Effects of Hypertonic Glucose and Mannitol on Plasma Volume.* (31211)

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Osmotic diuretics increase renal blood flow (1,2) and expand plasma volume(3,4). The relationship between these two phenomena was tested recently by administering mannitol in graded doses to dogs after one group had been primed with Ringer's solution while the second group was not(5). The animals that had been primed before mannitol was given experienced a greater increase of plasma volume than those which had not received a priming dose of Ringer's solution. There was, however, no significant difference between the renal blood flows of the two groups.

In another study(6) both glucose (25%) and mannitol (25%) increased cardiac output and renal blood flow in control and hypotensive dogs. The effect was greater in hypotensive than control dogs, and mannitol was more effective than glucose. It was concluded that the effects of mannitol and glucose on cardiac output and renal blood flow must result from an increased blood volume, although no direct evidence of an increase in blood volume was presented. The present studies were carried out to test this conclusion.

Methods. Twenty-nine non-fasted mongrel dogs, ranging in weight from 10-16 kg, were used. They were anesthetized with 10 mg/kg of morphine sulfate given subcutaneously, followed in 20-30 minutes by 15 mg/kg pentobarbital sodium intravenously. Cell volume was determined by the Cr^{51}-tagged red cell method and plasma volume by T-1824 dye as described elsewhere(7). Injections were made through a femoral vein and blood samples were taken from the right atrium through an indwelling cardiac catheter. A hematocrit (Wintrobe) was performed on all blood samples and corrected for trapped plasma by a factor of .95. Plasma protein concentration was determined by the falling drop method (8). In the bled dogs, mean carotid arterial pressure was measured with a mercury manometer. The following protocol was followed:

Control dogs. After completion of the initial determinations described above, 6 dogs were given an infusion of 1 g/kg of mannitol (25% solution) in 10 minutes, and 6 other dogs were given 1 g/kg of glucose (25% solution) in 10 minutes. Thirty minutes were allowed to elapse and the measurements were repeated.

Hemorrhage to 50 mm Hg. After the initial determinations, 17 dogs were bled from a femoral artery into a calibrated bottle system until their mean carotid arterial pressure reached the level of 50 mm Hg. This level of pressure was maintained for 30 minutes by raising or lowering the bleeding bottle as required. The tubing leading from the artery to the bleeding reservoir was then clamped, and in 6 dogs 1 g/kg of mannitol was infused (25% solution in 10 minutes) while in 5 other dogs 1 g/kg of glucose (25% solution) was given within 10 minutes. Thirty minutes later the determinations were repeated. The remaining 6 dogs were used for hemorrhage-controls. In this group the experimental procedure was the same except neither glucose nor mannitol was given.

Results. The injection of either hypertonic mannitol or glucose, 1 g/kg in control dogs, had no significant influence on any of the parameters measured (Table IA, B). In the group of dogs bled to 50 mm Hg mean arterial pressure, but not given hypertonic mannitol or glucose, the venous hematocrit increased significantly although the measured cell volume remained the same as expected. The increased venous hematocrit presumably resulted from release of previously tagged cells from the spleen as a consequence of the hemorrhage. Although the venous hematocrit increased, 4.6 ml/kg of fluid containing a small amount of protein entered the circulation (Table II). BVR_{cells} decreased from .94 to .83, primarily because of the in-

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TABLE I. Effects of Mannitol (1 g/kg) and Glucose (1 g/kg) on the Blood Volume.

		Venous hematocrit, %	Cell vol, ml/kg	Plasma vol, ml/kg	Circulatory hematocrit, %	BVR_{cells}	Plasma proteins	
							Concentration, g/100 ml	Total, g/kg
A. Mannitol*	Control	39.5 ± 2.49	28.9 ± 2.55	53.8 ± 2.11	34.8 ± 2.16	.88 ± .01	5.07 ± .17	2.72 ± .08
	After mannitol: Measured	39.9 ± 2.51	29.9 ± 2.75	52.1 ± 2.59	36.4 ± 2.61	.91 ± .01	5.19 ± .19	2.70 ± .08
B. Glucose†	Control	45.8 ± 2.19	33.6 ± 1.36	48.6 ± 4.20	41.3 ± 2.26	.89 ± .02	5.76 ± .34	2.80 ± .22
	After glucose: Measured	45.6 ± 2.48	35.6 ± 1.83	48.0 ± 3.86	42.5 ± 2.36	.93 ± .02	5.83 ± .30	2.80 ± .15

* Values are means ± S.E. for 6 dogs with a mean weight of 11.5 ± .62 kg.
† Values are means ± S.E. for 6 dogs with a mean weight of 12.3 ± .66 kg.

TABLE II. Effects of Hemorrhage to 50 mm Hg Mean Arterial Pressure on Blood Volume.

