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].

1 Acknowledgments. This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo and Conselho Nacional de Desenvolvimento Científico e Tecnológico.
2 O. Castro, M.W. Rosen and S.C. Finch, Proc. Soc. exp. Biol. Med. *147*, 106 (1974).
3 O. Castro, J. Orlin, M.W. Rosen and S.C. Finch, Proc. natl Acad. Sci. USA *70*, 2356 (1973).
4 O. Castro, G.W. Osbaldiston, L. Aponte, R. Roth, J. Orlin and S.C. Finch, J. Lab. clin. Med. *88*, 732 (1976).
5 O. Castro, J. Orlin and S.C. Finch, Yale J. Biol. Med. *47*, 55 (1974).
6 F.F. Costa, M.A. Zago and C. Bottura, Lancet *2*, 1302 (1979).
7 W.C. Mentzer, B.H. Lubin and S.Emmons, New Engl. J. Med. *294*, 1200 (1976).
8 T. Neveu and G. Biozzi, Immunology *9*, 303 (1965).
9 J.O. Almeida, Hospital *35*, 847 (1950).

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

R.P. Hof and A. Hof

Pharmaceutical Division, Cardio-Vascular Department of Preclinical Research, Sandoz Ltd, CH-4002 Basle (Switzerland),
22 April 1980

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

Table 2. Distribution of cardiac output in percent (left) and organ blood flows in ml/min per 100 g of tissue (right) measured with 15- and 8.5-μm microspheres

Organ	n	CO (%) 15-μm microspheres		8.5-μm microspheres		Flow/100 g 15-μm microspheres		8.5-μm microspheres	
		$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM
Brain total	6	1.72	0.19	0.06	0.01	73.52	2.55	2.73	0.54
Cortex	6	1.00	0.11	0.04	0.01	76.98	3.38	3.51	0.62
Cerebellum	6	0.29	0.03	0.01	0.00	84.62	2.80	1.91	0.62
Brain stem	6	0.42	0.04	0.01	0.00	61.43	2.75	1.61	0.44
Heart	6	2.40	0.20	0.97	0.07	150.83	15.9	68.2	7.80
Lungs	6	7.61	2.35	26.41	1.87	312.0	112	1083.7	137
Adrenales	6	0.05	0.01	0.02	0.00	72.25	9.58	27.72	4.98
Kidneys	6	16.59	1.34	8.95	0.72	398.0	37.8	246.7	40.1
Liver	6	1.63	0.18	30.46	2.89	10.29	2.07	201	25.2
Spleen	6	2.58	0.47	3.14	0.35	637.8	204	806.8	238
Stomach	6	6.32	0.57	2.65	0.31	101.5	10.3	48.95	8.67
Small/intestine	6	10.67	1.33	2.26	0.20	169.2	20.7	41.8	6.96
Colon	6	3.37	0.58	1.15	0.19	63.2	13.5	22.86	5.17
Cecum	6	4.42	0.94	0.64	0.08	74.98	16.1	12.63	1.29
Muscle		% CO/100 g							
	6	1.14	0.21	1.13	0.20	4.78	0.85	5.31	0.95

The smaller spheres were poorly extracted by many organs, especially the brain. The microspheres not trapped by the first capillary bed were found in the lungs (26% of all microspheres injected) and, in the case of the portal system, in the liver (31% of all microspheres injected). Except for muscle all differences were statistically highly significant ($p<0.01$, paired t-test). Samples of skeletal muscle were obtained from the legs, in all other cases the whole organs were counted.

identical in skeletal muscle (table 2). In all other peripheral organs many less of the 8.5-than of the 15-μm microspheres were trapped, resulting in extremely low flow estimates, especially to the brain.

The microspheres passing to the venous side were found either in the lungs or, in the case of the organs of the portal circulation, in the liver.

