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**RADIOLABELLED MICROSPHERE TECHNIQUE
IN CONSCIOUS SUBJECTS DURING ACCELERATION
EXPOSURES ON THE USAFSAM CENTRIFUGE**

**M. Harold Laughlin, Captain, USAF, BSC
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The methods used to apply the radiolabeled microsphere technique for the study of the effects of +G _z acceleration on regional blood flows are presented. A remote-control system designed to infuse suspensions of microspheres into the left atrium of conscious animals is outlined as is a device which allows the remote, sequential withdrawal of six blood samples. Results are presented which demonstrate that the cautious application of the radiolabeled microsphere technique using the outlined systems can produce good information about the effects of +G _z acceleration on regional blood flows.			

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RADIOLABELED MICROSPHERE TECHNIQUE IN CONSCIOUS SUBJECTS DURING ACCELERATION EXPOSURES ON THE USAFSAM CENTRIFUGE

INTRODUCTION

Radiolabeled microspheres were first used by Prinzmetal et al. (12) to study myocardial collateral circulation. This technique has since been used by many investigators and is now the preferred method for measuring the distribution of blood flow to and within organs. Specific limitations, problems, and possible sources of error have been studied and/or recognized for the use of this technique in usual laboratories (1,3,6,7,8,10,11,13,14,15) and during acceleration exposures (9). The purpose of this report is to present the systems and equipment which were devised and constructed (in house) specifically for the use of the radiolabeled microsphere technique in studies of acceleration physiology.

MATERIALS AND METHODS

The radiolabeled microsphere technique was applied using standard methods whenever possible (7). However, the application of this method in conscious animals by remote control on the USAF School of Aerospace Medicine (USAFSAM) centrifuge presented some unique problems (9).

Microsphere Infusion System

The system used to give intra-atrial infusions of radiolabeled microspheres during acceleration is illustrated in Figure 1 and shown in Figure 2. This infusion system consists of: two 0.75-cc microsphere suspension chambers (which are mounted on a high-frequency (60 Hz) vibration bar), two solenoid valves (Skinner, B. series), a Masterflex roller pump, and a normal saline reservoir. The microsphere chambers were designed as described by Heymann et al. (7). Each suspension of radiolabeled (^{46}Sc , ^{85}Sr , ^{141}Ce , and ^{125}I) microspheres ($9 \pm 0.8 \mu\text{m}$, 3M Company) consisted of between 10^6 - 10^7 microspheres suspended in 0.75 ml of 10% dextran, containing Tween-80. Suspension and non-aggregation of the spheres were maintained by continuous use of the vibration bar. An infusion of microspheres was performed by opening the appropriate solenoid valve, turning on the infusion pump, and pumping 20 cm^3 (0.8 Hz) of saline from the reservoir through the microsphere suspension chamber and into the animal's left atrial catheter. Approximately 5 - 10 sec prior to the microsphere infusion, withdrawal of a reference arterial blood sample was started at a constant rate of $10 \text{ cm}^3/\text{min}$ from the aortic catheter using the blood-withdrawal system discussed below. Withdrawal was continued for 1.5 to 2.0 minutes. In the typical experiment each reference arterial blood sample was withdrawn into two syringes. The first syringe represented the blood which entered the aorta during the first 50 sec of withdrawal, while the second syringe represented blood which entered the aorta during the last 40-70 sec of withdrawal. This method of withdrawal allowed a routine check for possible trapping of microspheres in the cardiac chambers during $+G_z$.

Blood-Withdrawal System

The system designed for the withdrawal of up to 6 sequential blood samples is illustrated in Figures 3 and 4 and shown in Figure 5. The requirement for the system was to fill from two to six syringes to a specified level automatically and in sequence. The approach was to use a pump to withdraw and apply the blood, under pressure, to a manifold supplying the syringes. To control the order, the filling of each syringe was opposed by the force from a pneumatic cylinder which could be vented to atmosphere to allow its associated syringe to fill.

