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# Body fluid expansion is not essential for salt-induced hypertension in SS/Jr rats

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**Qi, Nianning, John P. Rapp, Paul H. Brand, Patricia J. Metting, and Steven L. Britton.** Body fluid expansion is not essential for salt-induced hypertension in SS/Jr rats. *Am. J. Physiol.* 277 (Regulatory Integrative Comp. Physiol. 46): R1392–R1400, 1999.—To evaluate the importance of volume in the development of hypertension in inbred Dahl salt-sensitive rats (SS/Jr), we measured the changes in blood pressure (BP) that occurred with oral intake of food (salt) and water in rats whose body weight was permitted to increase versus those in which body weight was maintained constant with a servo-control system. We hypothesized that if volume expansion is essential in the development of hypertension, then BP would not increase if body weight was held constant. We found that oral presentation of chow containing 4% salt to SS/Jr rats caused BP to increase  $32.2 \pm 2.9$  mmHg over 4 days when body weight was controlled at its initial value. Plasma sodium increased from 142.0 to 145.2 meq/l during 4 days of high salt. Neither plasma volume, hematocrit, nor central venous pressure changed significantly on the high-salt diet. In contrast, the inbred Dahl salt-resistant rats (SR/Jr) did not increase their BP during body weight control when given 4% salt. This demonstrates that volume expansion is not an obligatory step in the pressure response to increased salt in SS/Jr rats. Our results obtained with oral presentation of salt, in contrast to intravenous, represent a physiological evaluation of the significance of volume changes in response to dietary salt because no potential regulatory reflexes have been bypassed.

Dahl rats; genetic models; plasma volume; blood volume; plasma sodium; extracellular fluid volume

SALT IN THE DIET appears to be of central importance to the increase in blood pressure in most forms of human and experimental hypertension. The exact connection between increased salt and hypertension has yet to be determined. A widely accepted model, originally conceived by Arthur Guyton and Thomas Coleman (13, 14), postulates that volume expansion in response to increased salt is an integral step in the development of hypertension. The theoretical importance of volume expansion is well founded and is an entirely logical hypothesis given the presently available data.

A previous study by Greene et al. (11) evaluated the importance of salt versus volume in Dahl salt-sensitive rats by control of body weight (and thus fluid volume) via feedback control of the intravenous delivery of salt and water. These critical experiments demonstrated

the potential importance of volume. That is, they found that if body fluid volume was not allowed to increase during delivery of excess salt, the blood pressure did not go up, despite hypernatremia. Nevertheless, the full extent of all physiological interactions may not have been revealed in these experiments, because body weight was controlled by intravenous delivery of salt and water. This route of delivery bypasses several important afferent reflexes that are known to influence the renal handling of salt. The most important of these "salt input reflexes" has been demonstrated in the intestine and the liver (2, 20–24).

With this information in mind, we designed experiments to again evaluate the importance of volume expansion in salt-sensitive rats, only this time we performed experiments at a higher level of integration. That is, we controlled the rat's body weight constant while they were allowed to take in salt and water orally. Oral presentation of food and water, in contrast to intravascular delivery, represents a more meaningful evaluation of the physiological and pathological significance of volume changes in response to salt because no potential regulatory steps have been bypassed. Our data support the hypothesis that net volume expansion is not an obligatory step in the pressure response to increased salt and suggest that the concentration of sodium in the plasma may play a critical role.

## METHODS

**Animal model.** Experiments were performed in male inbred Dahl salt-sensitive (SS/Jr) and inbred Dahl salt-resistant (SR/Jr) strains of rats. These strains have been selectively bred for their blood pressure response to a diet high in sodium chloride (salt). The SS/Jr rats develop hypertension on a diet high in salt, whereas the SR/Jr do not. The rats for this work were obtained from the colony of John Rapp, which is maintained at the Medical College of Ohio, Toledo, OH. Each of these strains has been bred for more than 70 generations of brother-sister matings. Thus measured differences for any given phenotypic trait within a strain derive mainly from environmental influences.

