma potassium and sodium ion concentrations occur.^{30, 31, 32, 33, 34, 35}

In vitro, a rapid diffusion of the potassium ion from the erythrocytes into plasma transpires.^{17, 36, 37, 38, 39} With this, there is an associated lowering of the sodium ion.^{19, 40, 41}

These changes were reinvestigated with the hope of ascertaining some underlying mechanism.

Analytical Procedure. Sodium was precipitated as uranyl zinc sodium acetate according to the method of Butler and Tuthill.⁴² Potassium by a modification of the argenticobaltinitrite method.^{33, 34, 43, 44} Ammonia nitrogen by the isothermal distillation method of Conway.^{20, 45}

Preliminary Analyses. Similar quantities of venous blood were collected from the same donor in three separate hematocrit tubes. In the first, there was no anticoagulant. In the second, there was placed exactly 1 milligram of sodium heparin. In the third, 0.5 cc. of 3.5 per cent sodium citrate solution was mixed with exactly 4.5 cc. of blood.

RESULTS OF PRELIMINARY ANALYSES FOR SODIUM

1	Serum sodium	321.7 mg. %	139.8 M. eq/L.
2	Heparinized plasma sodium*	317.9 mg. %	138.1 M. eq/L.
3	Citrated plasma sodium*	328.9 mg. %	142.8 M. eq/L.

* Corrected for sodium in anticoagulant.

Next, an attempt was made to establish a series of normal values, using each of the anticoagulants.

Experiment 1. Blood samples were collected from each of eight normal individuals into hematocrit tubes with an internal diameter varying between 6 and 7mm. and containing exactly 1 milligram of the sodium

TABLE II
PLASMA SODIUM IN NORMAL HEPARINIZED BLOODS

Corrected for Sodium in Anticoagulant		
<i>Number</i>	<i>Milligrams Per Cent</i>	<i>Milliequivalents Per Liter</i>
1	320.1	139.2
2	319.9	139.1
3	323.4	140.6
4	316.0	137.4
5	316.7	137.7
6	322.0	140.0
7	317.9	138.2
8	317.9	138.2
	<u>319.2</u>	<u>138.8</u>

TABLE III
PLASMA SODIUM IN NORMAL CITRATED BLOODS

Corrected for Sodium Added		
<i>Number</i>	<i>Milligrams Per Cent</i>	<i>Milliequivalents Per Liter</i>
1	317.3	138.0
2	318.0	138.3
3	337.8	146.9
4	316.8	137.8
5	315.5	137.2
6	309.8	134.7
	<u>319.2</u>	<u>138.8</u>

salt of heparin. The results of the plasma sodium analyses are recorded in Table II. These compare closely with the accepted values.

Experiment 2. In a manner similar to Experiment 1, the range of normal values was checked on citrated blood plasma. The results are listed in Table III.

TABLE IV

A COMPARISON OF THE RATE OF CHANGE IN THE PLASMA OF PRESERVED BLOOD OF AMMONIA, SODIUM, AND POTASSIUM ION CONCENTRATION

Serial Number	Initial Donor	Age of Blood in Hours	Ammonia—Nitrogen		Sodium		Potassium	
			Mg. Per Cent	M. eq./L	Mg. Per Cent	M. eq./L	Mg. Per Cent	M. eq./L
504	A	15	0.34	0.24	299.5	180.2	31.2	8.0
584	J	15	0.28	0.20	318.9	186.5	37.5	9.6
555	P	16	0.41	0.29	317.2	137.9	30.9	7.9
502	A	16	0.32	0.23	317.7	138.1	21.5	5.5
586	Y	18	0.49	0.35	314.8	136.9	34.0	8.7
532	B	18	0.46	0.33	305.2	132.7	32.6	8.3
507	B	19	0.49	0.35	320.7	139.4	32.6	8.3
509	N	20	0.30	0.21	319.2	138.8	27.4	7.0
533	J	38	0.57	0.41	297.8	129.5	34.0	8.7
542	C	68	0.87	0.62	283.5	123.3	55.5	14.2
484	P	69	1.05	0.75	83.7	21.4
592	W	98	1.08	0.77	261.0	118.5	133.0	34.1
541	L	116	0.99	0.71	285.6	124.2	132.0	33.8
557	K	140	0.92	0.66	251.9	109.5	95.8	24.5
483	H	140	1.06	0.76	123.0	31.5
534	Y	163	1.15	0.82	256.7	111.6	126.0	32.2
611	L	163	0.81	0.58	250.1	108.7	136.0	34.8
549	W	184	1.09	0.78	260.4	113.2	142.0	36.3
598	P	209	1.00	0.71	251.2	109.2	136.0	34.5

