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Arthur Grollman, … , Alvin P. Shapiro, Gordon Gafford

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Research Article

THE VOLUME OF THE EXTRACELLULAR FLUID IN EXPERIMENTAL AND HUMAN HYPERTENSION [1]

By ARTHUR GROLLMAN AND ALVIN P. SHAPIRO WITH THE TECHNICAL ASSISTANCE OF GORDON GAFFORD

(From the Department of Experimental Medicine, the Southwestern Medical School of The University of Texas and Parkland Hospital, Dallas, Texas)

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Several lines of investigation have indicated the existence of abnormalities in salt and water metabolism in hypertensive cardiovascular disease. It has been shown, for example, that the electrolyte and water contents of the tissues in the hypertensive animal deviate from that in the normal (1) and that there is an increased rate of excretion of antidiuretic substance in the urine in experimental and human hypertension (2). The effect of a sodium restricted diet on the blood pressure level (3) and the difference in the rate of urinary excretion of salt and water in the hypertensive subject as compared to the normal (4) also point to some fundamental deviation from normal in the salt and water metabolism of the hypertensive subject. Because of the importance of this problem to a better understanding of the basic mechanisms underlying hypertensive cardiovascular disease, the present study was undertaken to compare the extracellular fluid volume in experimental and human hypertension with that of the normal.

MATERIALS AND METHODS

The extracellular fluid volume was determined in a series of dogs rendered hypertensive by the application of a figure-of-eight ligature to the right kidney and ablation of the left organ (5). At least six months had intervened between the operation and the time of the experiments to permit the establishment of a stabilized blood pressure. Determinations were made simultaneously on a group of normotensive animals. Because of the uncertainty and difficulties attendant upon the determination of the extracellular volume, both mannitol and radioactive sulfate were utilized for this purpose. Although the use of inulin is considered to be the method of choice in man, erratic results are obtained when this substance is applied to the dog because of the large and variable blanks obtained in analysis of the blood under apparently constant conditions. The use of a constant infusion of mannitol (6) gave consistent and reproducible results. The radiosulfate method only was used in the experiments on the human.

In the mannitol procedure as carried out on the unanesthetized dog, a priming dose of 0.2 gram of mannitol per kilogram of body weight was injected intravenously followed after 20 minutes by a constant infusion at a rate of 0.2 gram of mannitol per kilogram of body weight per hour for 40 minutes. Samples of blood were drawn at the beginning of the infusion, and at 20 minute intervals thereafter until one hour after its cessation. The mannitol was analyzed by the procedure of Corcoran and Page (7) with minor modifications. The animals were fasted for 12 to 14 hours prior to the determination but were allowed access to water at all times. They were trained to lie quietly on the animal board during the course of the procedure with only slight restraint.

For the determination of extracellular volume by radiosulfate,[2] carrier-free S^{35} in the form of H_2SO_4 was used in doses of 100 microcuries as described elsewhere (8). The radioactivity of the blood serum or urine was determined directly with a flow counter. In the dog, the bladder was rinsed with distilled water by means of a catheter inserted at the beginning of the experiment and the excreted radiosulfate determined; in the experiments on the human, a constant correction of 4 per cent was used for the urinary loss during the 18 minutes required for equilibration. Corrections for serum water and the Donnan factor were made as previously described (8).

The studies on the human subject included 12 normotensive individuals and 14 hypertensive patients attending the Hypertensive Clinic of Parkland Hospital. The hypertensive group exhibited minimal or no evidence of renal or cardiac impairment and no apparent evidence of fluid retention. The normotensive subjects included ambulatory psychiatric patients and individuals from the laboratory personnel. The determinations were made in pairs, a hypertensive and a normotensive individual of the same sex and of the same approximate height and weight acting as subjects on the same day.

RESULTS

The pertinent data are summarized in Table I, which includes the data on the normotensive dog, Table II, on the hypertensive dog, and Table III, on the normotensive and hypertensive human

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[2] Supplied by the National Laboratory, Oak Ridge, Tennessee, on allocation from the U. S. Atomic Energy Commission.

TABLE I

The extracellular volume of the normotensive dog as determined by mannitol and radiosulfate

Dog No.	Body weight	Extracellular volume as determined by			
		Radiosulfate		Mannitol	
	Kg.	*per cent body wt.*	*liters per M.² body surface*	*per cent body wt.*	*liters per M.² body surface*
1	14.0			21.1	4.5
2	11.5			23.6	4.7
3	10.4			24.9	4.9
	10.9			21.1	4.2
	10.8			23.8	4.7
4	12.8	17.3	3.6	23.7	5.0
5	10.3			18.9	3.7
6	14.3			22.7	4.9
7	11.3	19.6	4.0	21.9	4.4
	9.9	18.9	3.6		
8	11.0	18.9	3.8		
9	11.8	21.0	4.3		
10	5.9	23.3	3.7		
	6.7	19.4	3.1		
11	13.6	15.9	3.4		
12	10.4	16.9	3.3		
13	16.2	17.5	3.9		
14	16.2			21.9	4.9
Mean		18.5	3.7	22.1	4.6
Standard deviation		±1.8	±0.3	±1.5	±0.4
Standard error		±0.8	±0.1	±0.7	±0.2