**Animal preparation.** All animals were weaned at 4 wk of age and raised on low-salt chow (0.2% NaCl) and tap water until 8 wk old (body wt  $300 \pm 20$  g). Rats were then anesthetized with a mixture of ketamine (80 mg/kg), xylazine (12 mg/kg), and atropine (0.032 mg/kg) via an intraperitoneal injection. MicroRenathane (0.040 OD  $\times$  0.025 ID) catheters extended with Tygon plastic tubing were inserted into the right carotid artery and the right jugular vein for the direct measurements of arterial pressure and central venous pressure and for the collection of blood samples. The free ends of the tubing were tunneled subcutaneously to exit the skin at the midscapular region. Antibiotic ointment (Bacitracin Zinc, Neomycin Sulfate, and Polymyxin B Sulfate) was used on the

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incisions after surgery to prevent infection and irritation. The exteriorized tubing was routed out of the cage through a stainless steel spring to protect the tubing. This spring was attached to a lighter extension spring that allowed maximum movement of the rat with a minimum tension on the springs. The tubing exiting from the heavier spring was connected to a fluid delivery system that provided constant flow of a heparin saline solution (50 U/ml).

A fluid delivery system was connected to each catheter. The fluid delivery system was made from a rubber bulb, a 0.2- $\mu$ m filter, and a hydraulic resistor (Fig. 1). The rubber bulb was connected in series with the filter and the hydraulic resistor for the continuous delivery of saline to each catheter. The bulb has a transmural pressure of  $\sim$ 500 mmHg when filled with 8 ml of saline and drives fluid through the filter and resistor without any appreciable decline in bulb pressure with a fluid loss of up to 4 ml. The resistor was made from glass tubing (25  $\mu$ m ID) that was fixed within a 21-gauge needle with Silastic glue. The length of the glass tubing was 3.2 cm and resulted in a delivery of  $\sim$ 1 ml/day for the arterial tubing and 1.5 ml/day for the venous tubing. This fluid delivery system enabled us to keep both catheters patent for extended periods.

The fluid delivery system was connected to the light tether spring from below and to a 360° swivel that connected to a support arm above the cage. The entire tethering and fluid system rotated freely and allowed essentially unrestricted movement of the rat.

**Control of body weight.** A servo-control feedback system was designed that controlled body weight constant while still allowing rats to take on food and water orally (Fig. 1). A modified metabolic cage was mounted on an electronic balance (Sartorius BP 6100) by which the total weight of cage and rat, including tether and fluid delivery system, was measured. The waste tray, water delivery line, and food holder were all supported outside of the cage so that their weight did not bear on the balance. When the rat had been

transferred into the cage for the start of weight control, the balance was tared to zero. The digital signal from the balance was fed to an 80386 microcomputer as the input signal for the servo-control algorithm that was written using Labtech Notebook software. The computer was connected to a peristaltic pump (Harvard Apparatus, model 1201). When the balance detected a decrease of 1 g or more (because of urination, defecation, and insensible loss of water), the algorithm put out an error signal to a digital-to-analog (D/A) channel on a Data Translation signal board (DT2801). This analog output activated the water pump that delivered water to a pan inside the cage. Once enough water was delivered to bring the balance back to zero, the pump automatically turned off. Therefore, the weight of the rat was kept at a constant value through this feedback control of its water supply, although the rat constantly had free access to food and water. Rats promptly drank essentially all of the water delivered into the pan with little or no spillage. Because rats would grasp onto the food holder for up to 2 min while they were eating, thus reducing the weight bearing on the balance by 10 g or more, the output signal from the computer was set with a 5-min delay. That is, only a decline in weight of  $\geq$ 1 g that persisted for 5 min caused the pump to deliver water to the rat's drinking pan. This delay avoided the errors in water delivery that would have resulted from balance changes due to the normal activity of the rats.

**Experimental measurements.** Arterial and central venous pressures were measured simultaneously from the indwelling catheters in the carotid artery and jugular vein using Statham strain gauge transducers (P23Db) that were placed at heart level of the rat. Each transducer was calibrated daily with a water manometer before the study. At least 30-min recordings of both pressures were performed at noon on each day when the rats were quiescent, and the most stable 10-min segment was extracted for analysis. The raw pressure signals (voltages) were amplified on a Sormedics R-611 polygraph, sampled at 300 Hz and then digitized by a Data Translation A/D board (DT 2801). The digital signals were averaged every second and stored on the hard drive of an 80386 microcomputer. The daily pressure was obtained by taking the average of the 10-min segment.

Plasma volume was estimated from the distribution of intravenously administered Evans blue solution. The plasma concentration of Evans blue was measured spectrophotometrically (Gilford, Response II) from a 0.2-ml sample of arterial blood withdrawn 5 min after the injection of 0.2 ml of 0.1% Evans blue solution, as described by Belcher and Harris (1). The plasma volume was calculated from the amount of Evans blue injected divided by its plasma concentration.

