The practical application of inosine to blood banking requires that consideration be given to the problem of formation of uric acid. This applies, of course, only to transfusion of whole blood since hypoxanthine would be removed with plasma in the preparation of packed red cells. While large amounts of uric acid can be excreted without harm, the extent to which multiple transfusions could be used remains to be determined.

Sodium Lactate as a Preservative for Whole Blood

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In 1943 a preserving solution for whole blood was described by C. Peeters. At that time a four-year experience had been gained: the results were excellent, but the dilution used was one to one. Later on the ACD solution came into general use with a lower dilution and undoubtedly good results. We made an attempt to get back to a greater dilution and perhaps longer preservation using the ACD solution and adding to it M/6 sodium lactate. We used lactate because it is an anticoagulant, though less powerful than citrate, and on the other hand it is a naturally occurring metabolite of the organism. A few bottles were transfused in 1950 but current use was started in 1951. At the Transfusion Congress in Lisbon (1951) this method was presented to Cohn who encouraged the idea. A few experimental figures obtained with this preserving solution and a summary of clinical experience with lactated blood will be given.

This gives 6.4 blood plus 3.6 solution. Compared to “ACD” which is 80% blood, our “ACDL” or “L” is 64% blood.

The experimental figures were obtained by taking blood from one donor into two bottles. 200 cc blood plus 50 cc ACD and 160 cc blood plus 90 cc ACDL. The time taken for drawing the blood was noted, as poorly taken samples are very likely to cause bad preservation. After collection sterile air was let into the bottle and the content divided over five 50 cc vacuum bottles on which determinations were performed.

Fig. 1

General Outline (Fig. 1)
Blood is taken according to the following rule:

ACD 50 cc  
M/6 lactate 40 cc  
Blood 160 cc  
Total 250 cc  
For clinical use the usual ACD bottle was supplemented with the required volume of M/6 lactate and used immediately for collection. For the moment ACDL bottles are prepared as such and need no further manipulation. All blood was kept at 4° C.

Critical Study in vitro

A. Drawing the Blood

Blood is collected by means of a dispensable plastic kit. Foaming is very slight in ACDL as compared with ACD. It is difficult to put this into figures, but the difference is so great that it allows to tell at once whether ACD or ACDL is present in the bottle. Part of the cause of this moderate foaming may be the change in viscosity of the blood proteins in presence of sodium lactate and is under investigation.

B. Préservation of Elements

Red Blood Cells

Hemoglobin and Number

Total hemoglobin is obviously lower in ACDL. It falls slightly in both solutions. Other methods of measuring hemoglobin will be used to explain this phenomenon.

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According to the greater dilution there are less cells in ACDL. During the preservation there is a slight fall which runs parallel to ACD. Irregularities are due to technical error. We shall see that the loss of hemoglobin into the plasma has a different shape in both solutions. There need not be a complete parallelism between loss of cells and appearance of plasma hemoglobin because diffusion of hemoglobin out of the cell without immediate cell destruction must be considered.

Hematocrit (Fig. 2)
The hematocrit figure is interesting as it points out a steady decrease in ACDL against a slight swelling in ACD. These differences cannot be attributed to a loss of cells as this loss is only very slight. Moreover, plasma hemoglobin will prove that, at least in ACDL, no hemolysis occurs before the fifteenth day. The hematocrit changes must be attributed to changes in cell volume.

We wish to stress individual differences between samples and their parallel evolution in ACD and ACDL.

Fig. 2. Hematocrit average of 6 cases.

Fragility

To give an account on fragility we considered both mechanical and osmotic resistance and did photometric evaluation of the color of the supernatant.

a) Mechanical Resistance (Fig. 3)

This is better for lactated blood as shown in a typical experiment. The first appearance of a red tinge on the plasma and the appearance of the definite red color were noted separately. Here again individual differences occur. Practice has shown that in the blood bank lactated blood keeps longer without trace of hemolysis than the usual ACD.

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Fig. 3. Mechanical resistance.

b) Osmotic Resistance (Figs. 4 and 5)

It is known that the effective osmotic resistance of the cell in a given solution is more important than the figure of osmotic pressure itself. We determined the osmotic resistance at definite salt concentration, pH, temperature and time and found consistently better figures for lactated blood especially as the preserving period grew longer. The difference is also more typical at salt concentration nearer the physiological range.