TABLE II

The extracellular volume of the hypertensive dog as determined by mannitol and radiosulfate

Dog No.	Body weight	Extracellular fluid volume as determined by			
		Radiosulfate		Mannitol	
	Kg.	*per cent body wt.*	*liters per M.² body surface*	*per cent body wt.*	*liters per M.² body surface*
1	11.3			29.1	5.9
	10.9			33.0	6.5
2	17.8	23.7	5.5		
	18.9	22.7	5.4		
	17.6	23.2	5.4		
3	10.3	19.8	3.8	27.1	5.5
	9.3	24.2	4.6		
	9.2	24.1	4.5		
4	12.1			30.6	6.3
	12.2	20.7	4.3		
5	9.1	20.8	3.9		
6	12.2	24.9	5.2		
	12.8	19.5	4.1	24.6	5.2
7	12.8			30.9	6.5
8	15.2	23.4	5.2	29.2	6.4
	15.1	22.0	5.0	29.4	6.5
9	12.9			31.6	6.1
	10.7	21.5	4.2	31.5	6.2
10	11.1	19.2	3.8	25.6	5.0
	9.8	22.7	4.4	31.3	6.0
11	19.6	25.2	6.0		
	19.2	19.2	4.6		
	18.8	20.0	4.8		
12	14.8	23.3	5.1		
Mean		22.0	4.6	29.2	6.0
Standard deviation		±0.9	±0.5	±2.2	±0.5
Standard error		±0.3	±0.2	±1.0	±0.2

TABLE III

Radiosulfate space in normotensive and hypertensive human subjects

Patient	Age/Sex	Blood pressure	Body weight	Height	Extracellular fluid volume		
		mm. Hg	*Kg.*	*cm.*	*liters*	*cc./Kg.*	*liters/M.²*
Normotensive group							
E. V.*	42/F	120/80	69.8	163	14.6	20.9	8.3
J. P.	62/M	128/78	68.7	156	9.7	14.1	5.3
A. P.	47/F	124/80	65.9	161	7.6	11.5	4.5
I. D.	28/F	110/70	66.2	168	8.1	12.2	4.6
L. S.	44/M	130/92	97.6	172	11.2	11.5	5.3
L. B.	44/F	130/94	59.4	166	9.3	15.6	5.6
S. C.	28/F	130/64	62.2	166	7.9	12.7	4.7
G. H.	46/F	124/84	55.8	161	7.6	13.6	4.8
J. J.	33/F	118/80	60.7	171	8.1	13.3	4.8
A. S.	31/M	120/80	77.4	177	9.7	12.5	5.0
L. M.	30/M	120/80	88.4	184	11.3	12.8	5.4
G. M.	32/M	110/64	83.9	187	11.4	13.6	5.5
Mean	38.6	122/79	71.5	169		13.0	5.0
Standard deviation						±1.1	±0.4
Standard error						±0.4	±0.1
Hypertensive group							
M. S.	54/F	220/130	83.0	158	11.6	14.0	6.3
D. R.	49/M	180/130	80.3	176	10.7	13.3	5.5
M. T.	39/F	180/120	77.8	166	10.0	12.9	5.6
E. L.	21/F	180/120	83.5	168	14.2	17.0	7.4
J. B.	42/M	192/156	81.7	184	10.6	13.0	5.2
M. E.	34/F	168/116	56.0	153	10.0	17.9	6.6
E. B.	44/F	220/130	81.0	161	12.0	14.8	6.3
R. S.	40/F	200/130	42.2	158	8.6	20.4	6.2
O. H.	56/F	230/120	68.1	164	10.5	15.4	6.0
O. C.	53/M	200/140	78.0	167	14.3	18.3	7.6
G. W.	39/M	220/160	72.7	173	10.8	14.9	5.8
L. M.	33/M	190/140	62.8	178	10.5	16.7	5.9
C. M.	52/F	165/118	65.3	152	12.1	18.5	7.5
R. M.	41/M	210/150	45.8	163	9.3	20.3	6.5
Mean	42.6	197/133	69.9	166		16.2	6.3
Standard deviation						±2.5	+0.8
Standard error						±0.7	±0.2

* Omitted from calculation of mean and standard deviation.

subjects. In calculating the means and statistical data of Tables I and II, the average values have been used where more than one determination was made on the same animal. The separate data are included in the table in order to indicate the consistency of results obtained at intervals of at least a month which intervened between the individual determinations. In Table III, the data on patient E. V. have been omitted from the calculated mean, since the observed extracellular fluid volume in this patient deviates from the mean by more than six times the standard deviation of the remainder of the group. Some technical error presumably vitiated the result obtained on this patient; its omission from the series is statistically valid. However, even if this result be included, the observed differences still remain statistically significant ($p < 0.02$).