The hematocrit and the concentrations of  $\text{Na}^+$  and  $\text{K}^+$  were measured from a 0.25-ml sample of arterial blood. Approximately 0.05 ml of blood was drawn into four microcapillary tubes and spun on a Fisher Micro-Hematocrit centrifuge (model 335) for 3 min; the four values were averaged to estimate the hematocrit. The remaining 0.2 ml of blood was centrifuged in an IEC MicroMax centrifuge at 3,000 rpm for 3 min, and 0.1 ml of plasma was separated for the determination of  $\text{Na}^+$  and  $\text{K}^+$  concentrations with an IL 943 Flame Photometer.

Salt consumption was estimated from the product of the mass of food eaten and the fractional content of salt in the chow. Food consumption was estimated by weighing the daily changes in the amount of food in the food holder and subtracting the food debris in the waste tray. Water consumption was recorded from the daily changes in volume of water in the syringe that served as a reservoir for the peristaltic pump.

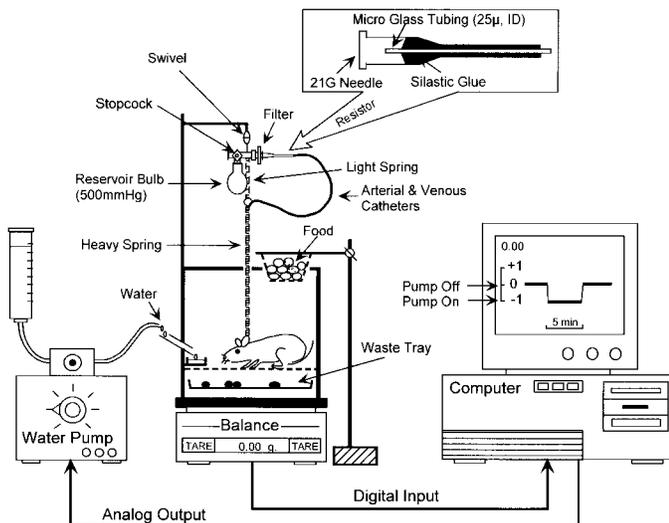


Fig. 1. Feedback control of body weight. Total weight of cage and rat, including tether and fluid delivery system, was measured by an electronic balance while waste tray, water delivery line, and food holder were supported outside of balance system. Changes of body weight were detected and set within range from 0 to  $-1$  g by computer that was connected to a peristaltic water pump, so that pump was turned on and off when changes of weight exceeded low and high limits. Thus input (food and water) was always equal to output (urination, defecation, and insensible loss of water), and weight of rat was kept constant.

**Experimental protocols.** Five groups of rats were chosen for study on the basis of strain, salt diet, and control or noncontrol of body weight. For SS/Jr rats, there were four groups: SS/Jr rats on 0.2% NaCl chow, body weight not controlled,  $n = 7$ ; SS/Jr on 0.2% NaCl chow, body weight controlled,  $n = 8$ ; SS/Jr on 4% NaCl chow, body weight controlled,  $n = 7$ ; and SS/Jr on 4% NaCl, body weight not controlled,  $n = 6$ . Only one group of SR/Jr rats was studied, SR/Jr given 4% NaCl chow while body weight was controlled at zero change ( $n = 6$ ). This is the only group that would provide new information because numerous studies have already shown that SR/Jr are resistant to salt-induced increases in blood pressure (26).

Seven days after surgery for instrumentation, rats were transferred into the modified metabolic cages described above. Then 4 days were allowed for them to adapt to the experimental environment while ingesting the low-salt chow. During this period, control measurements were performed to obtain the baseline data of hemodynamic variables. After the control period, rats were randomly chosen to have body weight either controlled or not controlled while receiving food containing either low salt (0.2% NaCl) or high salt (4% NaCl) for the subsequent 4 days of study. Body weight was recorded continuously throughout the experiments in all rats. Arterial and central venous blood pressures, hematocrit, plasma sodium and potassium concentrations, and food and water consumption were measured every day. The plasma volume was measured every fourth day to minimize blood loss and because ~48–72 h are needed for the rat to clear Evans blue solution from its blood. A maximum of 0.65 ml of blood was withdrawn for the measurement of plasma volume (including 0.2 ml for control reading before the injection of Evans blue), hematocrit, and plasma sodium and potassium concentrations on the same day. This amount of blood represents 2.5–3.0% of the estimated total blood volume. The erythrocytes from the blood sample were resuspended in isotonic saline and given back to the animal.

