PRO EXPERIMENTIS

An alternative decomplementation method for the measurement of the survival of human erythrocytes transfused into rats[1]

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Summary. An alternative technique for measuring the survival of ^{51}Cr-labelled human erythrocytes transfused into rats is described, in which aggregated human gamma-globulin is substituted for cobra venom factor as a decomplementing agent.

Human erythrocytes transfused into rats are rapidly cleared from the circulation as a result of complement-dependent intravascular hemolysis and phagocytosis by the reticulo-endothelial system (RES), particularly in the liver[2]. Decomplementation and blockage of the receptor's RES extend the survival of transfused heterologous erythrocytes and in this way the behaviour of normal and abnormal human erythrocytes can be compared[3,4]. This experimental model is also useful for evaluating the effects of different treatments on abnormal erythrocytes[5-7].

We report here a modification of the method for measuring the survival of human erythrocytes transfused into rats, substituting aggregate human gamma-globulin (AHGG)[8] for cobra venom factor (CVF) as a decomplementing agent.

Material and methods. The survival studies were carried out with erythrocytes from 8 normal subjects and 10 patients with sickle-cell disease. Blood was drawn into acid-dextrose-citrate and separated into 2 equal aliquots to which KCNO and KCl were added, respectively, to a final concentration of 0.1 mole/l, and oxygen was bubbled through the suspensions for 5 min. Normal samples were treated with KCl only. $Na_2\ ^{51}CrO_4$ (40 µCi/ml blood) was then added, and the mixture was incubated at 37 °C for 90 min with constant mixing. The erythrocytes were then washed 3 times with iso-osmotic saline and suspended in saline to a hematocrit of 0.33.

The transfusion recipients for these experiments were male Wistar rats weighing 150-300 g. 2 h before the transfusion each rat was given 0.5 g/kg b.wt of ethyl palmitate as an emulsion through the tail vein. Immediately before transfusion the animal received 440 mg/kg i.v. of a 150 mg/ml solution of heat-aggregated (60 °C for 30 min) human gamma-globulin (Boehringer), followed by 880 mg/kg i.p. 30 min afterwards and every 4 h for 12 h.

The ^{51}Cr-labelled human erythrocyte suspension (3 ml/kg) was injected through the dorsal vein of the penis. 15 min later and at 1-h intervals thereafter (for at least 6-8 h) 20 µl capillary blood samples were obtained from the tip of the tail. Percent survival was calculated by comparison of the radioactivity in each blood sample with that of the initial sample, obtained 15 min after the infusion of the labelled cells. In each experiment the same sample was transfused into 2 or 3 rats and the data averaged for the determination of the ^{51}Cr halflife ($T^1/_2$).

Serum complement levels were measured in 12 animals before AHGG injection[9]. Subsequent measurements were performed in groups of 3 rats bled by cardiac puncture 30 min after the first AHGG injection and every 4 h thereafter for 12 h.

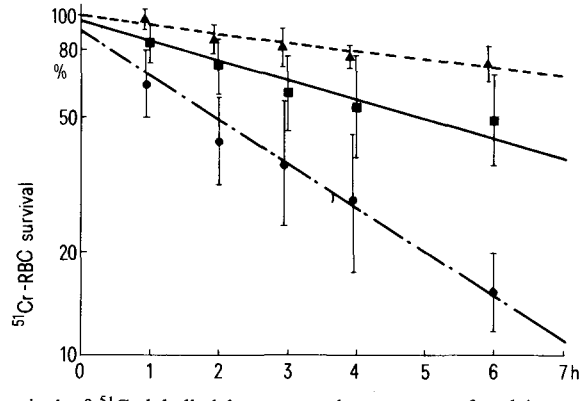
Results and discussion. The levels of serum complement in rats treated with AHGG were reduced to less than 11% of the initial values throughout the duration of the treatment (table). The survival of human erythrocytes transfused into rats previously treated with AHGG and ethyl palmitate is shown in the figure. Normal erythrocytes had a $T^1/_2$ of 14.8 ± 2.4 h (mean ± SD). Sickle erythrocytes had a $T^1/_2$ of 2.4 ± 0.5 h which increased to 5.6 ± 2.8 h after treatment with KCNO, a known antisickling agent. These values are lower than those reported by Castro et al.[3,5] using CVF, but the differences of the ^{51}Cr halflives of the 3 groups are statistically significant ($p < 0.05$). As expected[5], KCNO enhanced the survival of sickle erythrocytes but did not cause it to reach the normal range.

The results show that AHGG is an efficient agent to depress serum complement in rats, and that the combined administration of ethyl palmitate and AHGG results in a prolonged survival of transfused human erythrocytes. Probably, as a consequence of the profound blockage of the RES phagocytic function and complement depression pro-

Levels of serum complement in rats before and during treatment with AHGG

	Before treatment (12)	During treatment with AHGG 30 min (3)	4 h (3)	8 h (3)	12 h (3)
Mean	389.1	18.0	25.5	42.7	26.0
SD	48.6				

The values are in $C'H_{50}$ units/ml. The number of animals in each group is in parentheses. AHGG 440 mg/kg was given as an i.v. injection followed by 800 mg/kg i.p. 30 min afterwards and every 4 h for 12 h.