Experiment 3. Uniform samples of blood were obtained from each of nineteen flasks at the time of giving the preserved blood transfusions. After centrifuging, the plasma was analyzed for its ammonia, potassium, and sodium content. The corrected results are tabulated in Table IV.

Stored blood loses potassium at a constant rate from the cells. The extreme values recorded show a decrease of 30.7 milliequivalents for plasma sodium during the first week. Sodium, therefore, enters the red blood cells rapidly during the first five days and then approaches at a steady state. During the same period, there is an increase of 29.3 milliequivalents of potassium.⁴⁶ With these changes, ammonia nitrogen had increased to 0.58 milliequivalents per liter.

A possible explanation may lie in the work of Jacques⁴⁷ who demonstrated that changes in ammonia concentration alter the permeability of the sea algae, *Valonia macrophysa* Kütz, to both sodium and potassium.²⁴⁻²⁷

Conway^{20, 48} saw a sharp rise in ammonia concentration occur within the first few minutes after the shedding of blood; this was slowed by collecting blood under CO₂.

The permeability of the erythrocyte protoplasm to cations has been widely investigated.^{20, 48-60} Certain factors, however, such as an increase in CO₂ tension⁴¹ or change in fluid medium,⁶¹ or change in pH⁶² will markedly alter this state of "selective permeability."

Maizels⁶¹ in 1935 indicated that moderate shifts in pH do not alter the permeability of erythrocytes in respect to sodium and potassium; pronounced changes do affect the permeability, however.

It has been noticed that among the different preservatives used in blood storage, the one containing glucose and salt in the proportions suggested by Rous and Turner² prevented hemolysis but did not alter the outward diffusion of potassium.³⁹ The pH of the plasma which had been preserved with Rous' solution was 7.1. Others, reporting their results on blood stored in glucose, record a fall in the pH of such specimens.

Sheep's blood preserved with and without CO₂ revealed marked differences in both hydrogen and ammonia ion concentrations.³³

To test again the effect of CO₂ on human blood, the following controlled observations were made:

In each of the eight experiments, blood was obtained in the usual manner from a different individual, isotonic solution of sodium citrate being used as the anticoagulant. One half of the sample was drawn into

TABLE V

EFFECT OF CARBON DIOXIDE ON SODIUM, POTASSIUM, AMMONIA-NITROGEN, AND pH CHANGES
IN THE PLASMA OF PRESERVED BLOOD

Date	NH_4-N				Na				K				pH	
	Air		CO_2		Air		CO_2		Air		CO_2		Air	CO_2
	Mg. %	M. eq. /L	Mg. %	M. eq. /L	Mg. %	M. eq. /L	Mg. %	M. eq. /L	Mg. %	M. eq. /L	Mg. %	M. eq. /L		
9/11/39.....	0.10	0.07	0.01	0.01	322.1	140.1	337.4	146.7	17.1	4.4	17.4	4.5
9/12/39.....	0.37	0.26	0.08	0.06	324.0	140.9	336.0	146.1	31.3	8.0	25.4	6.5	7.76	7.48
9/16/39.....	0.55	0.39	0.18	0.18	302.6	131.6	333.0	144.7	56.4	14.4	34.6	8.8	7.58	7.22
9/19/39.....	0.77	0.55	0.30	0.21	296.4	128.9	317.0	137.8	73.9	18.9	49.9	12.8	7.65	7.31
9/26/39.....	0.94	0.67	0.44	0.31	276.8	120.4	312.5	135.9	91.6	23.4	62.1	15.9	7.69	7.17

an atmosphere of carbon dioxide while the control was collected in air. The details of the experiment have been previously reported.⁴¹

Results. The results of one of these experiments in which determinations of the concentrations of ammonia, sodium, and potassium ions were made at intervals during a two-week period is recorded in Table V. It is noted that the concentration of the ammonia ion in the blood taken under carbon dioxide was lower; the rates of potassium and sodium changes slower; and the pH nearer neutral at the end of the experiment.