**Statistical analysis.** Data are presented as mean values  $\pm$  SE. Mean arterial pressure and plasma sodium concentration were compared with the average of their control measurements within the group. The significance of differences within and between each group were evaluated by repeated-measures ANOVA followed by Student-Newman-Keuls test for multiple comparison with a significance level of 0.05. The relationship between mean arterial pressure and plasma sodium concentration was evaluated by linear regression analysis.

## RESULTS

The control measurements of mean arterial pressure, plasma sodium concentration, body weight gain, and salt and water intake for all five groups of animals are

summarized in Table 1. These values represent the average of the 4 days of control on low salt (individual daily values for these variables are represented in Figs. 2–6 as *days - 3* through *day 0*). The blood pressures for all the salt-sensitive (S) groups of rats were not significantly different from each other. The blood pressure of the salt-resistant (R) rats was on average, however, ~25 mmHg lower than the S rats. Plasma sodium concentration was significantly elevated in the R rats compared with all other groups. The S rats on 0.2% NaCl (+ body weight control) and the R rats ate a little less salt than the other groups during the control. There were no significant differences in water consumption or body weight gain between the five groups during the 4 control days.

**SS/Jr rats on high-salt diet, without body weight control.** As shown in Fig. 2, mean arterial pressure increased from an average of  $125.0 \pm 4.8$  mmHg during the 4 control days to  $139.8 \pm 3.9$  mmHg on the first day of the high-salt diet. This pressure increase continued and reached a peak of  $155.4 \pm 4.0$  mmHg on *day 4* of high-salt chow. All the pressures during 4 days of high salt were significantly higher than their control values on low salt, and the change in pressure from the control to *day 4* of the high-salt diet was 30.4 mmHg. Plasma sodium concentration also increased from an average of  $142.3 \pm 0.2$  meq/l during 4 days of the control to  $143.8 \pm 0.6$  meq/l on the first day of high salt and remained elevated for the next 3 days. Changing the food from low salt to high salt caused an approximate 18.7-fold increase in daily salt intake. Water intake was increased from the average of 24.8 to 67.6 ml/day in response to the increased salt intake. Body weight gain continued on a daily basis, however, without differences between low salt and high salt.

**SS/Jr rats on high-salt diet, with body weight control.** For this group, control of body weight at a constant value was started at the same time (*day 0*) that the food was switched from low salt to high salt. As shown in Fig. 3, the blood pressure increased from a control value of  $133.1 \pm 2.9$  to  $145.9 \pm 3.7$  mmHg on the first day of high salt. It continued to rise and reached  $165.2 \pm 2.8$  mmHg on *day 4*. All the pressures during 4 days of high salt were significantly higher than their control values, and the net increase in pressure was 32.1 mmHg from the control. The high salt plus body weight control caused plasma sodium concentration to

Table 1. Average of MAP,  $[Na^+]_p$ , body weight gain, salt and water intake for all 5 groups of rats during 4 days of control

Groups	Average of Control Measurements (0.2% NaCl)				
	S, 4% NaCl, no body wt control	S, 4% NaCl, +body wt control	S, 0.2% NaCl, no body wt control	S, 0.2% NaCl, +body wt control	R, 4% NaCl, +body wt control
MAP, mmHg	$125.0 \pm 4.8^*$	$133.1 \pm 2.9^*$	$135.3 \pm 6.7^*$	$137.1 \pm 3.9^*$	$107.6 \pm 2.6$
$[Na^+]_p$ , meq/l	$142.3 \pm 0.2^*$	$142.2 \pm 0.3^*$	$142.6 \pm 0.3^*$	$142.6 \pm 0.2^*$	$145.4 \pm 1.2$
Body wt gain, g/day	$6.1 \pm 0.4$	$4.8 \pm 0.6$	$4.0 \pm 0.8$	$5.5 \pm 0.9$	$3.0 \pm 0.8$
Salt in, meq/day	$0.90 \pm 0.05^*$	$0.92 \pm 0.06^*$	$0.86 \pm 0.06^*$	$0.80 \pm 0.08$	$0.73 \pm 0.06$
Water in, ml/day	$24.8 \pm 1.6$	$26.9 \pm 1.2$	$25.5 \pm 1.6$	$26.1 \pm 0.8$	$26.0 \pm 1.1$

Values are means  $\pm$  SE. \*Significantly different from value of SR/Jr (R) group ( $P < 0.05$ ). S, SS/Jr; MAP, mean arterial pressure;  $[Na^+]_p$ , plasma sodium concentration.

