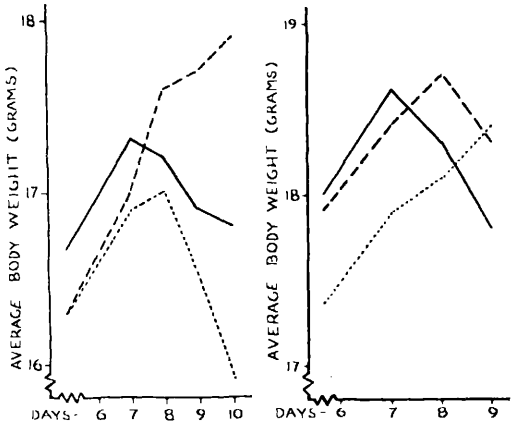

FIG. 1. (left). Showing influence of tumor implantation on controls (15 mice) and treated mice (15 each). Controls, ————; tumor-inhibiting fraction, – – – – (39.8% tumor wt decrease); tumor-stimulating fraction, ... (38.7% tumor wt increase).

FIG. 2. (right). Controls, ————; tumor-inhibiting fraction, – – – –, single dose (20.0% tumor wt reduction); tumor-inhibiting fraction, ..., double dose (53.0% tumor wt reduction).

period chosen. They reach a plateau at 24 g body weight.

By using single and double doses of the same material, a more accentuated response is obtained (Fig. 2). It is obvious that only fractions or substances with low toxicity can be used in this reaction. In cases in which we are dealing with a mixture of inhibiting and stimulating fractions the above reaction does not show, unless the 2 fractions are separated from each other. The evidence of such behavior can be obtained by using single and double doses of the same mixture (Fig. 2). A histological study‡ of the tumors revealed in the controls an invading tumor into the striated muscle, with rounded polyhedral and spindle shaped patterns, with widespread necrosis and reaction changes to necrosis. Treated animals (those on double dose were more accentuated) showed surprisingly more mitotic figures and more anaplasia. It may be mentioned that Cornman (1) reports, that the factors influencing mitosis may be different from those which cause reduction in tumor size.

Summary. Mice implanted with Crocker Sarcoma No. 180 invariably lose weight suddenly after 7-8 days. When injected with tumor-inhibiting fractions they continue to gain weight, with tumor-stimulating fractions the opposite effect takes place. Definite evidence of the two effects have been demonstrated.

‡ The histological work was done by Dr. A. Angrist, pathologist to the Queens General Hospital, N. Y., through the kind intervention of Dr. J. M. Brown. We wish to extend our thanks to both these gentlemen.

1. Cornman, I., *J. Nat. Cancer Inst.*, 1950, v10, 1123.

Received October 18, 1950. P.S.E.B.M., 1950, v75.

Effect of Sugars on Erythrocytes Preserved at 0° to –3°C.* (18303)

MAX M. STRUMIA, JOHN J. MCGRAW, Jr., MARGARET DOLAN, AND LOUISE S. COLWELL.

From the John S. Sharpe Research Foundation and the Laboratory of Clinical Pathology of the Bryn Mawr Hospital, Bryn Mawr, Pa.

Proper amounts of dextrose are known to improve preservation of stored erythrocytes. The use of other sugars has been reported by several workers(1). This report deals with the effect of certain sugars on the osmotic fragility of erythrocytes maintained at temperatures between 0°C and –3°C and the result of transfusions of blood thus preserved in humans. The fragility studies(2) were carried out for the purpose of general screen-

* This work was, in part, carried out with the support of the Army Medical Research and Development Board under contract with the Bryn Mawr Hospital

1. Rous, P., and Turner, I. R., *J. Exp. Med.*, 1916, v23, 219. DeGowin, E. C., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 481.