A sketch of the pump and two of the six syringe assemblies is shown in Figure 3. The whole assembly (Fig. 5), excluding the pump, is mounted on a 1/8" x 9" x 9" (0.3 x 22.9 x 22.9 cm) sheet of aluminum. The rails R-1 and R-2 are 3/8" x 1" x 9" (0.9 x 2.4 x 22.9 cm) aluminum bar stock and are fitted as shown to support the pneumatic cylinders (PC) and the sample syringes (SS). R-2 is drilled to accept #15 blunt needles which serve as sockets for the syringes, protrude through the rail, and connect to the blood manifold with tygon tubing. The blood manifold is a section of 1/8" (0.3 cm) O.D. copper tubing with six ports of #15 needle shanks soldered in place. CAPs-1 through 6 are blocks of Plexiglas that are machined to accept the plungers of the sample syringes and are attached to the push rods (PR). The push rods are made from 1/4" (0.6 cm) aluminum rods, whose length is fixed by the size of the syringes used and the withdrawal volume required. The "volume adjust" (VA) block is a block of Plexiglas bored to accept the 1/4" (0.6 cm) rod and locked on the rod by screw "A." Screw "B" provides an adjustment for correct deflection of the switch lever before the rail is encountered. The switch is a standard lever-operated microswitch and is used to trigger the next operation in the sequence. V-1 through V-6 are miniature solenoid-operated airvalves. They are manufactured by the Lee Company, Pettipaug Road, Westbrook, Connecticut, under the trade name "Epca," and provide a three-way "charge, vent action." When energized, they pressurize the pneumatic cylinders that in turn apply a force holding the sample syringes down. When deenergized, the PC is vented to atmosphere and the force is removed. The PCs are made from disposable 20-cm³ syringes by removing the piston from the plunger, discarding the plunger, and fitting the piston to a machined washer which is attached to the end of the push rod (PR). This washer is machined to fit and retain the piston of the specific syringe used. The air manifold is a duplication of the blood manifold and supplies a source air pressure of 15 psi to the valves. This is the maximum air pressure that the valves can control. The motor-driven pump used is supplied by the Cole Parmer Instrument Company, 7425 North Oak Park Avenue, Chicago, Illinois 60648, under the trade name Masterflex.

Figure 4 is a complete electrical schema of the device and shows S-7 in the "off" position. In this configuration, all the solenoid valves V-1 through V-6 are energized, and the pneumatic cylinders are charged, holding the sample syringes in an empty or discharged position with the pump not running. When S-7 is moved to the "start" position, the pump starts, and V-1 deenergizes, venting PC-1 to atmosphere. The pump pressure is felt in the blood manifold, and SS-1, being unopposed, starts to fill. As SS-1 continues filling, VA-1 actuates switch S-1 and deenergizes V-2, venting PC-2 to atmosphere. VA-1 contacts the mechanical limit (see Figure 3), and SS-2, now being unopposed, starts

to fill. This action continues through syringes 3, 4, 5, and 6 until VA-6 actuates S-6 and stops the pump. This sequence can be varied almost infinitely by changing the electrical wiring.

Prior to operation, some adjustments are necessary on the device. Consider the "volume adjust (VA)" (see Fig. 3) blocks. Loosen screw A, slide the block toward R-1, and adjust screw B so that when it encounters R-1 the VA block has activated the microswitch and flexed the spring lever of the switch lightly. Tighten the lock nut on screw B, and repeat the adjustment for the six syringes. To adjust the volume of blood each syringe receives, leave screw A loosened so that the VA block slides freely on the push rod, and disconnect the pump tubing from the manifold. Turn the +12VDC power source off to ensure that the PCs are vented. Hold the "CAP" firmly against the SS plunger, and move the plunger out until the piston reads the volume desired. Hold the CAP and plunger in this position, move the VA block firmly against R-1, and lock it in place by tightening screw A. When all the volumes have been adjusted, reconnect the pump tubing, turn on the 12VDC power supply, connect a regulated 15 psig air pressure to the air manifold, and operate the device by moving S-7 to "start" position. The device should function even though the pump is pumping air into the blood manifold. R-2 is secured to the plate with two thumb screws, and when the syringes are filled, the rail R-2 must be removed and the pump tubing disconnected to allow the complete sample syringe array to be removed from the device for processing. When pumping air, the device may be reset by disconnecting the pump from the manifold and moving S-7 to "off" position. S-7 is a single pole, double-throw, center-off (SPDTC) switch, and the "center-off" position provides for "remote" operation from another switch in parallel with S-7. However, one switch must be in the "center-off" position for the other to operate properly.