Fig. 4. Osmotic resistance.

Fig. 5. Osmotic resistance. Average of 4 cases.

c) Plasma Hemoglobin (Fig. 6)
Plasma was read in the photometer at 420, 540 and 660 mm and O.D. noted. A typical difference in the appearance of color change only on the fourteenth day in lactated blood against on the seventh day in ACD. This confirms better mechanical and osmotic resistance.

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Fig. 6. Plasma hemoglobin: average of 4 cases.

White Cells and Trombocytes

Both solutions show a progressive loss of elements which runs parallel in both solutions.

C. Plasma Components

Preliminary note: As plasma components vary because they get in or out of the red cell, or are used or produced by the red cell, we calculated for each constituent out of the average figures a theoretical curve for 100% blood, assuming the dilution to be ACD 80%, and ACDL 64% blood.

Electrolytes

1. Anions

a) Chloride

We found a temporary rise of chlorides half way the preserving period. Calculated for 100% blood the figures are alike (Fig. 7).

b) Phosphate

Total inorganic phosphate lies lower in ACDL but rises considerably in both solutions. For 100% blood the figures are alike (Fig. 8).

c) Lactate

We are doing determinations with a new specific lactic acid reagent which will also enable us to follow the fate of the lactic in the transfused patient. This will be published separately.

2. Cations

a) Sodium

This ion tends to diminish progressively entering the red cell as a compensation
for its loss of potassium. In lactated blood all the figures are lower (Fig. 9). Calculated for 100% blood it appears that curves touch each other and also that the

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Fig. 7. Chloride: average of 4 cases in meq/L.
Fig. 8. Total Phosphorus: average of 3 cases in mg/100 ml.
Fig. 9. Sodium: average of 4 cases in meq/L.
Fig. 10. Potassium: average of 4 cases in meq/L.

quantity which disappears during the period of storage is about identical for both preserves. This indicates that there is merely a diffusion of sodium out of the cell.

b) Potassium

The potassium content of both plasmas is a function of the quantity of potassium lost by the red cells. The increase is fivefold in both solutions in 35 days. The potassium rises sharper in ACD than in ACDL. If we consider both curves we see that the potassium in lactated blood of plus 28 days only reaches the level of plus 14 days ACD blood. If we calculate the potassium content for 100% blood, both curves (Fig. 10) are identical. Although there is less potassium in ACDL, no special mechanism is involved. It is merely a diffusion of potassium out of the red cell that is responsible for the level in both fluids.

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c) Sodium and Potassium

If we consider both kations together, the difference between ACD and ACDL is important because low sodium and low potassium occur simultaneously in ACDL, but the general trend is alike. In ACDL the sum of both kations rises from 140 to 145 meq/L, and in ACD from 160 to 166 meq/L. The lower salt content in ACDL might be the reason for the smaller fragility of red cells preserved in the lactated solution, and avoid the swelling found in ACD cells from the seventh day onward. Non-Electrolytes

Glucose (Fig. 11)

The absolute quantity present initially in both bottles is identical but less blood is added to ACDL bottles, so the initial level is lower. The glucose consumption
seems to develop faster in ACDL although theoretical figures for 100% blood cover each other by the end of the preserving period.

Fig. 11. Glucose average of 3 cases.

D. Blood Considered as a Whole

PH

a) Buffering Capacity (Fig. 12)

The buffering capacity of both preserving solutions is good but slightly smaller for ACDL especially on the acid side.

b) pH Measurements (Fig. 13)

The initial pH lies 0.1 higher and falls to the same point of 7.2. The pH of ACD shows a more rapid downfall than ACDL. No substantial difference was noted.

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Fig. 12. Buffercapacity.

Fig. 13. pH: 2 cases.

Fluidity (Fig. 14)

Dilution should of course enhance fluidity. Sluggishness is completely absent from lactated blood even after plus 30 days. This is an important practical advantage of dilution. To get an objective idea of this phenomenon we measured the flow time with a clinical viscosimeter using plastic transfusion equipment and needles of same length but different bore (15, 18 or 21).

For thick needles the difference is not great but for smaller needles, and thus for filters, the difference is important. Never is there any complaint on the rate of flow of ACDL blood, not even with European filters whose surface is far smaller.