ing; erythrocytes showing diminished resistance to hypotonic salt solution invariably showed poor survival when transfused. The *in vivo* studies included post-transfusion variations in the blood volume, erythrocyte count, hematocrit, bilirubin level, urobilinogen output and studies of survival by the non-agglutinable cell count. In some instances it has been necessary to choose, for the time being, an arbitrary value for some of the experimental conditions (such as pH, volume of resuspension fluid, etc.) on the basis of work previously done. The complex interrelationship of the various elements may require readjustment of many of the values here reported. Therefore, none of the data obtained so far can be considered as final. This preliminary report appears desirable in view of the fact that the *in vitro* and *in vivo* studies thus far carried out strongly suggest that, with proper technic, red cells can be preserved without hemolysis for periods of time much longer than now obtained with any of the acid-citrate-dextrose solutions and that such cells remain in circulation, when transfused, at least as long as the red cells of whole blood collected in acid-citrate-dextrose solutions and stored for less than 21 days.

Material and technic. Erythrocytes were used as whole acid-citrate-dextrose (A.C.D.) blood(3), as acid citrated packed erythrocytes, and as acid citrated packed cells resuspended in a 4% globin solution. The packed cells were obtained by bleeding in a cooled sodium citrate solution prepared as follows: Trisodium citrate dihydrate g 2.1; citric acid monohydrate 0.66 g; water to 100 ml. Seventy-five ml of this solution were used for each 500 ml of blood. Within 12 hours of collection, after storage at 1°C, the blood was centrifuged and 80% to 85% of the plasma removed. To the red cell residue, solutions of the various sugars with or without globin were added. All the concentrations noted are weight to volume of the suspension medium. The pH of the fresh A.C.D. blood, in all instances, was between 7 and 7.2. The cells were maintained in rubber stoppered test tubes or cylindrical bottles in aliquots of 10 to 60 ml at −3°C (±0.2°C) in the liquid state(4) or at higher temperatures up to +4°C. When the blood was intended for transfusion into humans, it was collected and preserved in the standard 500 ml bottles. Maltose, sucrose and lactose were tested in concentrations of 1 to 12%. In all instances the solutions of sugars were sterilized by Seitz filtration.

Experimental results. Early in the experimental work acid-citrated packed erythrocytes were found to deteriorate faster than erythrocytes preserved as whole blood or erythrocytes resuspended in a volume of 4% globin equivalent to the amount of plasma removed. Maltose, although exerting a favorable effect on preservation, was found to give inconsistent results.

The effect of temperature of storage was studied in relation to addition of sucrose and lactose. It was found that preservation was optimal, under the experimental conditions, with temperatures between 0°C and −3°C. Addition of sugars to blood preserved at +4°C improves preservation, but to a lesser degree.

When erythrocytes are preserved with the disaccharides, shrinkage of varying degree occurs, along with a striking change in the osmotic fragility curve (Fig. 1). The amount of shrinkage, however, does not necessarily have a direct relationship to preservation. Thus lactose, which produces in the same concentration less shrinkage than sucrose, provides a better preservation (Fig. 4).

It is notable that the results of the osmotic fragility are the same whether the cells are transferred directly from the suspension medium to the hypotonic salt solutions or whether they are previously swollen back to their normal size by immersion in normal plasma.

The optimal concentration of lactose and sucrose has not been determined but, with whole blood, good results are obtained with