Experimental Application

Experimental Animals--Miniature swine have been shown to have +G_z acceleration tolerances which are quite similar to those of man and exceed those of other experimental animals (2). Because a straining maneuver was necessary for the maintenance of such acceleration tolerance (2), these experiments were conducted on conscious miniature swine.

The microsphere studies were conducted on 27, 2-year-old, female, 35-50 kg, miniature swine (Pitman-Moore strain, Vita Vet Laboratories, Inc.). Using halothane and nitrous oxide anesthesia and sterile surgical techniques, chronic instrumentation was accomplished via a mid-sternal incision. Two Silastic catheters (0.062 in. ID/0.125 in. OD (0.15/0.30 cm)) were implanted: one in the left atrium and one in the arch of the aorta via the left internal thoracic artery. The heparin-filled (1000 USP units/cm³) catheters were then tunneled subcutaneously and exteriorized on the dorsum between the scapula. The animals were allowed 2-4 weeks of surgical recovery before the acceleration studies were performed.

Experimental Protocol--On the day of study, the animal was restrained, fitted with an anti-G suit (2), and placed feet down in a form-fitted, fiberglass couch on the USAFSAM centrifuge. The animals were positioned to receive positive head-to-tail directed inertial force (+G_z acceleration). After all instrumentation was connected and catheters cleared, the animal was allowed

15 minutes to reach a physiological steady state. When heart rate had remained constant for 5 minutes, an infusion of microspheres was made through the left atrial catheter to measure regional blood flow under resting conditions.

The microsphere infusion system was then reloaded and the animal exposed to +3 G_z for 15 sec with anti-G suit inflation. The purpose of this "warm-up" run was to assure that all systems would function properly during acceleration.

After heart rates had returned to control levels (5-10 min), the animal was exposed to either +3, +5, or +7 G_z with anti-G suit inflation for 60 sec. Withdrawal of the reference arterial blood sample was started after 10 sec of +G_z exposure. An infusion of microspheres was then made if the preparation attained a cardiovascular steady state (stable heart rate and aortic blood pressure \geq 100 mm Hg) during the next 20 sec of +G_z exposure. The average time of the start of microsphere infusion was 18 sec into the +G_z plateau and the range was 13-21 sec into the +G_z plateau. If a cardiovascular steady state was not attained during the first 30 sec of +G_z exposure, microspheres were not infused. If there was a significant change in the cardiovascular steady state at any time during acceleration after the start of microsphere infusion, the animal was excluded from the study. Microsphere infusions were also made 10 min after +G_z exposure. With a successful experiment, the protocol thus resulted in regional blood flows being measured under baseline conditions, during +G_z, and 10 min after +G_z exposure. Aortic and left atrial pressures and heart rates were recorded throughout each experiment.

The animals were sacrificed with a 10-cm³ injection of Euthanol-6 (I.V.) (Trico Pharmaceutical Co., Los Angeles, CA). The brain, heart, kidneys, and adrenal glands were removed and placed in 10% formalin for 24 hr. The heart was then divided into 120, 0.5-1.0 g tissue samples. The brain was transected longitudinally and the pons, medulla, and both cerebellum separated from the cerebral hemispheres. Tissue samples ranging from 1 to 2 g in weight were then taken from the following areas: (1) frontal, temporal, parietal, and occipital lobes of the left cerebrum; (2) left and right cerebellum; and (3) pons and medulla. Tissue samples ranging from 1 to 2 g in weight were also taken from the right and left adrenal glands and from the right and left renal cortex. The weight of all tissue samples was determined with a Mettler balance and recorded. The samples were then placed in counting tubes (Squibb, plastic, 13 mm x 100 mm) and counted for 10 minutes in a Packard Auto Gamma Scintillation well counter. The reference blood samples were divided into aliquots such that the counting geometry was similar to that of the tissue samples. Counting and isotope separation was performed utilizing standard techniques (10). Tissue blood flow (F_T) was calculated using equation:

$$F_T = F_R \times \text{cpm}_T / \text{cpm}_R \quad (1)$$

where F_R is the reference blood sampling rate (10 cc/min), and cpm_T and cpm_R represent the radioactive counts per minute for the tissue sample and reference blood sample, respectively. Flows were then expressed as ml/min/100 g of tissue.