The concentration of ammonia ion in the control, beginning at 0.07 milliequivalents per liter rose to 0.67; in the CO₂ environment, it began at 0.01 and rose to 0.31. The increase in the concentration of ammonia ion for the two-week period in the blood taken in CO₂ is, therefore, only 50 per cent of the increase noted in blood taken in air. Plasma sodium values decreased in the control 19.7 milliequivalents per liter; in CO₂, 10.8. Plasma potassium values increased in the control 19.0 milliequivalents per liter; in CO₂, 11.4.

The plasma pH value in CO₂ approached the normal more closely than did the samples taken in air. Hemolysis in the latter was greater than in blood collected in carbon dioxide.

CONCLUSIONS

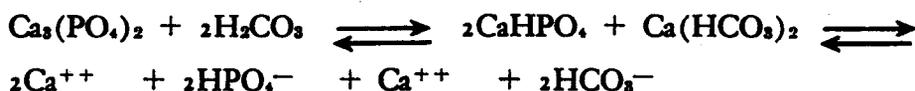
1. There is a rapid, constant decrease in sodium in the plasma of preserved blood.
2. This decrease is roughly inversely proportional to the increase in plasma potassium.
3. There is a suggestive evidence that, with an increase in ammonia content of blood plasma, permeability of the erythrocyte protoplasm to these two ions is changed.
4. Blood drawn under carbon dioxide maintains a plasma pH value nearer neutral than blood drawn in air.
5. Changes in the plasma concentrations of ammonia, sodium, and potassium ions in such blood collected in CO₂ is less than in air.
6. Hemolysis is retarded by collecting blood in an atmosphere of carbon dioxide.

CHANGES IN CALCIUM, MAGNESIUM, AND PHOSPHORUS CONTENT

Of the six minerals commonly present in living matter calcium has the greatest tendency to form insoluble salts.⁶⁸ In man, it occurs in the form

of the phosphate or carbonate and may be divided into two forms: diffusible and indiffusible. The former is capable of existing in the ionized state and only a small portion is ever actually dissociated from its very stable salts.⁶⁴

In the body it exists as tricalcium phosphate, a relatively insoluble compound; but under the influence of carbonic acid of the plasma, it is partly converted to the more soluble calcium bicarbonate and calcium hydrogen phosphate.



Methods. Calcium was determined by the method of Clark and Collip,⁶⁵ the final titration being done against a standard sodium oxalate solution.

Phosphorus, determined as phosphate, was done by an adaptation of the methods of Clark and Collip⁶⁵ and Fiske and Subbarow.⁶⁶ Following the suggestion of Gamble⁶⁷ the base equivalence is 1.8 times the molecular concentration of HPO_4 . An adaption of several methods^{65, 66, 68} was used in determining magnesium.

Procedure. From a voluntary donor, 450 cc. of blood was drawn in air into a bottle containing 50 cc. of 3.5 per cent sodium citrate solution, and then divided into twelve equal portions and kept in a refrigerator. On the plasma of one sample, calcium, magnesium, and phosphorus analyses were done on the day of collection and on the following day. Then, on every second day, two tubes were taken. From one, the plasma was immediately removed; from the other, only after inverting five times and centrifuging for thirty minutes.

Results. The results are presented in Table VI and Table VII and indicate the changes which took place during nine days. Each is an average of at least two separate determinations by two individuals.

Discussion. Joseph and Meltzer⁶⁹ reported in 1910 that the toxicity of the chlorides of magnesium, calcium, potassium, and sodium varied in almost inverse proportion to the quantities found in blood, particularly in the plasma. Since plasma potassium concentration is greater than plasma magnesium concentration, significant increases in the latter, according to this theory, would be more toxic. These results indicate that during the nine day observation period, there is very little outward diffusion of magnesium.