2. Parpart, A. K., *et al., J. Clin. Invest.,* 1947, v26, 636.

3. Strumia, M. M., *et al., J. Clin. Invest.,* 1947, v26, 678.

4. Strumia, M. M., *Science,* 1949, v110, 398.

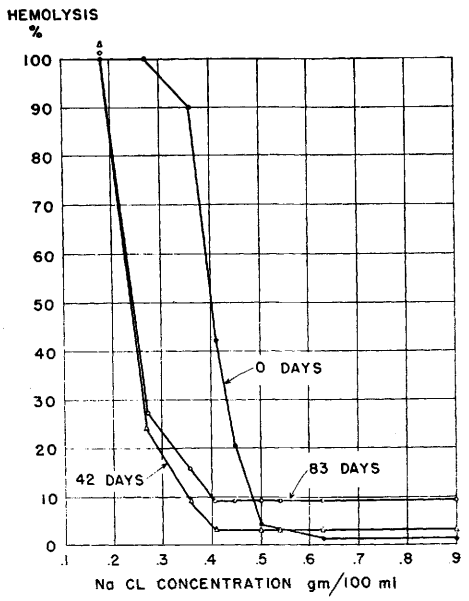

FIG. 1

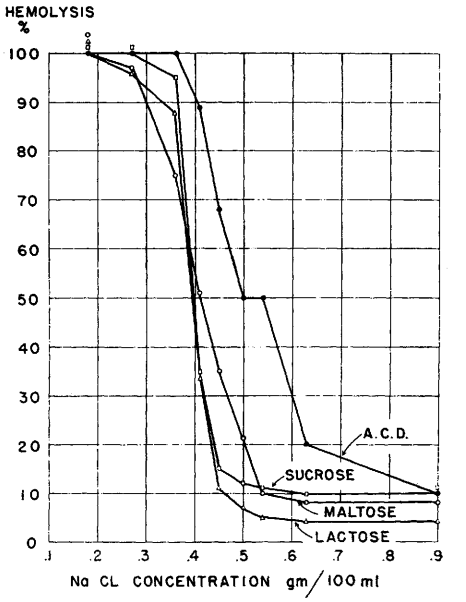

FIG 2

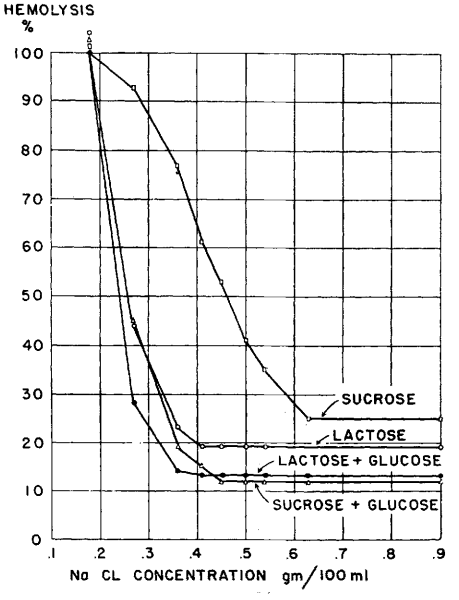

FIG 3

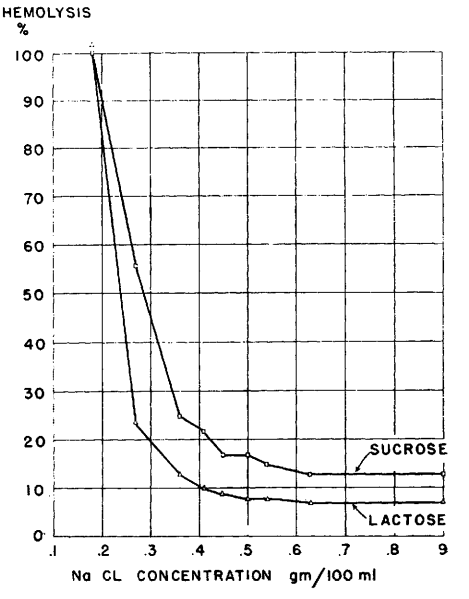

FIG. 4

FIG. 1. Changes in osmotic resistance of erythrocytes resuspended in: Globin 4%, lactose 5%, dextrose 250 mg %—storage at ±0°C.

FIG. 2. Effect of addition of 5% of sugars on the osmotic resistance of erythrocytes preserved at —3°C for 35 days (as packed red cells).

FIG. 3. Effect of glucose (250 mg/100 ml of plasma) on the osmotic fragility of erythrocytes when added to whole blood containing 5% sucrose or lactose. Blood preserved at —3°C for 49 days.