Student's paired and unpaired t-tests were used for statistical analysis when appropriate. A difference was considered significant only at the 95% confidence level ($p \leq 0.05$).

Cardiac Chamber Transit Times for Microspheres

The inertial forces produced by $+G_z$ acceleration could cause small areas of low and/or zero flow within the cardiac chambers. If this occurred, it could cause microspheres to become trapped and/or sequestered in these areas during the acceleration exposure. The entrance of some of the microspheres into the circulation could then actually occur after $+G_z$ stress had ceased. This would result in errors in the blood flow calculations. The possibility of this error occurring was tested in a pilot study in which the reference arterial blood sample was taken in a sequential manner such that one sample was taken every 12 sec. This was done under resting baseline conditions and during exposures to $+G_z$ acceleration.

Since it is possible that $+G_z$ exposure might have an effect on cardiac microsphere washout in some animals and not in others, we felt it necessary to check for this possibility routinely in each experiment. This was accomplished by taking each reference arterial blood sample in two sequential syringes. The first syringe represented the blood which entered the aorta during the first 50 sec of withdrawal while the second syringe represented blood which entered the aorta during the last 40-70 secs of withdrawal. Thus, from microsphere infusions performed during acceleration, the first reference blood sample syringe was withdrawn during $+G_z$ acceleration and the second for 40-70 sec after $+G_z$. The percent of total arterial reference sample microspheres that were in the first syringe withdrawn during acceleration was then compared with the percent of total arterial reference sample microspheres that were in the first syringe withdrawn under baseline conditions.

RESULTS

The results of the regional blood flows obtained with the microsphere technique are presented in Table 1. Although acceleration exposure produced minor changes in cerebellar, cerebral, adrenal, and brain stem blood flows, none of these changes were statistically significant. Acceleration exposure produced dramatic decreases in renal cortical blood flow and dramatic increases in myocardial blood flow.

The data do not indicate any nonrandom distribution of the spheres. This is illustrated by the lack of an effect of acceleration on brain blood flow and by the fact that no differences were observed between left and right cerebellum or adrenal gland flows and by the reasonable distribution of myocardial blood flow.

Cardiac Chamber Transit Times for Microspheres

The results of the pilot study on the effects of $+G_z$ on cardiac chamber transit times for microspheres infused into the left atrium are presented in Figure 6. It can be seen that not only did all the microspheres enter the aorta during exposure to $+3 G_z$, $+5 G_z$, and $+7 G_z$, but there was no obvious effect of acceleration on the rate of microsphere appearance time in the aorta. The observation that $+G_z$ had no significant effect on microsphere washout from the cardiac chambers was supported by the routine comparisons of the percent

of microspheres which were contained in the first half of the reference arterial samples withdrawn during acceleration and during baseline conditions. The results of this comparison from all 20 animals demonstrated that at least $97\% \pm 2\%$ of the microspheres in reference arterial blood samples was in the first syringe both during control conditions and during all $+G_z$ exposures.

DISCUSSION

Results obtained with the microsphere technique are more easily interpreted when the spheres are injected during a cardiovascular steady state (9). Therefore, we only used the microsphere technique, during acceleration, when aortic blood pressure and heart rate had reached a relatively steady state and when heart level aortic blood pressure was ≥ 100 mm Hg.

The regional blood flow results presented in Table 1 indicate that, within the guidelines given above, the microsphere technique can be used in studies of acceleration physiology. Comparisons of the number of microspheres per gram of tissue in various areas of the body provides a check for adequacy of mixing and/or significant fluid-sphere separation (7). Therefore, the results from the heart, brain, kidney, and adrenal glands (Table 1) are consistent with adequate mixing during $+G_z$ and during control conditions.

