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Fluid Volumes in One-Kidney 30-Day Renal Artery Stenosis Rabbits (41278)

KENNETH D. KURZ,[1] J. ALAN JOHNSON,[2] SHUICHI ICHIKAWA,[3] WAYNE L. FOWLER, JR., AND CHARLES G. PAYNE

Harry S. Truman Memorial Veterans Administration Hospital and Department of Physiology, University of Missouri School of Medicine, Columbia, Missouri 65201

Abstract. Body fluid volumes were measured in 14 one-kidney control rabbits and in 12 one-kidney rabbits with renal artery stenosis of 30 days duration (one-kidney, one-clip). Plasma volume (*PV*), extracellular fluid volume (*ECFV*), and total body water (*TBW*) were determined by the distribution volumes of radioiodinated serum albumin, $^{35}SO_4$, and ^{3}HOH, respectively. Mean arterial pressure was significantly ($P < 0.01$) elevated in the rabbits with renal artery stenosis; plasma renin activity was the same in the two groups. *PV* was the same in the control and renal artery stenosis groups, but the *ECFV* and *TBW* were significantly ($P < 0.05$) elevated in the rabbits with renal artery stenosis. The results of this study are in keeping with the hypothesis that volume expansion may play a role in the development and maintenance of chronic renal hypertension, but do not rule out the possibility that the hypertension may result from mechanisms not related to increases in fluid volumes and that the expansion of these volumes may occur simply because of decreased ability of the sole artery-stenosed kidney to excrete sodium and water.

Clinical studies of fluid volumes in hypertensive patients have not produced consistent results (1–9), possibly because many of the studies failed to match adequately the control subjects with their experimental subjects as to age, race, sex, and body size, and because the type of hypertension in the experimental group was not defined adequately. Also, most studies have measured only one or two of the fluid compartments, which does not allow the examination for possible subtle shifts of fluids from one compartment to another in hypertension. The present study examined the plasma volume (*PV*), extracellular fluid volume (*ECFV*), and total body water (*TBW*) in a group of rabbits with experimentally induced renal hypertension and in a matched control group of rabbits.

Methods. Male New Zealand white rabbits were used in these studies. The rabbits were housed one per cage and were kept at a constant environmental temperature of 26°. Room lights were controlled by an automatic switch which allowed illumination from 7:00 AM to 7:00 PM daily. The rabbits were allowed free access to a commercial rabbit diet (Purina Lab Rabbit Chow HF5326) containing 0.165 meq of Na^+ and 0.457 meq of K^+ per gram of feed. Water was available *ad libitum*.

All experiments were performed on conscious rabbits. In each experiment the rabbits were placed in a narrow rectangular box which served to limit their movements. The rabbits were not restrained in any way except by the size of the box. The rabbits appeared calm and undisturbed when in the box. Simultaneous measurements of *PV*, *ECFV*, and *TBW* were obtained by the distribution volumes of ^{125}I-radioiodinated serum albumin (RISA), $^{35}SO_4$, and ^{3}HOH, respectively, as described by Bauer *et al.* (10). All experiments were performed at the same time of day to avoid any possible errors due to circadian variations. Two series of experiments were performed.

Series 1: Replicate volume determinations in normal rabbits. The purpose of these experiments was to determine the reproducibility of the volume measurements in

[1] Present address: Lilly Research Laboratories, Division of Eli Lilly & Co., Indianapolis, Ind. 46206.

[2] Address reprint requests to: Dr. J. Alan Johnson, Research Service (151), Harry S. Truman Memorial V.A. Hospital, Columbia, Mo. 65201.

[3] Present address: 2nd Department of Internal Medicine, School of Medicine, Gunma University, Maebashi, 371 Japan.

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rabbits by these techniques. To evaluate this, volume measurements were obtained in six normal rabbits, and the measurements were repeated 2 days later.