FIG. 4. Effect of sucrose and lactose in a 5% concentration on the osmotic resistance of erythrocytes preserved at —3°C for 98 days. (As cells resuspended in globin).

concentrations of 3% to 7%. When erythrocytes resuspended in 4% globin solution are used, somewhat higher concentrations of sugars appear to exert a better effect.

Fig. 2 shows the effect of sugars in a concentration of 5% on the fragility curve of whole acid-citrated blood stored, without addition of glucose, for a period of 35 days at −3°C. The fragility curve of a standard ACD blood, stored under identical conditions, is shown for comparison. Fig. 3 shows the effect of the addition of 250 mg of glucose 100 ml of plasma containing 5% sucrose or lactose. The added glucose shows a definitely beneficial effect on the preservation of blood. The absence of glucose is felt more when sucrose is used than when lactose is used. Glucose, in concentrations comparable to those of sucrose and lactose, produced gross hemolysis within one week of storage.

A number of experiments were carried out to test the relative value of sucrose and lactose when glucose is added (250 mg/100 ml of suspension medium). A difference is noted only when the experiment is carried over a period of 1½ to 3 months or longer. In order to carry the preservation over the three months' period it is necessary to remove plasma and replace it with an equal amount of a protein solution. A 4% solution of globin has been found very useful for this purpose. Fig. 4 gives the result of one of the experiments carried out for 98 days.

Preliminary studies with decalcified blood, using either a modified phenol-formaldehyde sulfonic type of resin ("Amberlite")* or a sulphonated aromatic hydrocarbon polymer ("Nalcite")† show that under the experimental conditions preservation of erythrocytes with or without sugar is not as good as when acid-citrated blood is used. A comparison was also carried out between wettable and non-wettable apparatus, using plastic tubing, silicone coated glassware and stainless steel cannulas coated with dicocodimethyl ammonium chloride ("Arquad 2C").‡ The extent of this experimental work is not enough to justify any conclusion but, so far, no difference was found between the two types of apparatus on the preservation of erythrocytes.

* Rohm and Haas Co., Philadelphia, Pa.

† National Aluminate Co., Chicago, Ill.

‡ Armour Co., Chicago, Ill.

Experiments in vivo. In all, 64 transfusions of about 500 ml of blood have been given to humans, using blood preserved with the aid of sugars. In 36 instances, lactose was used and in 28 sucrose. The concentration of sugars has been between 3% and 5%. To all but 3 specimens dextrose was added in concentration of 250 mg/100 ml. Thirty-four of these transfusions were made with

TABLE I. Variations in the Hematocrit Following 50 Consecutive Transfusions of 500 ml of Whole Blood or a Similar Amount of Resuspended Erythrocytes.

Whole blood or resuspended erythrocytes stored at 0° to −3°C with the aid of sugars and globin solution		Whole ACD blood stored at +4°C	
Days of preservation	Hematocrit variation	Days of preservation	Hematocrit variation
21	4.0	2	1.3
21	4.1	5	3.1
21	2.6	6	2.1
21	3.0	11	3.9
23	2.1	15	−0.5
23	0.5	15	3.0
24	0.9	16	1.8
25	2.4	16	0.4
26	0.5	17	3.3
26	0.6	17	2.2
28	2.0	17	4.0
31	3.3	17	2.2
32	4.1	17	2.5
33	1.2	17	3.4
35	2.8	18	6.3
35	1.7	18	2.9
35	1.7	18	2.1
40	0.1	19	−0.1
40	0.4	19	2.7
41	0.3	20	−4.0
41	1.2	20	2.1
41	1.5	20	2.3
42	0.8	20	5.2
42	3.9	22	2.1
47	5.0	23	0.0
Avg 31.7	2.02	14.2	2.08