The observed values of cerebral blood flow are in good agreement with the values of cerebral blood flow in miniature swine reported by Foreman et al. (4) and Hamlin and Leverett (5). The causes of the dramatic decreases in renal cortical flow during $+5$ and $+7 G_z$ are not clear. However, it is believed that the large increase in intraabdominal pressures caused by the anti-G suit inflation and the $+G_z$ cardiovascular reflex-induced increased alpha constrictor tone may be important factors (9). The increase in myocardial blood flow observed during acceleration is believed to be due to the increased myocardial metabolic demand which results from $+G_z$ -induced, sympathetic stimulation and increased left ventricular systolic pressures (9).

We have shown that aortic flow velocities during acceleration are more than sufficient to maintain microsphere suspension (9). However, it is possible that acceleration exposure could cause small areas of low and/or zero flow within the left cardiac chambers. If this occurred, microspheres could be trapped in these areas, therefore remaining in the heart throughout the $+G_z$ exposure, and not washed out until acceleration had ceased. The results shown in Figure 6 and the method of taking the arterial withdrawal samples in this study demonstrate that the microspheres infused into the left atrium during $+G_z$ entered the aorta during $+G_z$. Therefore, the blood flow measured from these microsphere infusions represents blood flow during $+G_z$ exposure.

The results of this study indicate that the systems designed for applying the radiolabeled microsphere technique are adequate. In future studies systems which are similar to those presented here should be used. Although these systems are not ideal, they have made it possible to accurately use the radiolabeled microsphere technique in the study of regional blood flows during $+G_z$ acceleration.

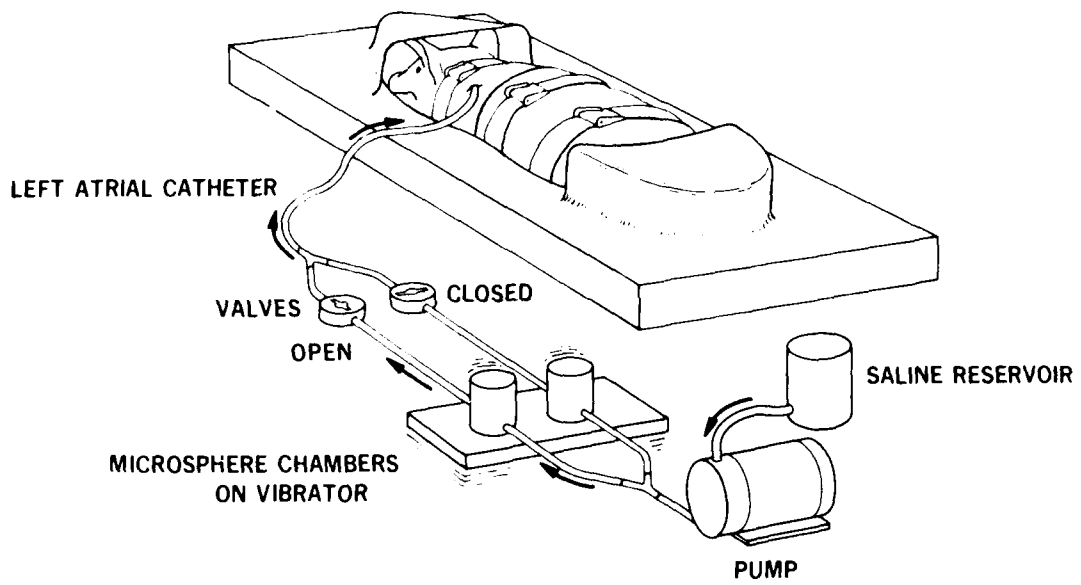


Figure 1. Illustration of the microsphere infusion system.



Figure 2. Microsphere infusion system.