	Venous hematocrit, %	Cell vol, ml/kg	Plasma vol, ml/kg	Circulatory hematocrit, %	BVR_{cells}	Plasma proteins		Mean arterial pressure, mm Hg
						Concentration, g/100 ml	Total, g/kg	
Control	45.3 ± 1.58*	36.3 ± 3.43	47.6 ± 2.30	42.8 ± 2.26	.94 ± .03	5.16 ± .16	2.46 ± .18	93 ± 2.8
Hemorrhage to 50 mm Hg†	44.4 ± 1.84	16.7 ± 2.09	20.6 ± 1.80			5.19 ± .18	1.07 ± .05	
After hemorrhage: Expected		19.3 ± 1.40	26.1 ± 2.05	42.6 ± 2.95		5.32 ± .21	1.39 ± .11	
Measured	48.0 ± 1.59	20.5 ± 1.47	30.7 ± .79	39.8 ± 2.19	.83 ± .02	4.73 ± .16	1.43 ± .08	61 ± 4.7

* Values are means ± S.E. for 6 dogs with a mean weight of 12.6 ± .61 kg.

† Samples withdrawn included.

TABLE III. Effects of Glucose (1 g/kg) After Hemorrhage to 50 mm Hg Mean Arterial Pressure.

	Venous hematocrit, %	Cell vol, ml/kg	Plasma vol, ml/kg	Circulatory hematocrit, %	BVR_{cells}	Plasma proteins: Concentration, g/100 ml	Plasma proteins: Total, g/kg	Mean arterial pressure, mm Hg
Control	47.4 ± 1.95*	36.0 ± 1.61	50.3 ± 2.81	41.7 ± 1.52	.88 ± .02	5.24 ± .21	2.63 ± .23	105 ± 2.3
Hemorrhage to 50 mm Hg†	47.6 ± 1.86	19.3 ± 1.33	21.2 ± 1.41			5.11 ± .14	1.08 ± .08	
After hemorrhage and glucose:								
Expected		16.7 ± 1.04	29.1 ± 2.07	36.5 ± 1.50		5.33 ± .29	1.55 ± .19	
Measured	39.2 ± 3.06	16.5 ± 1.47	36.2 ± 2.07	31.3 ± 3.25	.80 ± .02	4.25 ± .23	1.54 ± .19	53 ± 8.1

* Values are means ± S.E. for 5 dogs with a mean weight of 12.1 ± .87 kg.

† Samples withdrawn included.

TABLE IV. Effects of Mannitol (1 g/kg) After Hemorrhage to 50 mm Hg Mean Arterial Pressure.

	Venous hematocrit, %	Cell vol, ml/kg	Plasma vol, ml/kg	Circulatory hematocrit, %	BVR_{cells}	Plasma proteins: Concentration, g/100 ml	Plasma proteins: Total, g/kg	Mean arterial pressure, mm Hg
Control	45.5 ± 1.21*	36.1 ± 1.68	47.5 ± 1.92	43.1 ± 1.34	.95 ± .01	5.08 ± .21	2.41 ± .12	92 ± 4.9
Hemorrhage to 50 mm Hg†	41.8 ± 2.09	16.6 ± .71	23.6 ± 2.41			4.96 ± .15	1.18 ± .14	
After hemorrhage and mannitol:								
Expected		19.5 ± 1.15	23.9 ± 2.05	45.1 ± 3.06		5.15 ± .34	1.23 ± .05	
Measured	40.4 ± 1.72	19.4 ± 1.27	36.1 ± 1.09	34.7 ± 1.77	.86 ± .03	4.26 ± .12	1.54 ± .02	58 ± 7.0

* Values are means ± S.E. for 6 dogs with a mean weight of 12.4 ± .50 kg.

† Samples withdrawn included.

creased venous hematocrit and decreased circulatory hematocrit.

Hypertonic glucose administered after the hemorrhage to 50 mm Hg resulted in a 7.1 ml/kg increase in plasma volume. The fluid entering the circulation was protein-free. The addition of this fluid decreased both hematocrits, but the decrease in the circulatory hematocrit was greater than that of the venous hematocrit; consequently, the BVR_{cells} fell from .88 to .80 (Table III).

Mannitol, when administered after hemorrhage, produced the same general effects as glucose, but the amount of fluid entering the vascular system was significantly more, amounting to 12.2 ml/kg. The fluid was accompanied by 0.31 g/kg of protein (Table IV). Thus, the protein concentration of the fluid entering was 2.5 g/100 ml.

For the 3 groups of dogs that were bled to 50 mm Hg mean arterial pressure, the mean bleeding volume was 42-44% of their control blood volume.

Discussion. In this study no evidence has been found to indicate that hypertonic glucose or mannitol injected into control dogs in the amout of 1 g/kg results in an increase in plasma volume, although earlier Detmer *et al* (5), using a lesser amount of mannitol, had reported a small increase in plasma volume. The increase was not so great, however, for control dogs as for those receiving a priming dose of Ringer's solution.

When mannitol was given to dogs that have been bled to 50 mm Hg mean arterial pressure, the plasma volume was 12.2 ml/kg more than expected after the hemorrhage. The total increase in plasma volume cannot be ascribed to mannitol, however, because in another group of dogs, with hemmorhage alone, 4.6 ml/kg of fluid entered the circulation (Table II). If it is considered that the volume of the infusion was 4 ml/kg and all this stayed in the circulation, then only 3.6 ml/kg of fluid was recruited by mannitol from extravascular sources.