There is a small loss of erythrocytes in the preparation of resuspended cells. An exact measure of this loss is not available because the technic has so far been somewhat variable. In addition, the subjects receiving blood preserved at 0° to −3°C have generally been patients suffering from terminal forms of carcinoma or leukemia when determination of hematocrit as a measure of survival of red cells is more likely affected by hemorrhage or excessive hemolysis. The relatively small gains in both series are due to the fact that the post transfusion hematocrit was taken 24 hr after transfusion at a time when most of the transfused plasma had been retained, reducing the percentual rise of erythrocytes.

whole blood, 30 with erythrocytes resuspended in 4% globin containing sucrose or lactose. The period of preservation varied from 14 to 47 days and the temperature of storage varied from −3°C to +4°C. In all instances the blood was maintained in the liquid state except for one specimen which was maintained in the solid state at −3°C (4). In this series there were 2 reactions, one due to pyrogens and one to Rh incompatibility in a known Rh negative patient receiving repeated injections of Rh positive blood. Neither reaction can be attributed to the specific method of blood preservation used. In the majority of cases the changes in the hematocrit and hemoglobin concentrations, the variations in the serum bilirubin content and urobilinogen output were those expected from the transfusion of ACD blood preserved up to 21 days. (Table I.) Blood volume studies were carried out in a number of occasions and in 5 patients, survival studies by the non-agglutinable cell count were carried out using the technic suggested by Young, Platzer and Rafferty(5). One patient suffering from a hypoplastic anemia received two 500 ml lots of blood preserved with 5% sucrose at −3°C for 14 and 21 days respectively, and two 500 ml of lots of blood preserved with 5% sucrose at 0°C for periods of 31 and 40 days. Evidence from studies of the blood volume, hematocrit, hemoglobin concentration, serum bilirubin and non-agglutinable cell count showed good preservation of the transfused cells.

One patient recovering from coronary thrombosis received 500 ml of blood preserved at −3°C with 5% sucrose for 25 days. The same studies as carried out in the preceding case indicated good retention of the transfused cells. These findings are contrary to those reported by Mollison and Young(6). The lower temperature of preservation and the addition of glucose is possibly responsible for this difference. From the *in vivo* studies, packed erythrocytes resuspended in 4% globin with 5% lactose and stored at 0° to −3°C, gave the most encouraging results. For this reason, blood stored in this manner was used in detailed experiments using 3 healthy recipients. These 3 recipients were normal, young male volunteers who were bled approximately 500 ml and then transfused an amount of stored blood containing the same quantity of red cells removed. The period of preservation of the blood used was 30, 32 and 39 days respectively. In all instances 80% to 85% of the plasma was replaced by 4% globin solution containing 5% lactose and 250 mg/100 ml of dextrose. Preservation was carried out at 0°C (±0.2°C).

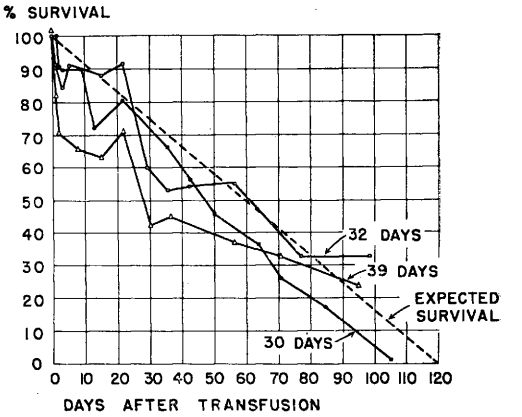

FIG. 5.

Survival of transfused erythrocytes preserved with 5% lactose at 0°C with the non-agglutinable cell count. 100% represents the ideal value on non-agglutinable cells if no transfused cells were lost.