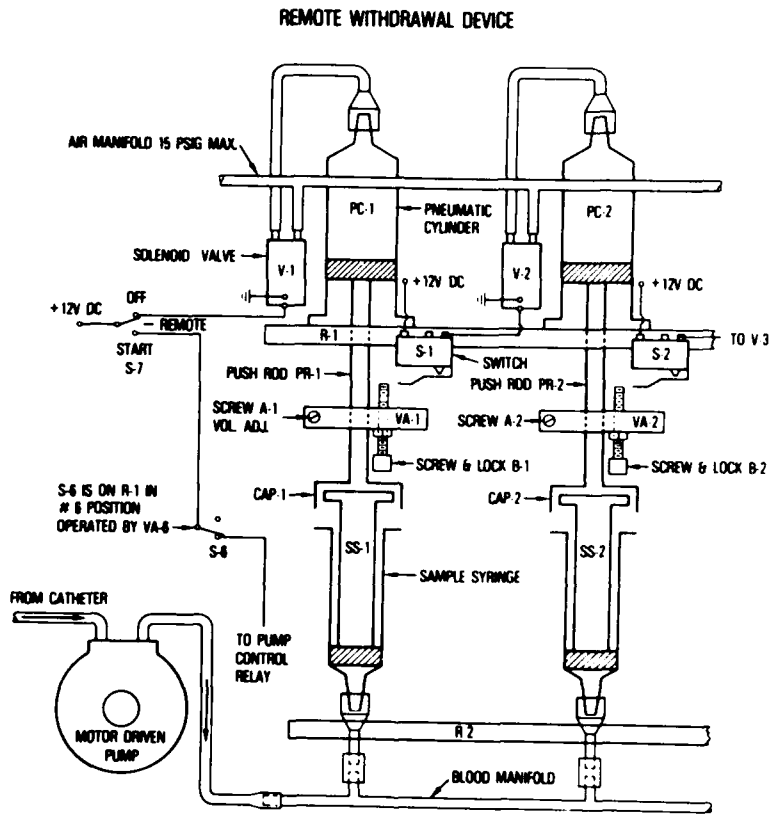


Figure 3. Illustration of the syringe assemblies for the withdrawal system.

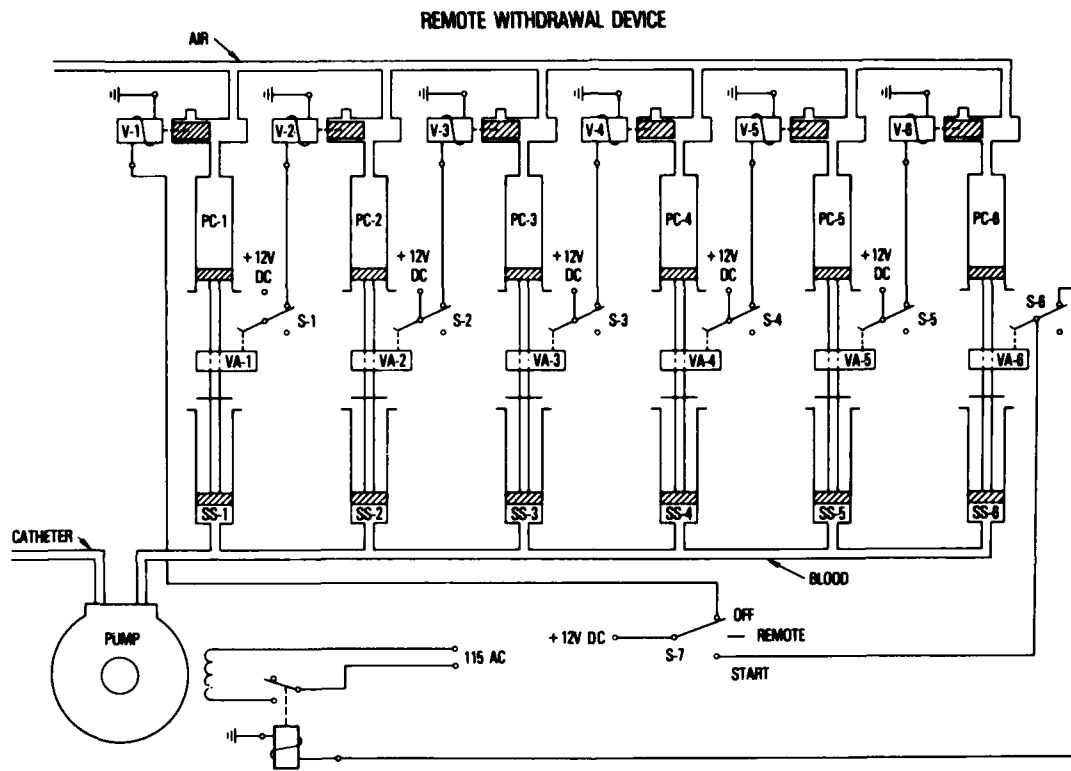


Figure 4. Electrical schema of the withdrawal system.