The data for glucose, compared to mannitol after hemorrhage, are equivocal. The total amount of fluid mobilized with glucose was 7.1 ml/kg, only 2.5 ml/kg more than after hemorrhage alone. Further, in these animals the volume of infusion was also 4 ml/kg. Thus, it seems only 2.5 ml/kg of this remained in the circulation after hemorrhage and no fluid was recruited by glucose from extravascular spaces. Camishion and Fishman(6) found that glucose and particularly mannitol significantly increased cardiac output and renal blood flow in hypotensive dogs, and they suggested plasma volume expansion as the mechanism. The data presented here confirm their conclusion about an increased plasma volume, although the role of glucose as a plasma volume expander is questionable. It is possible that most of the fluid mobilization after glucose was due to the compensation after hemorrhage alone. Fluid mobilization of similar magnitude has been reported previously after a hemorrhage to 50 mm Hg mean arterial pressure(9). The lesser effect of glucose compared to mannitol in causing an expansion in plasma volume probably can be accounted for by its more rapid metabolism and excretion.

In all 3 groups of bled animals the plasma protein concentration was significantly reduced from that expected after hemorrhage. When the total circulating amount of protein was calculated, it was found that in the control hemorrhage and in the hemorrhage-glucose groups the expected and measured amount were in good agreement, indicating that the fluid mobilized in either of these groups of dogs did not contain any protein. Mannitol, however, increased the total amount of circulating protein after hemorrhage by 0.31 g/kg, and the protein concentration of the fluid mobilized with mannitol was 2.54 g/100 ml.

Summary. The influence of hypertonic glucose and mannitol on blood volume was tested in normovolemic-normotensive and hypovolemic-hypotensive dogs. Neither glucose nor mannitol had any effect on the blood volume of normovolemic dogs. In hypovolemic-hypotensive dogs, mannitol produced a significant increase in the plasma volume by mobilizing fluid from the extravascular spaces. The fluid entering the circulation was accompanied by a significant amount of protein. Also, plasma volume increased after hemorrhage with glucose, but the increase was small and was not

accompanied by protein. Thus, both glucose and mannitol, but particularly the latter, increased the blood volume in hypovolemia.

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Effect of Acetylsalicylic Acid on Lysosomes.* (31212)

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Since the description of lysosomes over 10 years ago(1), their occurrence in various tissues and important role in cellular metabolism have been the subject of much investigation(2,3). A number of agents, particularly corticosteroids(4) and chloroquine(5) have been found to stabilize lysosomes *in vitro* and this action has been proposed as an explanation of certain of their therapeutic effects in various clinical diseases.

Duthie(6) has hypothesized that salicylates might stabilize lysosomes. This study was carried out to investigate the influence of acetylsalicylic acid (ASA) on rat liver lysosomes.

Materials and methods. Adult white rats (Wistar strain, Charles River Laboratories) were sacrificed by a blow to the base of the skull. The liver was dissected free, weighed, and placed in ice cold 0.25 M sucrose. In most experiments, 2 livers were used. The livers were homogenized in a Rosett grinder chilled with ice(7). All centrifugations were done in a Servall RC-2 centrifuge with a SS-34 rotor at 5°. The liver homogenate was centrifuged at 2880 rpm for 10 minutes (10,000 *g* minutes) to remove unruptured cells and nuclear debris. The residue was discarded and the supernate was centrifuged at 14,400 rpm for 10 minutes (250,000 *g* minutes) to obtain a lysosomal residue. The supernate was discarded, the residue resuspended in cold 0.25 M sucrose and centrifuged again at 14,400 rpm for 10 minutes (250,000 *g* minutes). Acetylsalicylic acid (USP-Mallinckrodt) was dissolved in water in a 10^{-2} M concentration and in 95% ethanol in a 10^{-1} M concentration and serially diluted with water and 95% ethanol, respectively, to 10^{-4} M. ASA was prepared fresh prior to each experiment to minimize hydrolysis. Hydrocortisone sodium succinate (Solu-cortef, Upjohn) and chloroquine phosphate (Winthrop) were prepared in aqueous solutions similarly. The lysosomal residue was resuspended in 0.25 M sucrose and 0.9 ml of the lysosomal suspension was added to 0.1 ml of water or 0.1 ml of 95% ethanol as a control (final ethanol concentration 9.5%). 0.1 ml of various concentrations of ASA, chloroquine, and hydrocortisone in water and ASA in ethanol were added to 0.9 ml aliquots of the lysosomal suspension. After mixing, the lysosomal suspensions were incubated in a waterbath at 37° for 2 hours to labilize the lysosomes(8). Following incubation, the lysosomal suspensions were centrifuged at 14,400 rpm for 10 minutes (250,000 *g* minutes) to remove mitochondria, unruptured lysosomes,

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