Discussion. In the conscious rabbit good extraction of 15-μm microspheres by most peripheral organs was demonstrated by Warren and Ledingham[5]. We have extended some of their work to smaller microspheres. Our experiments demonstrated that 8.5-μm microspheres were poorly extracted by most vascular beds and cannot be used for measuring the distribution of cardiac output or blood flow to most peripheral vascular beds. They were however completely and permanently extracted by the lungs. The heart and the kidney are organs that receive a high flow and extract these small microspheres relatively well. They would have trapped microspheres, had any been released by the lungs. Recirculation will therefore not occur and 8–10-μm microspheres may well be used for the determination of cardiac output by the reference flow method[10]. The animals tolerate large numbers of these small spheres. Satisfactory accuracy for the cardiac output measurement with a smaller withdrawal sample and/or several successive determinations of cardiac output (with the same isotope if desired) therefore become possible.

The accuracy of measurements with microspheres is influenced by many factors[5,8]. One of the most important ones, which should be kept under control, is the number (N) of microspheres trapped, in the case discussed here, trapped in the reference sample[11]. We shall try in this section to translate the statistical aspects into practical guidelines. This number follows the Poisson distribution according to which the % accuracy (A) of a measurement will be

$$A=\frac{196\sqrt{N}}{N}$$ at the 95% confidence level and

$$A=\frac{258\sqrt{N}}{N}$$ at the 99% confidence level.

If a 95% confidence interval is accepted, then $N=\frac{196^2}{A^2}$ spheres will be needed: thus about 400 spheres will permit an accuracy of $\pm 10\%$ and about 1500 microspheres will be necessary to achieve an accuracy of $\pm 5\%$.

If a rough estimation of cardiac output (CO) is possible, then the number of microspheres needed for injection (N_i) is: $N_i=\frac{CO\times Ns}{W}$

where Ns is the number of spheres desired in the syringe and W the withdrawal rate of the reference sample in the same units as the estimated cardiac output. The CO of rabbits will normally be in the order of 150–300 ml/min/kg and for repeated CO determinations withdrawal rates of about 3 ml/min are reasonable. $\pm 10\%$ accuracy will be achieved with $2\text{–}4\times 10^4$ microspheres per kg and $\pm 5\%$ accuracy with $7.5\text{–}15\times 10^4$ microspheres per kg of body weight. The withdrawal rate and time depends on the dimensions of the catheter and the site of implantation. It must be determined in pilot experiments.

Following these guidelines 2–4 relatively accurate determinations of cardiac output can be performed in rapid succession and of course with the same isotope. An even greater number of determinations is possible, if they are performed at intervals of hours to days.

1 R.H. Phibbs, F. Wyler and J. Neutze, Nature *216*, 1339 (1967).
2 R.H. Phibbs and L. Dong, Can. J. Physiol. Pharmac. *48*, 415 (1970).
3 J.M. Neutze, F. Wyler and A.M. Rudolph, Am. J. Physiol. *215*, 486 (1968).
4 R.K. Creasy, K.V. Kahanpää and M. de Swiet, Acta physiol. scand. *90*, 252 (1974).
5 D.J. Warren and J.G.G. Ledingham, Cardiovasc. Res. *8*, 570 (1974).
6 M.A. Katz, R.C. Blantz, F.C. Rector, Jr, and D.W. Seldin, Am. J. Physiol. *220*, 1903 (1971).
7 J. Utley, E.L. Carlson, J.I.E. Hoffman, H.M. Martinez and G.D. Buckberg, Circulation Res. *34*, 391 (1974).
8 M.A. Heymann, B.D. Payne, J.I.E. Hoffman and A.M. Rudolph, Prog. cardiovasc. Dis. *20*, 55 (1977).
9 W. Schaper, P. Lewi, W. Flameng and L. Gijpen, Basic Res. Cardiol. *68*, 3 (1973).
10 J.P. Archie, Jr, D.E. Fixler, D.J. Ullyot, J.I.E. Hoffman, J.R. Uttley and E.L. Carlson, J. appl. Physiol. *35*, 148 (1973).
11 G.D. Buckberg, J.C. Luck, D.B. Payne, J.I.E. Hoffman, J.P. Archie and D.E. Fixler, J. appl. Physiol. *31*, 598 (1971).