istic feature of the dairy cow and related to the higher rate of milk production.

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HÆMATOLOGY

Recovery and *in vivo* Survival of Rabbit Erythrocytes

Human and other mammalian red cells have been frozen and thawed with limited hæmolysis using a variety of cooling conditions and protective additives. Slow cooling in high concentrations (30 per cent) of glycerol[1], moderately rapid cooling in plasma containing sugars[2], and ultra-rapid cooling in low concentrations (6 per cent) of glucose[3] have allowed high recoveries (90–95 per cent) of red cells of which 80–90 per cent survive at least 24 hr. when transfused.

Polymeric solutes, including polyvinylpyrrolidone (PVP), dextran, polyethylene glycols, and albumin, afford effective protection for red cells as measured by *in vitro* recoveries[4]. Under conditions of rapid cooling and warming, polymers are the most efficient of all protective agents[5]. To our knowledge, however, only one investigator has reported *in vivo* survival studies on red cells frozen in the presence of polymers. Sloviter[6] observed poor viability (20 per cent) of rabbit and human red cells frozen with polyethylene glycols unless thawed cells were washed free of the polymer. Washing, however, lysed some 80 per cent of the initially recovered cells.

We have observed, in work described in this report, that rabbit blood containing a low concentration of PVP, a neutral, hydrophilic polymer, can be rapidly frozen and thawed with erythrocyte recoveries of greater than 90 per cent. Thawed blood was transfused directly, without removal of the polymer, and the red cells exhibited a 24-hr. survival averaging 93 per cent. Table 1 shows results obtained in four experiments using 24 separate blood specimens.

Rabbit blood was collected in acid-citrate glucose (3 vols. blood, 1 vol. ACD). Polyvinylpyrrolidone of average molecular weight 40,000 (*K*-30 PVP, Oxford Laboratories, San Francisco) in saline was added (4 vols. blood-ACD, 1 vol. PVP) to give a final concentration of 7 per cent in the mixture. Twenty to forty ml. of the mixtures was frozen rapidly in capped aluminium tubes of 17–33 mm. diameter by mechanical shaking in liquid nitrogen. After storage at − 170° C. for several days, samples were thawed by mechanical shaking in water at 45° C.

Recoveries of red cells were determined using hæmatocrits and colorimetric measurements of supernatant and total hæmoglobin as oxyhæmoglobin in 1 : 100 dilutions in aqueous sodium carbonate.

Aliquots of thawed blood were labelled with chromium-51 (ref. 7), (Abbott Laboratories, sterile sodium 'Rachromate') and an accurately measured volume was infused through an ear vein. Total infused radioactivity as red cells was measured on a separate aliquot. All chromium-51 measurements were made by scintillation counting using a Nuclear Chicago *DS5*–5 well counter. Blood samples were taken from the opposite ear into oxalate at 30–60 min. and again at 24 hr. after infusion. The percentage survival of red cells at each sample time was calculated using apparent red cell volumes previously determined on each rabbit by auto-transfusion of labelled unfrozen blood.

Table 1. Recovery and Survival of Red Cells in Rabbit Blood containing Polyvinylpyrrolidone

Exp.	No. of samples	Container diameter (mm.)	Red cell recovery (per cent)	Red cell survival (per cent) 30–60 min.	24 hr.
1	6	17	92 ± 3	110 ± 15	98 ± 12
2	6	17	87 ± 6	97 ± 4	98 ± 6
3	6	17	93 ± 1	93 ± 16	89 ± 17
4	6	33	96 ± 1	97 ± 7	85 ± 7

In experiment 4, samples also were taken at 2–3 day intervals up to 12 days. Percentage survival at each sample time was corrected for labelled cells removed by previous sampling and chromium elution. Identical measurements were made on six additional rabbits which received unfrozen blood. Frozen cells were lost more rapidly during the first 48 hr.; subsequent rates of loss of frozen and control cells were equal (about 1·8 per cent per day). Approximately 85 per cent of the transfused frozen cells exhibited normal *in vivo* viability.

Results indicate that polyvinylpyrrolidone, long studied as a plasma expander, may prove useful as a protective agent in the low-temperature preservation of whole blood suitable for direct transfusion after thawing.

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PATHOLOGY

An Iso-Antigen (γB^A) of Mouse γ-Globulin Present in Inbred Strains

Hereditary iso-antigens (allotypes) of rabbit γ-globulin were discovered in 1956 by Oudin[1], and independently in 1958 by Dubiski *et al.*[2]. Since then many publications have appeared reporting the serological, genetical and immunochemical characteristics of rabbit γ-globulins[3–6]. More recently, antigenically different types of serum globulins have been described in guinea pig[7], man[8] and baboon[9].

In the present experiments, two inbred strains (*BALB/c* and *C57BL*) of mice were used; the *BALB/c* mice were first immunized, by Dubiski's method[3], with *Proteus vulgaris* (*X* 19). *Proteus* cells coated with anti-*Proteus* sera were afterwards injected subcutaneously twice a week (15 times) into individuals of the *C57BL* strain. In this way, five pairs of mice were immunized with five individual sera (more particularly the γ-globulin selected by bacteria) of

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