Fig. 5 shows the survival of transfused cells by the non-agglutinable cell count technic. Erythrocyte and plasma volume studies were carried out before and 48 hours after the transfusion by the dye method and air turbine hematocrit; the non-agglutinable cell count was determined repeatedly prior to transfusion; the volume of cells to be transfused was measured and from these 3 figures the expected 100% survival was calculated as shown in the following example:

Red cell volume (avg of 2 determinations) = 2602 ml

Volume of transfused red cells 138 ml (30 days old)

5. Young, *et al., J. Lab. and Clin. Med.*, 1947, v32, 49.

6. Mollison, P. L., and Young, I. M., *Brit. Med. J.*, 1941, v2, 797.

Percentage of non-agglutinable cells before transfusion 1%

$$100\% \text{ survival} = 1 + \frac{13800}{2602} = 5.3\% \text{ non-agglutinable cells.}$$

As seen from Fig. 5, the survival was that which might be expected from fresh blood with a full life expectancy.

Summary. Sucrose and lactose exert a preserving effect upon erythrocytes when stored at 0°C to −3°C. Sixty-four transfusions of blood thus stored for periods of time up to 47 days have shown good survival of the transfused cells. In the experiments *in vivo* the concentration of sugars varied from 3 to 5%. The experimental conditions were not always necessarily optimal for red cell preservation, and therefore the limits of storage are not maximal and will most likely be extended in future work. No attempt has been made to study the mechanism of action of the sugars on the preservation of red cells.

Received October 18, 1950. P.S.E.B.M., 1950, v75.

Fat Emulsions for Oral Nutrition. II. Failure of Phosphatide, Tween 80, or Choline to Influence Fat Absorption.* (18304)

MILTON SHOSHKES, ROBERT P. GEYER, AND FREDERICK J. STARE.

From the Department of Nutrition, Harvard School of Public Health and the Department of Biological Chemistry, Harvard Medical School.

This laboratory has studied the absorption of fat from fat emulsions given orally, and has investigated the effect of emulsification of fat on its intestinal absorption, using the rat as the experimental animal(1). It was felt that studies of this type would be of value as preliminary observations toward the use of orally administered fat emulsions in humans with normal and abnormal digestive systems. It was found that gelatine-stabilized emulsions of oil in water were unstable in the rat stomach, but were stable when instilled in the ligated rat intestine. The oil in such emulsions was absorbed more rapidly than when present in the same unemulsified systems. This increased rate of absorption was related to the particle size of the emulsions. Other workers(3) have reported that relatively large amounts of lecithin (16.7% in relation to the fat) increased the absorption of fat given orally to the rat in experiments using approximately twice the amount of fat this laboratory used. Tidwell(4), using 20% lecithin in relation to physiological amounts of orally fed fat, found an increase in the chylomicronemia of rats over controls. It has also been reported(5) that lecithin increased the blood fat levels in normal human subjects and in a small series of idiopathic sprue patients. Tween 80 (polyoxyethylene sorbitan monooleate), a synthetic emulsifier, has been stated to reduce the fecal fat excretion in patients with steatorrhea(6). Irwin *et al.*(7) have investigated the effect of the addition of various substances to fat meals upon fat absorption in the rat and found that they had little or no positive effect. It was also reported(8) that the physical condition of the animal determined

* Supported in part by grants-in-aid from the National Dairy Council, Chicago, Ill.; the Upjohn Company, Kalamazoo, Mich.; the Nutrition Foundation, New York City; and the Cancer Research Grants Branch, National Cancer Institute, Bethesda, Md.

1. Shoshkes, M., Geyer, R. P., and Stare, F. J., *J. Lab. and Clin. Med.,* 1950, v35, 968.

2. Geyer, R. P., Mann, G. V., Young, J., Kinney, T. D., and Stare, F. J., *J. Lab. and Clin. Med.,* 1948, v33, 163.

3. Augur, V., Rollman, H. S., and Deuel, H. J., Jr., *J. Nutrition,* 1947, v33, 177.

4. Tidwell, H. C., *J. Biol. Chem.,* 1950, v182, 405.

5. Adlersberg, D., and Sobotka, H., *J. Nutrition,* 1943, v25, 255.

6. Jones, C. M., Culver, P. J., Drummey, G. D., and Ryan, A. E., *Ann. Int. Med.,* 1948, v29, 1.