TABLE VI
PLASMA CALCIUM AND PHOSPHORUS CHANGES IN PRESERVED BLOOD

Age in Days	Calcium				Phosphorus			
	Before Shaking		After Shaking		Before Shaking		After Shaking	
	Mg. %	M. eq./L.	Mg. %	M. eq./L.	Mg. %	M. eq./L.	Mg. %	M. eq./L.
0	9.2	4.6	3.6	2.1
1	8.8	4.4	8.7	4.4	3.5	2.0	3.4	1.97
3	8.9	4.5	8.7	4.4	3.4	1.97	3.4	1.97
5	8.6	4.3	9.2	4.6	3.5	2.0	3.6	2.1
7	8.7	4.4	8.7	4.4	3.8	2.2	3.6	2.1
9	9.1	4.6	9.1	4.6	3.7	2.1	4.0	2.3

TABLE VII
PLASMA MAGNESIUM CHANGES IN PRESERVED BLOOD

Age in Days	Magnesium in Milliequivalents per Liter	
	Before Shaking	After Shaking
0	2.3	..
1	2.3	2.35
3	2.5	2.4
5	2.6	2.4
7	2.5	2.5
9	2.4	2.4

SUMMARY

1. The plasma calcium ion concentration of preserved blood remains constant for a period of nine days and is not increased by shaking.
2. There is no definite increase in the plasma phosphorus content. This is not accentuated by shaking, even in the nine-day old blood.
3. Magnesium diffuses out of the erythrocytes of stored blood at a very slow rate, if at all. Shaking apparently does not increase this.
4. The actual increase in magnesium at the end of nine days' storage appears to be too small to account for any toxic manifestation following transfusions of such preserved bloods.

TABLE VIII
ACID-BASE COMPOSITION OF FRESH BLOOD PLASMA

(Expressed in Milliequivalents per Liter)

<i>Base</i>			<i>Acid</i>		
<i>Ion</i>	<i>Gamble</i>	<i>Gutman</i>	<i>Ion</i>	<i>Gamble</i>	<i>Gutman</i>
Na'	142	142	HCO ₃ '	27	28
K'	5	5	Cl'	108	104
Ca''	5	5	HPO ₄ ''	2	2
Mg''	3	2	SO ₄ ''	1	1
			Org. Ac.	6	1
			Protein	16	18
Total	155	154		155	154

CHANGES IN THE TOTAL ELECTROLYTE STRUCTURE OF THE PLASMA OF PRESERVED BLOOD

With these observed alterations in the potassium, sodium, ammonia, calcium, phosphorus, magnesium, and hydrogen ion concentrations, the status of total ionic balance in aging blood needs investigation.

To this end, freshly drawn blood was set aside at approximately monthly intervals for four months. The cations were determined as in the previous section; and of the anions, the bicarbonate, chloride, phosphate, and hydrogen ions were analyzed. The sulphates, organic acids, and proteins were omitted, as there is not a complete unanimity of opinion concerning the equivalent values to be assigned to the organic acid and protein components on the acid side of the equation.

The chlorides were determined by the method of Van Slyke⁷⁰ and the carbon dioxide and oxygen by the method of Van Slyke and Neill.⁷¹ The pH measurements were carried out by means of the glass electrode potentiometric method of MacInnes and Longworth.⁷² The bloods which were examined on the fifth, sixty-eighth, and one hundred seventeenth days of preservation had been stored in narrow-waisted dumb-bell-shaped flasks which contained 50 cc. of 3.5 per cent sodium citrate and 450 cc. of blood. The interface diameter between the settled cells

TABLE IX
CHANGES IN TOTAL CATION STRUCTURE
IN THE PLASMA OF PRESERVED BLOOD

(Expressed in Milliequivalents per Liter)

Cations	Normal Gutman	Age in Days					
		5	21***	68†	93*	117†	117***†
Na'.....	142	122.4	106.3	119.0	89.7	105.2	120.7
K'.....	5	28.2	35.0	29.2	54.2	40.6	28.8
Ca''.....	5	5.3	5.5	5.7	5.7	5.8	5.4
Mg''.....	2	1.9	2.5	2.2	2.4	2.7	2.1
Total.....	154	157.8	149.3	156.1	152.0	153.9	157.0

CHANGES IN ANION COMPOSITION IN
THE PLASMA OF PRESERVED BLOOD††

Anions

HCO ₃ '.....	27	16.6	11.9	15.4	12.4	10.7	16.5
Cl'.....	103	99.3	99.7	100.5	80.0	99.7	107.0
HP ₂ O''.....	2	1.9	6.0	5.2	8.2	7.5	3.4

* Commercial bottle
Interface 10.4 cm.