Survival of ^{51}Cr-labelled human erythrocytes transfused into rats treated with ethyl palmitate 0.5 g/kg i.v. 2 h before the transfusion and AHGG 440 mg/kg given as an i.v. injection immediately before the transfusion followed by 880 mg/kg i.p. 30 min afterwards and every 4 h thereafter. Percent survival was calculated by comparison of the radioactivity in each blood sample with that of the initial sample, obtained 15 min after infusion of labelled cells. Each sample was transfused into 2 or 3 rats, and the points represent the means of the groups (vertical bars, ± 1 SD). (–·–●–·–) Sickle erythrocytes treated with 0.1 mole/l KCl (N = 10 samples). (—■—) Sickle erythrocytes treated with 0.1 mole/l KCNO (N = 10 samples). (––▲––) Normal erythrocytes treated with 0.1 mole/l KCl (N = 8 samples).

duced by this treatment, the removal of transfused erythrocytes from the circulation becomes more dependent on the retention of distorted and injured cells in the microvasculature. This animal model seems to be very sensitive to sickling and to the action of antisickling drugs, and it represents an alternative to the method employing CVF. It provides a preliminary approach for the in vivo investigation of drugs with potentially antisickling effects by evaluating the results of the treatment of either the transfused erythrocytes or the recipient animal[6].

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Very small microspheres are useful for the determination of cardiac output but not organ blood flow in conscious rabbits

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Summary. Very small microspheres with a diameter of 8–10 μm can be used for the measurement of cardiac output, by the reference flow method, in conscious rabbits. However, they were found not to be suitable for the determination of the distribution of cardiac output or blood flow to most organs. These small microspheres offer certain advantages for the measurement of cardiac output; the numbers required to achieve a specified accuracy of measurement are discussed.

Rabbits were among the first animals in which radioactive microspheres were used[1] and excellent validation studies have been reported for this species[2–5]. Little is known, however, about the usefulness of microspheres smaller than 15 μm. In many experimental situations it is desirable to use the smallest possible microspheres for the following reasons: the number of microspheres that can be injected is limited by their size; if more than about 1×10^5 50-μm or about 3×10^5 25-μm microspheres are injected into conscious rabbits, hemodynamic disturbances may occur[5]. There is also evidence that large microspheres will show preferential streaming[2,5] since the specific weight of all non-biodegradable microspheres currently available is of the order of 1.3 g/cm^3. The smallest microspheres will behave most like erythrocytes[2]. Furthermore, it has been shown that in the kidney[6] and the heart[7] intraorgan distribution of microspheres may depend on their size. In these studies too, the smallest possible spheres gave the best results. Therefore, we investigated, whether 8–10-μm microspheres could be used in rabbits.

Materials and methods. Microspheres of 8.5 ± 0.7 μm and 15.1 ± 0.9 μm (3M Company) were prepared for use according to the methods of Rudolph and Heymann[8]. We used a Packard 9012 gamma-counter with a 1024 channel pulse height analyzer. The data were recorded on a Kennedy 1610 360 R incremental tape and processed on a HP 21MX computer. For the isotope separation calculations we followed the simplified scheme of Schaper et al.[9].

In a 1st series of experiments in 5 New Zealand white rabbits about 2×10^5 8.5-μm microspheres were injected into a marginal ear vein and the animals were killed at the intervals shown in table 1. The lungs as well as the heart and the kidneys were counted to establish whether microspheres could cross the pulmonary bed and reach the periphery.

6 further New Zealand albino rabbits of 2.5–3.5 kg were anaesthetized with Nembutal, 35 mg/kg injected into an ear vein. They were intubated and ventilated with a Loosco MK2 infant ventilator. Polyvinyl catheters were inserted into the carotid artery and the jugular vein under aseptic conditions. The chest was opened through the left 4th intercostal space and a catheter was tied into the left atrium. The animals were allowed to recover for 1 week. Then about 2×10^5 15-μm microspheres were injected into the left atrium while a reference sample was obtained from the carotid artery. 24 h later the same procedure was repeated with about 2×10^5 8.5-μm microspheres.

The animals were killed with an overdose of Nembutal, dissected and the tissues were placed in plastic vials for counting.

Results. Extraction of 8.5-μm microspheres by the lungs. In all 5 rabbits, independently of the duration of the experiment, the radioactivity was confined to the lungs (table 1). The heart and the kidneys contained no radioactivity. 8.5-μm microspheres are therefore a 'non-recirculating indicator' in conscious rabbits and may be used for the determination of cardiac output.

Extraction of 8.5-μm microspheres by the peripheral organs. The extraction of both sizes of microspheres used was

Table 1. Trapping of 8.5-μm microspheres injected into the ear vein of 5 conscious rabbits. No relevant activity was found in the kidneys or the heart. The lungs presumably extracted all the circulating microspheres during their 1st passage

Experiment number	Interval from injection to death	Activity in the lungs (cpm)	% of lung activity Kidneys	Heart
1	1 day	217,961	0.0	0.0
2	3 days	184,630	0.0	0.0
3	1 week	111,858	0.0	0.0
4	2 weeks	110,227	0.0	0.0
5	5 weeks	245,667	0.0	0.0