One hour before the initial volume determinations, catheters of polyethylene tubing (PE 50) were inserted into a marginal ear vein and into a central ear artery under local anesthetization with 2% lidocaine. A three-way stopcock was attached to the arterial catheter, and an iv catheter plug with a rubber diaphragm was attached to the venous catheter. At time zero, 1.00 ml each of RISA (Mallinckrodt Nuclear, approximately 1 μCi, in a citric acid, sodium citrate, dextrose solution), $^{35}SO_4$ (New England Nuclear, approximately 5 μCi, in water), and ^{3}HOH (New England Nuclear, about 5 μCi in water) were injected iv through the catheter plug and were flushed through with 3 ml of saline. The volume of each isotope injected was measured by drawing it up, with the aid of a magnifying glass, into a graduated 1-ml tuberculin syringe. The same volume of each isotope was added to individual volumetric flasks (100 ml for RISA, 1000 ml for $^{35}SO_4$ and ^{3}HOH), and the flasks were diluted to volume with distilled water; these served as the standards. Arterial blood samples of 3 ml each were obtained through the arterial catheter at 60, 90, 120, and 150 min after injection of the isotopes. Prior to the collection of each blood sample a tare volume of 1 ml was withdrawn through the arterial catheter and was discarded. Immediately after the collection of each blood sample, 4 ml of unlabeled blood from a donor rabbit, collected by cardiac puncture, was injected iv to replace the blood removed for the tare and the sample. The blood samples were placed in heparinized tubes and were centrifuged to obtain plasma samples. The processing of these samples and the volume calculations are described later. At the completion of these experiments the catheters were removed, and each rabbit was returned to its cage where it received free access to food and water.

For the second volume determination 2 days later, polyethylene catheters were placed in the marginal ear vein and central ear artery of the opposite ear. Volume measurements were obtained in the same manner as the initial determinations, except that a 3-ml arterial blood sample was obtained from each rabbit prior to injection of the isotopes, in order to determine the residual concentrations of these isotopes in the plasma; any residual background activity was subtracted from the activity of each sample obtained following administration of the isotopes.

Series 2: Volume measurements in control and renal artery stenosis rabbits. The purpose of this study was to examine for possible alterations in the volumes of body fluid compartments, or shifts in fluids between these compartments, in rabbits with hypertension produced by renal artery stenosis. Sixteen rabbits, averaging 2.7 kg in body weight, were subjected to renal artery stenosis by the method of Brooks and Muirhead (11). Each rabbit was anesthetized with halothane plus nitrous oxide (12), and the abdomen was opened by a midline incision, with sterile surgical procedures. A small silver clip with a gap size of 0.6 mm was placed around the left renal artery to produce a stenosis; the right kidney was removed. The incision was closed, and each rabbit was returned to its cage. An additional 14 weight-matched rabbits received only a unilateral nephrectomy; these rabbits served as controls. Thirty days later, an acute experiment was performed on each rabbit.

At the time of the acute experiment, polyvinyl catheters (Fr 5 infant feeding tubes) were placed in the femoral artery and vein under anesthesia with halothane and nitrous oxide (12). The rabbits then were allowed several hours to recover. In conscious rabbits, an arterial blood sample of 2 ml was collected for determinations of hematocrit, plasma renin activity (*PRA*), and serum urea nitrogen. The samples were placed in chilled tubes containing ethylenediaminetetraacetate (EDTA) and were cooled by placing them immediately in a container of ice. After removal of the aliquot for hematocrit determination, the sample was spun in a refrigerated centrifuge, and the plasma was removed and stored at $-14°$ until later when it was processed and assayed for PRA. Arterial blood

pressure was recorded through the femoral arterial catheter by a pressure transducer (Statham P23Db) and an oscillographic recorder. Mean arterial pressure was recorded for 30 min. Volumes were determined as described in the Series 1 experiments, except that the isotopic markers were injected through the femoral venous catheter, and blood samples were obtained through the femoral arterial catheter.

Analytical procedures and fluid volume calculations. *PRA* was determined by radioimmunoassay of generated angiotensin I, by a modification of the method of Cohen *et al.* (13). The results are expressed as nanograms of angiotensin I per milliliter of plasma per hour of incubation. This modification, as used in our laboratory, has been described in detail previously (14). Serum urea nitrogen was determined by the method of Fawcett and Scott (15). Hematocrit measurements were obtained by microhematocrit.