** Undisturbed plasma
† Interface 3.5 cm.

*** Wide-mouth flask
Interface 8.6 cm.

and plasma was 3.5 cm. All but one of these flasks were inverted to mix thoroughly the cells and plasma, before the sample was centrifuged and the plasma removed for analyses. The values in the last column were obtained from the plasma of a blood one hundred seventeen days old, which had not been disturbed during the entire period. The twenty-one day old blood was kept in a wide-mouthed flask (interface 8.6 cm.) while the ninety-three day old blood (column six from the left) was collected in a commercial bottle (interface 10.4 cm.) which contained 70 cc. of 2.5 per cent sodium citrate in physiologic saline under vacuum of 24-26 inches of mercury. All values are corrected for dilution and added sodium or chloride.

Discussion. The greatest changes are seen in the sodium and potassium ion concentration, particularly in the bottle in which the blood

†† We are indebted to A. B. and E. B. Gutman for permission to use their figures for normal human serum.

was collected under vacuum.

Calcium in these bloods stored for longer periods, acted similarly to that in bloods stored for shorter periods and showed relatively little change; nor did mixing a one hundred seventeen day old blood increase the plasma calcium content.

Magnesium, as the second largest constituent of the cells, might have been expected to show a greater outward diffusion.

The total average number of milliequivalents in the six bloods amounts to 154.3 compared with the control normal value of 154.0.

The alkali reserve of the plasma as measured by the CO₂ combining power decreases with age. The chloride ion concentration decreases, but not to the extent of the sodium ion. The plasma chloride concentration of the blood which had been collected in a commercial vacuum bottle and which contained an additional 70 cc. of normal saline was strikingly low when compared with the high chloride values in the samples taken under atmospheric pressure without the addition of saline.

The plasma phosphate concentration gradually increases in the blood with increasing age, but never as great as that of potassium.

The pH of stored blood after mixing with the plasma varied between 7.1 and 7.34.

The quantity of the determined anions in these six bloods ranged from 100.6 to 126.9 milliequivalents per liter, with an average of approximately 117. These figures are exclusive of the sulphate, protein and organic acid anions.

Summary. In the plasma of bloods stored in an electric refrigerator thermostated at 4°C. for a period ranging from five days to four months, the following changes were observed:

1. Potassium increases.
2. Sodium decreases.
3. Calcium remains practically constant.
4. Magnesium shows little change.
5. Bicarbonate decreases.
6. Phosphate increases, particularly following agitation.
7. Chlorides decrease in plasma intimately mixed with the cells: remain constant or slightly increased when left undisturbed.
8. The total cation concentration remains constant, despite great variations in the plasma content of individual cations.
9. The observed loss of determined anions suggested that balance

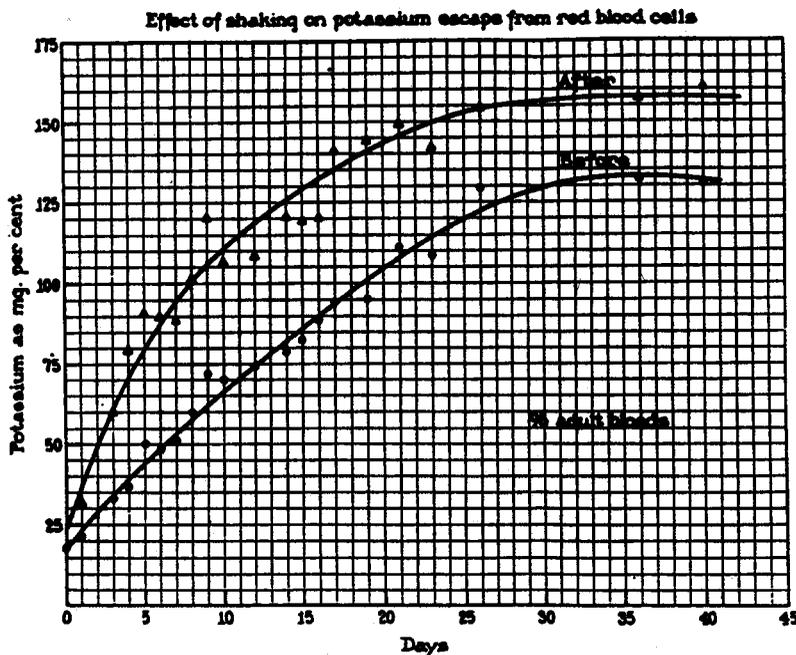


Figure 6

is maintained by a gradual increase in the organic acid ion component and a decrease in albumen.

