Influence of Mixing Time on Determination of Red Cell Volume in Normal and Shocked Rats. (24894)

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There is widespread use of radioactive chromium^{51} tagged red blood cells for measurement of red cell volume(1). Application of this technic to the rat has been described (2,3). Reliability of this method in the rat, as evidenced by blood volume determinations with varied mixing times of the injected, tagged cells, has not been established. Further, reliability of the method has not been established in rats in advanced hypovolemic shock. The purpose of this paper is to report red cell volumes, as determined with Cr^{51} rat tagged red cells, with varied mixing times in normal rats and in rats subjected to hypovolemic shock.

Methods. Details of the chromium^{51} erythrocyte tagging and dilution technics employed have been reported(3). Rat red cells were incubated in isotonic $Na_2Cr^{51}O_4$ solution at 4°C overnight. They were then separated, washed 4 times in isotonic saline and resuspended in their previously separated plasma. One milliliter aliquots of the tagged blood were pipetted into lustroloid tubes and counted in the well of a scintillation counter. Approximately .1 ml heparin and 0.4 ml of the counted blood were injected into the femoral vein. The syringe was rinsed once with water and the rinse put into the counted tube from which the injection aliquot was taken and the tube recounted. The difference in counts was taken to represent counts injected. Upon checking the syringe after the single rinse to determine residual counts which might have been lost, it was found that this represented less than 0.5% of the dose and no correction was made for such an insignificant loss. At varying times after Cr^{51} injection, the left common carotid artery was cannulated with polyethylene tubing and the animals were bled through cannulae. This blood was used for microhematocrit determinations and for isotope counting. Arterial hematocrit was used in all calculations and was determined in duplicate in capillary tubing spun in a Phillips-Drucker microcentrifuge. Albino rats of the Holtzman Farms weighing between 190 and 290 g were used. Weight was taken as the morning weight after food had been removed from the cages at about 5:00 p. m. of the previous evening. In a normal series of animals, mixing times of 5, 10, 20 and 30 minutes after isotope injection were allowed and red cell volumes calculated. Eight animals were used in each time period (Exp. 1). In another series of animals bilateral hind limb tourniquets were applied for 4 hours(4). Three hours after release of the tourniquets, when the animals were in profound shock, the tagged cells were injected. Mixing times of 15, 30 and 60 minutes (Exp. 2) and 5 and 10 minutes (Exp. 3) were allowed. Red cell volumes were then calculated. Again, 8 animals were used in each time period.

Results. Exp. 1. When 5, 10, 20 and 30 minute mixing times were allowed in normal animals, the values for red cell volumes were essentially the same for each mixing time period (Table I). The standard deviations were remarkably stable.

Exp. 2. In the tourniquet shocked animals values for red cell volumes were essentially the same for each mixing time period (Table II). When red cell volumes obtained at 30 minutes in the shocked animals were compared to values obtained in the normal animals in Exp. 1 after the same mixing time, no apparent difference was seen. Statistical analysis also showed low probability ($0.10 < p < 0.20$ by Student "t").

Exp. 3. The fact that mean red cell volumes in shocked and normal animals were not different in Exps. 1 and 2 led to the conditions of the third experiment. Here, determinations of red cell volume were made, using short mixing times (5 and 10 minutes) in shocked groups. An untraumatized control group with a mixing time of 5 minutes was included for direct comparison (Table III). It is apparent that the red cell volume obtained in

TABLE I. Red Cell Volume as Measured in Normal Rats after Various Mixing Times.

Wt (g)	No.	Mixing time (min.)	Hematocrit	Red cell vol (ml/100 g)	P
200.0	8	5	47.8 ± 1.6	2.66 ± .17	
208.5	8	10	48.0 ± 2.0	2.58 ± .10	$.20 < p < .30$
216.8	8	20	47.0 ± 1.6	2.62 ± .17	$.50 < p < .70$
199.6	8	30	46.8 ± 1.2	2.69 ± .15	approaches 1

TABLE II. Red Cell Volume as Measured in Hypovolemic Shock Employing Long Mixing Times.

Wt (g)	No.	Mixing time (min.)	Hematocrit	Red cell vol (ml/100 g)	P
206.2	8	15	66.8 ± 2.3	2.60 ± .13	
193.3	7	30	64.4 ± 1.9	2.51 ± .15	$.20 < p < .30$
199.9	7	60	66.7 ± 1.2	2.58 ± .13	$p = .80$

the normal untraumatized group is within the narrow range established in Exp. 1. It is further demonstrated that in the 2 shocked groups determinations of volume show values indistinguishable from each other and from the normal untraumatized animals.