Volume measurements from the collected plasma samples were determined in the following manner. A 1.50-ml aliquot of each plasma sample and a 1.50-ml aliquot of the RISA standard were counted for 5 min each in a well-type gamma counter. The counts were calculated as counts per minute per milliliter (cpm/ml). A linear regression analysis of sample cpm/ml vs sample collection time for each experimental determination revealed a highly negative correlation ($r < -0.95$) with a slope unequal to zero ($P < 0.05$). The ordinate intercept was calculated to determine the theoretical equilibrated sample cpm/ml at zero time. *PV* was calculated as

$$PV \text{ (ml)} = \frac{100 \times (\text{cpm } ^{125}\text{I/ml of RISA standard})}{\text{zero-time cpm } ^{125}\text{I/ml of plasma}},$$

where 100 is the dilution volume of the RISA standard, in milliliters. By precipitating the protein in plasma samples to which standard amounts of RISA had been added and then counting the supernatant, we determined that over 99.8% of the ^{125}I was bound to albumin.

After the plasma volume determinations, the protein in each sample tube was precipitated with 3.00 ml of 10% trichloroacetic acid (TCA), and the tubes were centrifuged. One milliliter aliquots of the supernatant, in triplicate, were added to scintillation counting vials; 1-ml aliquots of the $^{35}SO_4$ and ^{3}HOH standards, in triplicate, also were added to individual counting vials, and 60 μl of 100% TCA also was added to these vials to produce the same quench as the sample vials. Liquid scintillation counting fluid was added, and the vials were counted in a dual-window, automated liquid scintillation system. An online data reduction system automatically corrected for quenching and crossover. Linear regression analysis of disintegrations per minute (dpm) of the samples vs sample collection time indicated a highly negative correlation ($r < -0.95$) with a slope unequal to zero ($P < 0.05$) for $^{35}SO_4$. Therefore, the ordinate intercept was used as the equilibrated plasma $^{35}SO_4$ concentration. The plasma concentrations of ^{3}HOH did not change significantly with time; thus, the average ^{3}HOH concentration for the four plasma samples in each experiment was used in the calculation of *TBW*.

ECFV was determined as sulfate space, calculated as

$$ECFV \text{ (ml)} = \frac{0.837 \text{ (dpm } ^{35}\text{S/ml of standard)} \times 1000}{3 \times \text{(zero-time dpm } ^{35}\text{S/ml of plasma)}},$$

where 0.837 is a constant to correct for the Donnan factor and the plasma protein concentration, 1000 is the dilution volume of the $^{35}SO_4$ standard, in milliliters and 3 is the dilution factor due to TCA deproteinization. *TBW* was determined as the distribution volume of ^{3}HOH in the following manner,

$$TBW \text{ (ml)} = \frac{0.93 \text{ (dpm } ^{3}\text{H/ml of standard)} \times 1000}{3 \times \text{(mean dpm } ^{3}\text{H/ml of plasma)}},$$

where 0.93 is a constant to correct for the protein concentration of plasma, 1000 is the dilution volume of the ^{3}HOH standard, in milliliters, and 3 is the dilution factor due to TCA deproteinization of the plasma.

Interstitial fluid volumes were calculated by subtracting the *PV* from the *ECFV*. Intracellular fluid volumes were determined by subtracting the *ECFV* from the *TBW*.

Statistics. Values for body weight, mean arterial pressure, *PRA*, serum urea nitrogen, *PV*, *ECFV*, *TBW*, interstitial fluid volume, intracellular fluid volume, and volume ratios between the control and the renal artery stenosis groups of rabbits were compared by Student's *t* test for group (unpaired) observations (16). Values for the replicate volume determinations in normal rabbits (series 1) were compared by Student's *t* test for paired observations.

Results. *Series 1: Replicate volume determinations in normal rabbits.* Table I gives the average values (±SEM) for the two replicate volume determinations, with the means ±SEM for the absolute differences (disregarding the directional changes) in the two determinations. All volumes are expressed as milliliters per kilogram of body weight. Due to an error in the initial *PV* measurement in one rabbit, there are only five replicate measurements of *PV*. Since the replicate determinations were made only 2 days apart, it is assumed that the fluid volumes were constant over that period of time. Of the directly determined volumes, TBW was measured most reproducibly, varying an average of less than 2% between the two measurements. *PV* measurements varied by an average of 4%, and *ECFV* by less than 8% between the two replications. Student's *t* test for paired observations revealed no significant differences between the two determinations for any of the volumes.