10. The pH changes of the whole blood after mixing are slight.

SOME FACTORS GOVERNING TRANSPORT OF BLOOD

In a previous report trauma to blood in the form of shaking caused loss of potassium from the cells and rapid laking.³⁹

During the winter of 1938-1939, the blood from ninety-six voluntary donors at the Mt. Sinai Hospital* was collected in mason jars containing 2.5 or 3.0 per cent sodium citrate. From these, five to six cubic centimeters were removed and stored in identically shaped centrifuge tubes. These tubes were transported at once to the Presbyterian Hospital, a distance of six miles.

There was no gross hemolysis in these tubes containing freshly shed blood, whereas old blood transported at the same time in partially filled mason jars revealed striking hemolysis.

At varying intervals, the blood was removed from the refrigerator and mixed by inverting the tube 20 times. The results are depicted in Fig. 6.

* We express our gratitude to N. Rosenthal and his staff for their coöperation in this investigation and to the Mt. Sinai Hospital for permission.

TABLE X

INCREASING EFFECT OF TRAUMA WITH INCREASING AGE OF BLOOD

Time in Weeks	Plasma Potassium				Plasma Ammonia Nitrogen			
	as Milligrams Per Cent		as Milliequivalents Per Liter		as Milligrams Per Cent		as Milliequivalents Per Liter	
	Before	After	Before	After	Before	After	Before	After
0.....	25.4	26.0	6.5	6.7	0.09	0.151	0.064	0.11
1.....	68.1	149.2	16.1	38.2	0.58	0.126	0.41	0.90
2.....	73.6	172.6	18.8	44.1	0.87*	1.45*	0.62	1.04

* Volume of blood, 25 cc.

All other experiments, volume of blood, 50 cc.

Discussion. The curve representing the diffusion of potassium in the unshaken blood is the same order of magnitude as previously reported for citrated blood.³⁹ In each instance, agitation caused both potassium and hemoglobin to leave the cells. During the first few days, this was not as great as later. As the plasma potassium concentration approached that within the cells (i.e., decrease in concentration gradient) shaking dislodged less.

To check these findings a controlled experiment was set up.

Method. The blood of two voluntary donors, type A and type B, was drawn at weekly intervals and placed in 50 cc. colorimeter tubes with an internal diameter of 2 cm. containing 5 cc. of 3.5 per cent sodium citrate solution.

At the end of two weeks following the drawing of the last blood, the previously drawn samples were removed from the refrigerator where they had been kept, stoppered and sealed, at a temperature of 5 to 6° C.

A small sample of the plasma was taken from each of the six tubes for potassium and ammonia determinations. After this, all of the tubes were rotated end over end on a specially devised piece of apparatus for twenty minutes, centrifuged, and from each, samples were taken for potassium and ammonia determinations after the rotating.

The diffusion of potassium from red blood cells to plasma following trauma increases rapidly with increasing age of the blood. This suggests that if transportation of blood is contemplated, it should be done while the blood is fresh for the damage incurred by the cells is less at this time than when the blood is older.

The effects of shaking can be greatly reduced by filling the container completely with blood. Durán Jordá¹⁰ employed this principle during the recent Spanish Civil War in which preserved blood was used on an extensive scale.

It would appear, then, that in the transportation of preserved blood, factors which would minimize the loss of intracellular substances, such as: decreasing the interface between cells and plasma; obviating any interface between liquid and gas by filling the container completely; should be also considered in addition to proper refrigeration, etc.

Summary. 1. The diffusion of intracellular substances (potassium and hemoglobin) is accelerated by shaking and factors which limit this should be employed in blood preservation.

2. Transportation of preserved blood adequately refrigerated in suitable containers completely filled should be done early after shedding.