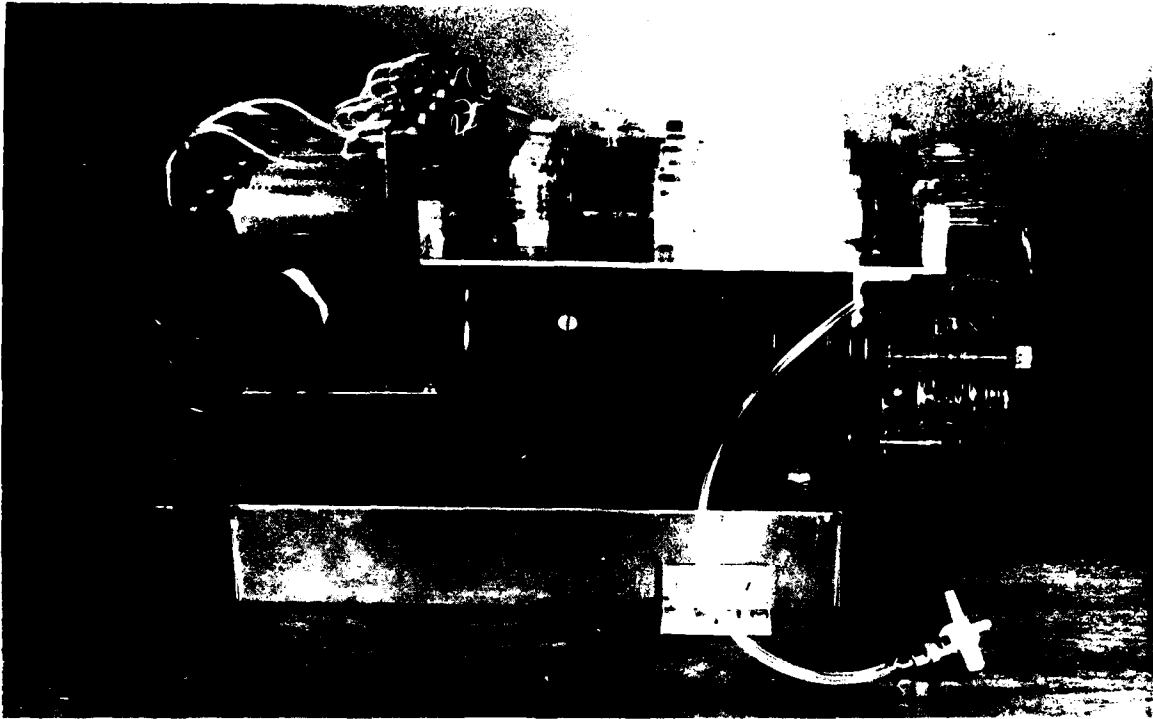


Figure 5. Blood withdrawal system.