Series 2: Volume measurements in control and renal artery stenosis rabbits. Four of the rabbits with chronic renal artery stenosis had abnormally high values for *PRA* (exceeding 20 ng/ml/hr) and for serum urea nitrogen (over 40 mg%). Greatly elevated values for *PRA* and serum urea nitrogen generally indicate that the renal artery stenosis animal is developing malignant hypertension. Because the purpose of the present study was to examine volume factors in rabbits with chronic renal hypertension and not malignant hypertension, the data from these four rabbits were discarded.

The values for body weight, mean arterial pressure, *PRA*, and serum urea nitrogen for the control and for the renal artery stenosis rabbits are summarized in Table II. The body weights were very similar between the two groups of rabbits. The rabbits with renal artery stenosis were hypertensive, having significantly ($P < 0.01$) higher mean arterial pressures than the controls, although there was some overlapping of the mean arterial pressure values between the two groups. Values for *PRA* in the renal artery stenosis group were not significantly different from the values for the control group. Figures 1–3 describe the values for *PV*, *ECFV*, and *TBW* for the control and for the renal artery stenosis groups of rabbits. *PV* in the renal artery stenosis group averaged 33.5 ± 1.5 (SEM) ml/kg, which was not significantly different from the average of 31.1 ± 1.2 ml/kg for the control group. The hematocrit values for five control and five renal artery stenosis rabbits were 0.43 ± 0.015 and 0.39 ± 0.008, respectively; thus, blood volumes also were quite similar. Average values for *ECFV* and *TBW* for the renal artery stenosis group of rabbits were 201 ± 7 and 659 ± 13 ml/kg, respec-

TABLE I. VALUES FOR REPLICATE DETERMINATIONS OF FLUID VOLUMES IN SIX NORMAL RABBITS

	PV	*ECFV*	*TBW*	*ISFV*	*ICFV*
Trial 1	29.8	198	647	170	449
	±1.1	±11	±15	±14	±6
Trial 2	30.5	192	650	161	458
	±1.6	±4	±14	±5	±11
Absolute differences[a]	1.2	15	11	17	13
	±0.4	±5	±3	±5	±5

Note. Values are means ± SEM. *PV*, Plasma volume; *ECFV*, extracellular fluid volume; *TBW*, total body water; *ISFV*, interstitial fluid volume; *ICFV*, intracellular fluid volume. All volumes are in ml/kg of body wt. There were no significant differences between the two trials for any of the volume factors, when tested by Student's *t* test for paired observations.

[a] Mean ± SEM of the differences between the two trials, disregarding the direction of the differences.

TABLE II. VALUES FOR CONTROL AND ONE-KIDNEY, ONE-CLIP 30-DAY RENAL ARTERY STENOSIS RABBITS

	Weight (kg)	*MAP* (mm Hg)	*PRA*	SUN (mg%)
Controls	3.25	90	4.5	19
(n = 14)	±0.06	±2	±1.4	±1
Renal artery stenosis	3.14	111*	3.0	31*
(n = 12)	±0.09	±4	±0.8	±3

Note. Values are means ± SEM. *MAP*, Mean arterial pressure; *PRA*, plasma renin activity, in ng of angiotensin I/ml/hr; SUN, serum urea nitrogen.

* $P < 0.01$ that the value for the renal artery stenosis group is greater than the value for the control group.

tively, which were significantly ($P < 0.05$) higher than the values of 182 ± 4 and 623 ± 8 ml/kg for the corresponding volumes in the control group. The interstitial fluid volumes were significantly ($P < 0.05$) greater in the renal artery stenosis group of rabbits than in the control group (see Table III). Intracellular fluid volumes between the two groups were quite similar. Ratios of *PV/ECFV*, *PV/TBW*, and *ECFV/TBW* were not altered between the control and renal artery stenosis groups of rabbits (Table III). There were no significant correlations between mean arterial pressure and any of the volumes for either the control or the renal artery stenosis groups.