PLASMA

These studies indicate that degenerative changes occur as soon as blood leaves the vascular system, and progress with age. The large changes in the electrolyte composition might indicate its unsuitableness in those pathological states which are known to be associated with disturbances in the mineral metabolism, such as dehydration,^{32, 33} adrenal insufficiency,^{31, 34} and traumatic and hemorrhagic shock.^{33, 35} Should large amounts of old blood be given rapidly in these conditions, dangerous sequelae might ensue.

Since the work of Bowditch⁷⁸ in 1871, increasing attention has been directed to both serum and plasma as possible substitutes for whole blood transfusion.^{21, 74, 75}

Amberson⁷⁶ in his review on this subject has pointed out some of the advantages of plasma.

Using the electrophoretic method of Tiselius,⁷⁷ as modified by Longsworth,⁷⁸⁻⁸⁰ the stability of the various protein components in plasma has been investigated.²¹ These observations confirmed the previous work of Knoll⁸¹ who reported a decrease in albumin, a change in the albumin-globulin ratio, and an increase in gamma globulin.

To ascertain more exactly the magnitude of these alterations, blood was drawn from a single donor at varying intervals, and the plasma separated from the cells on the same day.

These values indicate a decrease in the albumin and a shift in the

TABLE XI

ELECTROPHORETIC PATTERN OF PLASMA FROM SAME INDIVIDUAL²³

Age of Blood Days	TYPE A												Remarks
	Composition						Mobilities, $U \times 10^{-5}$						
	Albumin Per Cent	A/G	α/A	β/A	ϕ/A	γ/A	pH	Albumin	Globulin				
α									β	ϕ	γ		
Fresh	3.96	2.27	0.08	0.18	0.08	0.18	7.72	5.95	4.2	2.9	1.6	0.2	Citrate
12	3.81	1.92	0.11	0.21	0.07	0.20	7.71	6.15	4.3	2.9	1.6	0.1	Citrate
20	3.66	1.72	0.11	0.21	0.09	0.26	7.73	6.25	4.6	3.2	1.7	0.2	Citrate
28	2.89	1.54	0.12	0.27	0.08	0.26	7.75	6.18	4.5	3.1	1.7	0.3	Citrate

CONDITIONS OF EXPERIMENT

In brief, a four times diluted portion of plasma is dialyzed in a bag made from cellophane tubing constructed in such a manner as to give a large surface to volume relationship. The buffer with a pH at 7.8 to 25° C. consisting of 0.025 M. lithium diethyl barbiturate, 0.025 M. diethyl barbituric acid, and 0.025 M lithium chloride is used. The dialysis is carried out from 48 to 72 hours in a two liter flask containing fresh buffer at a temperature between 0° and 2° C. in a thermostatically controlled electric refrigerator. During the dialysis some precipitate separates out. It is, therefore, necessary to clear the protein solution in an angle centrifuge operated at 0° C. before its introduction into the electrophoresis cell. The pH measurement is determined with the glass electrode of MacInnes and Longworth.²³ The conductivity cell is of special design as well as the screened bridge used for the measurement of electrolytic conductance.²³ The establishment of a Donnan equilibrium is assumed when further dialysis produces no change in conductance of the protein solution and the outside solution has the conductance of the original buffer. The manner of obtaining the protein patterns and of computing the different mobilities of the protein constituents has been published by Longworth, Shedlovsky, and MacInnes.²³

albumin-globulin ratio, accompanied by an increase in gamma globulin in the supernatant plasma of stored blood.

In order to compare two common methods of desiccating plasma, blood was drawn from the same individual and the plasma of one portion was dried under vacuum from the frozen state, while the second portion was dried at body temperature as has been suggested by Edwards, Kay, and Davie.²⁰ Electrophoretic patterns of the former gave a sharper picture than the one derived from the latter, indicating that the plasma reconstituted from the frozen state appears to be more normal.²¹

Electrophoretic studies on liquid plasma preserved for five weeks, and in one instance for a year, showed evidence of some alterations, particularly in the beta globulin region.

PRACTICAL CONSIDERATIONS

Healthy donors, free from communicable diseases, are to be chosen. Blood obtained from cadavers is to be rejected, both on account of marked electrolytic changes and degradation products.^{21, 46}

In the collection of blood, strict surgical asepsis is to be observed. *Cleansing the skin* is the most important step on account of the danger of contamination. The skin of the antecubital fossa should be scrubbed with soap for two minutes. This is removed with 70 per cent alcohol. Three and a half per cent tincture of iodine is painted over the area and allowed to dry.