There was no correlation evident between the extracellular fluid volume and the level of the blood pressure. However, this is not surprising since the variation in the hypertensive group of dogs was only between 150 and 180 (mean pressure) and the observed levels in the human need not reflect accurately the severity of the disease.

The blood volume which was determined in the dogs simultaneously with the extracellular fluid volume was found to average 1.64 liters per square meter of body surface in the normotensive group and 1.69 liters in the hypertensives. This difference is of questionable significance, but it is possible that the vascular space is also increased in

the hypertensive proportionately to the observed increase in the extracellular fluid volume.

It is evident from the results of Tables I and II that the extracellular fluid volume of the hypertensive dog as measured either by mannitol or by radiosulfate is greater than in the normal. Essentially the same difference is observed in Table III in the human subject with radiosulfate. The observed differences are all statistically significant as shown by the statistical data appended to the tables.

The observed discrepancy between the extracellular fluid volume as determined by the use of mannitol and by radiosulfate in the dog may be attributed to expansion of the extracellular volume by the osmotic effect of mannitol. It is also evident in Table III that the radiosulfate space as determined in man is less than that obtained by the use of other agents (mannitol, inulin, thiocyanate, sucrose). Because of this discrepancy, which has been discussed elsewhere (8), all determinations were made on paired normotensive and hypertensive subjects of similar body build.

The observed differences, expressed as liters per square meter of body surface, of the radiosulfate spaces of the hypertensive and normotensive dogs are 0.9 liter and of the mannitol spaces, 1.4 liters. The standard errors of these differences are $\pm$ 0.2 and $\pm$0.3, respectively. The probability, calculated from the "t" test, that these observed differences occurred by chance is less than one in 1000; "t" 4.50 and 4.78, respectively; $p < 0.001$. The observed difference of 1.3 liters per square meter of body surface for the human subjects is also highly significant, "t" 4.33, $p < 0.001$.

DISCUSSION

The significance of our observed increase in extracellular volume in hypertension must at present remain a matter of conjecture. Serum sodium has been claimed to be slightly higher in essential hypertension as compared to the normal (9). One might conclude, accordingly, that the observed increased extracellular volume in hypertension reflects an abnormal retention of salt and an attempt to maintain osmotic homeostasis. It is tempting to proceed further and assume that this retention of salt with expansion of the extracellular fluid volume reflects an abnormal steroidal activity since desoxycorticosterone also causes a similar change with an elevation in blood pressure when administered with excessive sodium chloride (4). However, the available evidence (10) opposes this simple theory. The observed increase in antidiuretic hormone activity (2) is more logically to be considered as a compensatory phenomenon which maintains a normal tonicity at the expense of an expansion in the extracellular fluid volume (11).

Braun-Menéndez and Covián (12) have also noted an increase in extracellular fluid volume in nephrectomized rats following bilateral nephrectomy, accompanied by an elevation in blood pressure. However, the fact that their animals were permitted to ingest salts and water renders their observed increase in extracellular fluid volume inevitable (13). The rapidity with which the rise in blood pressure occurred renders it questionable also if this represents true hypertensive disease. The fact that the animals increased enormously in weight (15 grams) and were obviously edematous is evidence of their highly abnormal state to which any rise in blood pressure may be attributed. To designate this rise in pressure as hypertensive disease is unjustifiable; it is more likely analogous to the transient rise in blood pressure observed often in congestive heart failure. Moderate increases in extracellular volume induced by the intravenous injection of isotonic solutions fail to alter the blood pressure in the nephrectomized dog (14).

An increase of 7 per cent in the blood volume and 12.5 per cent in the extracellular volume (thiocyanate space) has also been demonstrated in the hypertensive rat by Braun-Menéndez and Martínez (15) and in the extracellular volume in the muscle of the hypertensive dog by Eichelberger (16). The presently reported observations indicate that this increase is a general property of the hypertensive state whether occurring spontaneously, as in man, or induced experimentally by various procedures in the rat or dog.

The conclusion that the extracellular fluid volume is increased in hypertension assumes that the mannitol and radioactive spaces represent this body compartment both in the normal as well as in hypertension. Alterations in cell permeability of tissues normally impermeable to mannitol or radiosulfate would result in apparently higher values,

but there is no evidence to indicate that such changes occur in hypertension.

It has been demonstrated that the blood-free tissues of the hypertensive rat contain more sodium and less potassium than the normal (1). It is evident from the present findings that this may be in part or entirely due to alterations in extracellular volume rather than to an assumed alteration in the intracellular content of these cations.

SUMMARY

The extracellular fluid volumes of a series of normotensive and hypertensive dogs and patients suffering from "essential" hypertension were determined by the use of mannitol and radiosulfate. The results indicate an appreciable expansion of the extracellular space in hypertension as compared to the normal, the significance of which, in the mechanism of hypertension, is discussed.

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