Spontaneous Sedimentation after Shaking

Sedimentation rates after one or two hours soon fall to zero. Instead blood was shaken every three days and left to settle on its own. It appears that the height of the red cell column runs parallel in both solutions and is a function of the number
of cells.

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Fig. 14. Flow time in sec for 10 ml.

Blood Clotting

P.T. Factor V and VII. Howell Time and H.S.T. with 0.5y heparine were all measured. All these figures run down for both preserving solutions but in the face of greater dilution, ACDL preserves these factors a little better.

General Conclusions In Vitro

ACDL blood has less fragile red cells, contains less sodium and potassium and shows better fluidity than ACD blood. On no single point is lactated blood inferior to ACD. A specific action of lactate was not found, and the parallelism between both preserves does not call for a new mechanism.

Clinical Results

More than 9000 transfusions have been given with ACDL (table I). The number of reactions is exceedingly low. Errors in grouping and cases of S.H. are not considered. The clinical impression of those who transfused the blood was excellent. We did not transfuse a single bottle ourselves but only provided the blood.

Table I

Statistic of Nearly 9000 Transfusions with Blood Stored in Lactate Solution

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The plus days (Fig. 15) of 1500 consecutive transfusions are given and with reference to + days no difference in reaction rate was observed. We never received complaints about “old” blood. The advantage of lactated blood is greater dilution, smaller red cell fragility and low kation content. These qualities act together and allow to use the blood one month. Bleeding is easier as there is less foaming. Better fluidity has several interesting features.

- age of the blood is less important
- mixing cells and plasma is easy
- sludging of filters does not occur
- rapid flow is obtained without mechanical devices with small needles.
Fragility of the red cells is smaller and allowed for practical use of old blood without hemolysis in vitro nor complaints about transfusion reactions. The organism receiving ACDL blood receives smaller quantities of potassium and this certainly accounts for a smaller number of reactions as at least part of these are due to intoxication of the myocard by a sharp rise of potassium level. The sodium level is nearer to normal. Massive transfusions have been done with excellent results because the organism received a more suitable intravenous solution together with the red cells.

Fig. 15. Storage-time of 1500 consecutive transfused bottles of blood stored in lactate solution.

As to absolute red cell count, dilution is a disadvantage if one single bottle is considered. For absolute replacement five bottles ACDL equal four bottles of ACD. The argument against dilution is the lower absolute value of hemoglobin and red cells. This difficulty did not show up in practice. In many cases which are not acute hemorrhage, transfusion is used to restore blood volume and avoid collapse; and in such cases blood containing less, but better preserved, elements and in which the electrolytes have a better pattern, is an advantage.

Conclusion

In vitro studies on ACDL blood showed some favourable aspects. In vivo survival has not yet been done but the lack of reactions with old blood seems to be a favourable argument. Clinical experience of the blood bank in 9000 cases was uniformly favourable. The way is open to replace citrate, at least partially, by lactate. This could be done either maintaining the dilution as we personally prefer, or preparing a more concentrated formula, if dilution is not considered an advantage. A specific action of lactate was not found, but lactate is a good preservative for whole blood and might supersede citrate in the future.

References


The Immediate Collection Damage to Red Cells*
The damage sustained by red cells during the process of collection and storage of blood results from two orders of lesions: the first is the sudden and complex physico-chemical change in the environment, which is followed by a slower biological deterioration, resulting from continuance of metabolic activity in vitro. This activity progressively exhausts components essential to the maintenance of the dynamic requirements of the red cells.

In a series of 35 autotransfusions of blood collected with ACD solution (NIH formula A) as anticoagulant and transfused within one hour of collection the average loss of radioactivity of red cells tagged with Cr51 during the first day after transfusion is 6.4%. From the second to the tenth posttransfusion day this apparent loss is constant and averages 2.2%/diem, resulting in a linear disappearance curve (fig. 1).

Mollison et al.1 found a similar excessive loss of radioactivity of Cr81 tagged transfused cells in the first day post transfusion. These authors favor the hypothesis that the loss is only apparent, and is due to loss of Cr51 and not destruction of cells. Since a similar phenomenon is noted when red cell survival is determined with the

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