Discussion. As an animal ages there is an increase in the relative contribution of fat to the body weight (17). Relative to most tissues of the body, adipose tissue has less plasma and less extracellular fluid (18). Thus, while older and larger animals may have greater absolute values for *PV* and *ECFV*, these volumes on a per body weight basis are decreased in larger animals (19, 20). Therefore, in studies investigating the effects of hypertension on fluid volume factors it is important to use control and experimental subjects or animals of the same weight, or to express the fluid volumes as per unit weight of lean body mass. The present study used experimental and control rabbits that were weight matched, so the weights of the two groups were very similar; also, the present study used only male rabbits to avoid the differences in body fat content which occur between males and females.

Volume studies of human subjects with hypertension have yielded varying results. *PV* and blood volume have been measured more than other fluid volumes and have been reported to be normal (1, 5, 7, 8) or

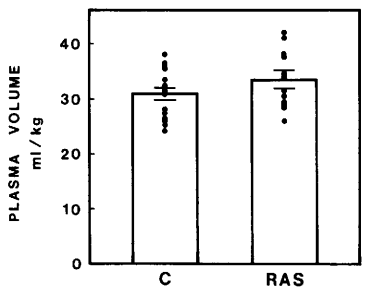

FIG. 1. Plasma volumes (ml/kg body wt) in 14 control (C) rabbits and in 12 one-kidney, one-clip rabbits with 30-day renal artery stenosis (RAS). Dots indicate individual values. Bars represent mean values; lines above and below bar heights indicate SEM. Values for the control and renal artery stenosis groups were not significantly different when compared by Student's t test for group (unpaired) observations.

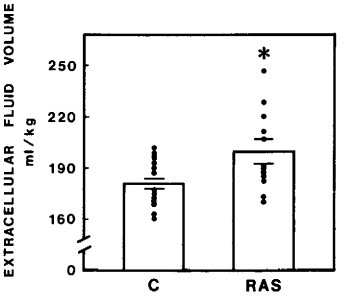

FIG. 2. Extracellular fluid volumes (ml/kg body wt) in 14 control (C) rabbits and in 12 one-kidney, one-clip rabbits with 30-day renal artery stenosis (RAS). Dots indicate individual values. Bars represent mean values; lines above and below bar heights indicate SEM. *$P < 0.05$ that the value for the renal artery stenosis group was significantly greater than the value for the control group, by Student's t test for group (unpaired) observations.

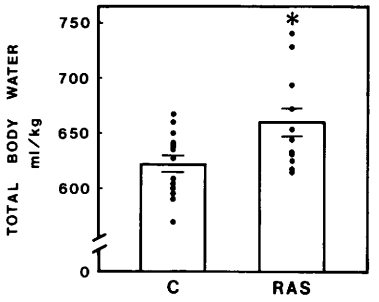

FIG. 3. Total body water (ml/kg body wt) in 14 control (C) rabbits and in 12 one-kidney, one-clip rabbits with 30-day renal artery stenosis (RAS). Dots indicate individual values. Bars represent mean values; lines above and below bar heights indicate SEM. *$P < 0.05$ that the value for the renal artery stenosis group was significantly greater than the value for the control group, by Student's t test for group (unpaired) observations.

decreased (3, 4, 6, 9). Studies of *ECFV*, on the other hand, have indicated that it is increased (2, 8) or normal (1, 3, 5, 6) in hypertensive subjects. Two studies (3, 6) have reported a decrease in *PV/ECFV* ratio in hypertension. *TBW* in hypertensive patients has been found to be either increased or normal (1, 8). These variable results among different investigators may be due to several factors, including failure to match the control subjects with the hypertensive patients with respect to age, race, sex, and body size, and failure to determine adequately the types of hypertension in the experimental groups.

Volume studies with animal models of hypertension have advantages over clinical studies in that with animal studies the control and experimental groups can be weight and sex matched easily, and animals can be obtained that are genetically very similar. However, there have been relatively few volume studies in animals with experimental hypertension. Studies in dogs with experimental renal hypertension have shown increases in *PV* (21, 22), *ECFV* (21), and *TBW* (8), and studies in one-kidney renal hypertensive rats have found that the *PV* is increased (23) and that the *ECFV* is increased (24) or normal (23).