Venipuncture. Prior to the venipuncture, the skin is again swabbed with 70 per cent ethyl alcohol. A wheal is raised over the selected vein by injecting 1 per cent novocain, in the center of which a small nick is made by using a number eleven blade. A large needle of number thirteen or fifteen gauge is used for the phlebotomy.

The blood is collected by the closed system. This is superior to an open one for not only are chance contaminations decreased but also the loss of CO₂ is lessened. The keeping qualities of blood are further enhanced by its collection in an atmosphere of carbon dioxide.

As chemical changes are a function of temperature, the nearer zero the blood is stored, the slower will be these changes. Freezing is to be avoided as it causes rupture of the red cells. As chemical reactions are also a function of the surface area, it is natural that blood kept in specially designed bottles in which the interface between the plasma and the cells is small, will enhance its keeping qualities.

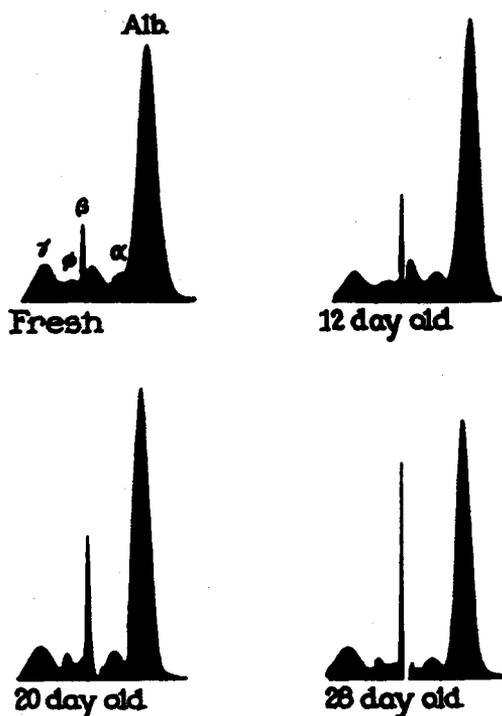


Figure 7—Electrophoretic patterns of preserved plasma from same donor. Four blood samples taken at different times; collected in 35 per cent sodium citrate and stored in four tubes in electric refrigerator at 4° C. Type A blood.

In the preparation of plasma, either the settling or the centrifuge method may be employed. With the latter, a bottle capable of containing the whole donation of blood (500 cc. in one bottle) is ideal for two reasons: 1) this halves the chance of contamination, and 2) the yield of plasma is greater. (Number three International General Electric centrifuge bottle—62 per cent citrated plasma yield vs. 46 per cent yield by settling seventy-two hours in a dumbbell shaped flask.)

In the removal of plasma, the procedure should be carried out in a dustproof, air-conditioned room, the air of which has been sterilized by ultraviolet light radiation. This will prevent possible airborne contaminants that have been found in plasma. The removal of the plasma should be carried out in a cabinet, thus minimizing further chance of infection.

Pooling and culture. The plasma from six to eight donor bottles is

siphoned off by suction and pooled in a two-liter flask. Cultures, both aerobic and anaerobic, are taken.

Final container. The plasma is not considered suitable for final processing until a two-week negative report has been received. The pool is then broken down into the final containers, 500 cc. of plasma may be mixed with 500 cc. of saline. The last portion of the pool is collected in a pilot bottle so that the concentration of the plasma mixed with saline is similar to that of the larger bottles. This material serves as a test on the sterility of the final container as well as furnishing another check on the sterility of the plasma.

Filtration: The safety of the plasma is enhanced if it has been passed through a clarifying and sterilizing filter. This step may be carried out after the initial pooling in a Seitz filter.

Dried Plasma: As proteins are more stable in a dried state, the keeping qualities of the plasma may be enhanced if it is reduced to such a condition by a suitable lyophile process. The dried plasma can then be dispensed in glass sealed ampoules.

Use: Dried plasma is turned into the liquid state by the addition of distilled water. It may be reconstituted in either an isotonic or hypertonic form, depending upon the amount of diluent added.

One abnormality, however, of such plasma is its extreme alkalinity.

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