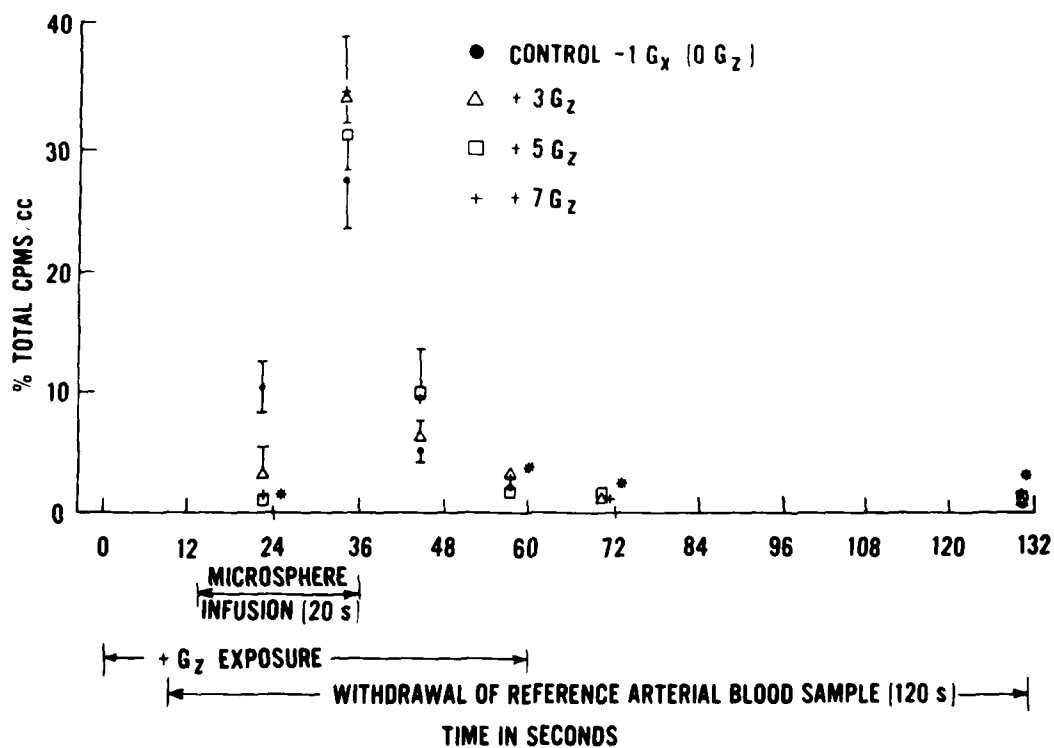


Figure 6. Illustration of the effects of +G_z stress on the rate of washout of radiolabeled microspheres from the cardiac chambers. Means from two animals are presented. \pm SD is shown where possible. *SDs were too small to be shown (9).

TABLE 1. EFFECTS OF $+G_z$ ACCELERATION ON REGIONAL BLOOD FLOW

A. Effects of $+3 G_z$ exposure. N = 6		Cerebellum		Brain Stem	Renal Cortex		Myocardium		Adrenal	
	Left	Right	Cerebrum		Left	Right	LV	RV	Left	Right
Control	29±3	30±3	32±4	15±2	480±52	452±63	124±23	88±22	--	--
During $+3 G_z$	24±4	23±3	27±3	14±2	178±38	*78±41	*274±50	*186±34	--	--
10 MP $+G_z$	25±3	21±3	29±3	13±2	*326±48	*218±33	110±17	78±11	--	--
B. Effects of $+5 G_z$ exposure. N = 7		Cerebellum		Brain Stem	Renal Cortex		Myocardium		Adrenal	
	Left	Right	Cerebrum		Left	Right	LV	RV	Left	Right
Control	35±4	36±5	40±5	18±3	451±68	466±49	92±10	55±6	216±20	248±35
During $+5 G_z$	43±5	43±5	38±5	23±2	*1±1	*1±1	*292±41	*201±30	178±42	217±73
10 MP $+G_z$	26±4	26±4	28±4	14±2	*266±50	*245±36	97±16	67±19	165±25	178±18
C. Effects of $+7 G_z$ exposure. N = 7		Cerebellum		Brain Stem	Renal Cortex		Myocardium		Adrenal	
	Left	Right	Cerebrum		Left	Right	LV	RV	Left	Right
Control	31±3	33±3	39±6	19±3	628±46	642±641	131±10	78±7	176±22	178±34
During $+7 G_z$	26±8	30±12	23±7	21±8	*0	*0	*289±20	190±21	109±33	112±19
10 MP $+G_z$	26±2	27±3	29±3	18±3	*205±32	*216±39	125±19	72±12	224±30	214±80

Means \pm SE's are presented. All values expressed as ml/min/100g tissue. * indicates significantly different from control, with $p < .05$.

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