Discussion. While a 20-minute mixing time allows for reproducible red cell volume determinations in the dog(5), such data are not available in the rat. It is apparent from the data presented that red cell volume determinations measured by radiochromium dilution are reliable and reproducible in the rat. In fact, working in groups of 8 animals the mean values reported are reproducible within a little more than 1% variation. From the data reported, an adequate mixing time for the normal rat is 5 minutes from time of tag injection, which is the shortest mixing time employed in the experiments.

It was surprising to find that in rats in hypovolemic shock, characterized as it is by hematocrit elevation, concomitant increase in blood viscosity and lowered arterial pressure, a relatively short mixing time (15 minutes) for radiochromium tagged cells allowed a reliable red cell volume determination. Since there was no *a priori* knowledge of the order of magnitude of red cell loss into the injured extremities following tourniquet trauma, it did not seem reasonable at first to compare red cell volume of these animals with that found in normal animals. It was felt that by using relatively long mixing times under these circumstances, adequate distribution and dilution of the tagged cells would take place and would allow utilization of this technic in shocked animals. As a result, in this experiment, relatively long mixing times (15, 30, and 60 minutes) were allowed and the red cell volumes compared with each other to determine at what time stabilization had occurred.

The finding that absolute red cell volumes in shocked and normal animals were in the same range then suggested simultaneous comparison of the 2 groups with use of shorter mixing times. These again showed values of essentially the same magnitude. It is true that there is some suggestion from the scatter of the 5 and 10 minutes means in the tourniquet treated rats, as well as a probability of 0.02 in the 5 minute mixing time in the tourniquet treated group, that it might be more reliable to use a 15 minute mixing time in deeply shocked rats.

Summary. Determinations of red cell volume using radiochromium 51 tagged red cells

TABLE III. Red Cell Volume as Measured in Hypovolemic Shock Employing Short Mixing Times.

Wt (g)	No.	Mixing time (min.)	Hematocrit	Red cell vol (ml/100 g)	P
245.8	10	5 (controls)	47.4 ± 1.8	2.66 ± .11	
254.5	9	5 (shock)	65.2 ± 3.0	2.51 ± .15	$p = .02$
263.1	9	10 "	66.7 ± 4.8	2.76 ± .17	$.10 < p < .20$

have been made in normal and tourniquet shocked rats using varied mixing times. Red cell volume determinations were significantly reproducible in the normal rat using mixing times of from 5 to 30 minutes. A 15 minute mixing time in the tourniquet shocked rats allowed significantly reproducible determinations of the red cell volume.

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Nephron Reabsorptive Site for Calcium and Magnesium in the Dog.* (24895)

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The technic of stopped flow, introduced by Malvin, Wilde and Sullivan(1), consists in collecting consecutive small volumes of urine discharged from a mannitol-loaded kidney immediately following release of ureteral occlusion of a few minutes' duration. A plot of concentration of any substance in consecutive urine fractions against ordinal number of the fraction, generally shows that the concentration curve passes through a minimum or a maximum according to whether the substance is reabsorbed or secreted by the nephron. Relative locations of maxima and minima of a group of substances in such a plot constitutes a sequence of transport processes which should correspond to the relative order in which these exist anatomically in the nephron. The validity of any sequence established in this way rests upon its agreement with the sequence established by analysis of fluid obtained directly from various segments of the nephron by micropuncture. Moving toward the glomerulus, direct analysis indicates, in the rat at least, that sodium is reabsorbed strongly in first portions of distal tubule(2) while glucose is reabsorbed and dyes secreted in proximal convoluted tubule(3,4) In the stopped flow analysis as described above, the same sequence is observed: concentration minimum for Na (and K), followed by a minimum for glucose and a maximum for p-aminohippurate secretion, the last 2 occurring simultaneously(1). The above substances, whose anatomical site of transport is at least approximately known, can tentatively serve as reference points in stopped flow analyses for other substances which are transported by renal epithelium and which for one reason or another have not been examined by micropuncture. Application of this concept to locate approximate site of transport of calcium and magnesium in the nephron is reported here. The technic was that described by Malvin *et al.* using dogs as experimental animals. The urine reinfusion technic was employed to attain a steady state of renal function and body fluid composition following appropriate priming(5). Methods for measurement of sodium, potassium, p-aminohippurate, inulin, creatinine, calcium and magnesium are those regularly employed in our laboratory(6-9).

Results. Three successful experiments were in complete agreement. Fig. 1 illustrates results of one experiment. Both Ca and Mg are reabsorbed in the same region of the nephron. The area of reabsorption of these cations appears to coincide with the area of K reabsorption and to coincide either with or lie slightly "proximal" to the area of maximal Na reabsorption. On the other hand, the Ca

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