The present study demonstrated that one-kidney rabbits with renal artery stenosis of 30 days duration (one-kidney, one-clip) were hypertensive and had significant increases in *ECFV*, *TBW*, and interstitial fluid volume. The increase in *TBW* was due mainly to increases in *ECFV*, as the intracellular fluid volumes were quite similar between the renal artery stenosis and control groups. Although the *PV* values were slightly larger in the renal artery stenosis group than in the control group, these differences were not statistically significant. It is of interest to compare the results of the present study with the findings of Bauer and Brooks (1), who used the same techniques to measure simultaneously the *PV*, *ECFV*, and *TBW* in human subjects with essential hypertension. Their studies in white males with normal-renin essential hypertension, in which the control subjects were carefully matched to their hypertensive patients, revealed normal values for *PV* and *ECFV*, with an increase in *TBW* reflecting an increase in intracellular fluid volume. The findings of the present study in rabbits would suggest that different mechanisms are involved in producing the fluid volume alterations in this rabbit model than are functioning to produce the volume

TABLE III. VALUES FOR DERIVED VOLUMES AND VOLUME RATIOS IN CONTROL RABBITS AND IN ONE-KIDNEY, ONE-CLIP 30-DAY RENAL ARTERY STENOSIS RABBITS

	ISFV (ml/kg)	*ICFV* (ml/kg)	*PV/ECFV*	*PV/TBW*	*ECFV/TBW*
Controls	151	448	0.170	0.049	0.290
(n = 14)	±3	±8	±0.006	±0.002	±0.007
Renal artery stenosis	167*	456	0.168	0.051	0.310
(n = 12)	±7	±12	±0.007	±0.002	±0.009

Note. Values are means ± SEM. *ISFV*, interstitial fluid volume; *ICFV*, intracellular fluid volume; *PV*, plasma volume; *ECFV*, extracellular fluid volume; *TBW*, total body water.

* $P < 0.05$ that the value for the renal artery stenosis group is higher than the value for the control group.

changes in humans with normal-renin essential hypertension.

Sodium retention and expansion of body fluid volumes and the roles they may play in the development and maintenance of hypertension have been studied extensively (21, 24–26). The results of many of these studies are supportive of the "whole body autoregulation" hypothesis advanced by Guyton and Coleman (27). This hypothesis maintains that fluid retention following renal disturbances results in expansion of *PV* and *ECFV*. The increased *PV* induces increases in venous pressure, venous return, and cardiac output, which in turn increases blood flow to the tissues of the body. In response to tissue overperfusion, peripheral arterioles constrict to autoregulate the tissue blood flow, thus raising the total peripheral resistance. The findings of the present study of increases in *ECFV* and *TBW* in the renal artery stenosis rabbits are in keeping with the hypothesis of whole body autoregulation. However, earlier studies from this laboratory with this rabbit model have failed to demonstrate increases in cardiac output during the acute phase of the hypertension which occurs during the first 24 hr following renal artery stenosis (14); also, increases in cardiac output were not seen at 3 days after renal artery stenosis (28), or in chronic hypertensive rabbits with renal artery stenosis of 30 days duration (28, 29). Similarly, studies from this laboratory (30), as well as studies by others (31), have failed to observe changes in cardiac output in rabbits with perinephritic hypertension. Thus, the role of expansion of the ECFV and whole body autoregulation in the development and maintenance of renal hypertension in this rabbit model is unclear.

There has been interest recently in a possible role for volume expansion in mediating pressor and vascular hyperresponsiveness to vasoactive substances in hypertension. It has been proposed (32) that volume expansion in hypertensives could result in increased secretion of natriuretic hormone, which then would increase the sodium content of cells, including arteriolar smooth muscle cells, by inhibiting Na-K ATPase. The enhanced contractions of the arterioles then would result in elevated arterial pressure. It is of interest that previous studies from this laboratory (28) have shown that one-kidney, one-clip rabbits with renal artery stenosis of 30 days duration, the same rabbit model as the present study, did indeed have exaggerated pressor responses to infusions of norepinephrine.

It should be pointed out that although the expanded *ECFV* and *TBW* observed in the present study are in keeping with the hypothesis that increased volumes may play a role in the maintenance of chronic renal hypertension, these studies do not rule out the possibility that renal artery stenosis in one-kidney rabbits may produce chronic hypertension by mechanisms not related to the increases in fluid volumes and that the expansions of these volumes may occur simply because of decreased ability of the single stenosed kidney to excrete sodium and water.

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