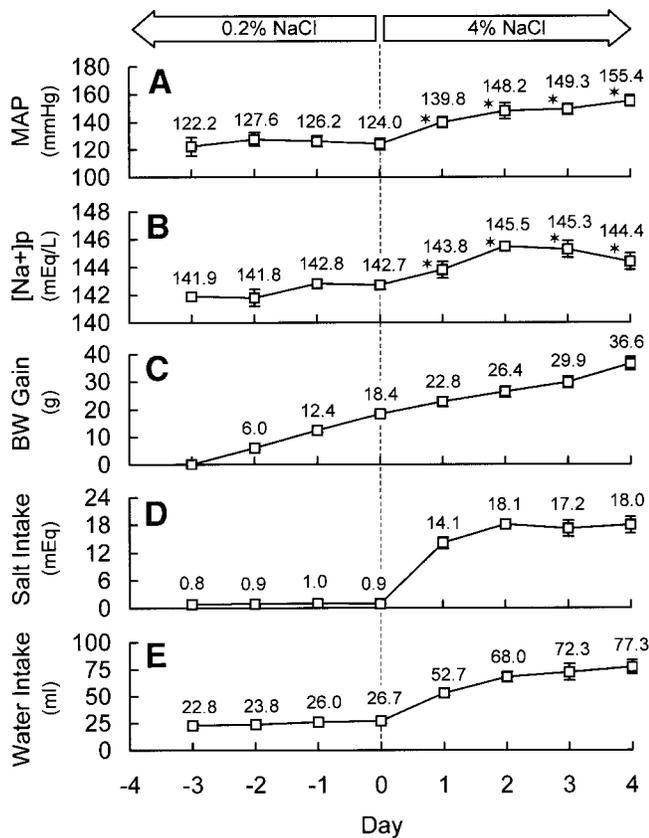


Fig. 2. Changes in mean arterial pressure (MAP), plasma sodium concentration ( $[Na^+]_p$ ), body weight (BW) gain, salt and water intake in SS/Jr rats that were on high-salt (4% NaCl) diet while their body weight was not controlled during 4 days of experiments. \*Significantly higher than average of control measurements ( $P < 0.05$ ).

increase from the control of  $142.2 \pm 0.3$  meq/l to significantly higher levels of  $145.2 \pm 0.7$  meq/l on day 2 and  $146.8 \pm 0.8$  meq/l on day 3 of the test. These increases in blood pressure and plasma sodium concentration were not different from those of the SS/Jr rats that were on high salt but without body weight control (Fig. 2). Body weight increased on a daily basis over 4 days of low salt, but of course the body weight remained essentially constant over 4 days of high salt because of the efficient feedback control of body weight. Changing food from low salt to high salt resulted in an approximate 14.6-fold increase in daily salt consumption, even though total daily food consumption decreased by 25%. Water intake increased from  $26.9 \pm 1.2$  to  $50.9 \pm 3.5$  ml/day in response to the high salt intake.

*SS/Jr rats on low-salt diet, without body weight control.* As expected, there were no changes in arterial blood pressure or plasma sodium concentration in the SS/Jr rats when on low salt for 8 days. Body weight increased at a normal rate on a daily basis with an average increase of  $3.8 \pm 0.4$  g/day. Salt and water intakes were similar on each of the 8 days (Fig. 4).

*SS/Jr rats on low-salt diet, with body weight control.* When the control of body weight began at day 0 while the SS/Jr rats were continued on low salt, blood pressure gradually increased over 4 days until it was 19 mmHg higher than during the control period

(Fig. 5). This pressure increase was not statistically significant. Nevertheless, a change of this magnitude suggests that the increase in pressure could have resulted from a greater autonomic response to 1) the limitation on drinking water and 2) the greater hypernatremia that occurred with body weight control in the SS/Jr strain compared with the SR/Jr. Plasma sodium concentrations varied slightly, but not significantly, over 4 days of body weight control. Body weight increased as usual during the 4 days of control, but body weight remained essentially constant when feedback servo-control was started on day 0. Salt intake was not different on any of the 8 days. Water intake did, however, decrease a little in the last 3 days of the control of body weight, but the decline was not significant.

*SR/Jr rats on high-salt diet, with body weight control.* No significant changes in arterial pressure or plasma sodium concentration were found in the SR/Jr rats when placed on 4 days of high-salt diet (Fig. 6). Body weight gain continued on a daily basis over 4 days of low salt, and body weight remained constant over 4 days of high salt when servo-control was instituted. Daily salt intake increased 18.2-fold from the control. Water intake increased from a control of  $26.0 \pm 1.1$  to  $39.6 \pm 2.3$  ml/day in response to the increased salt intake. This increase in water intake was significantly

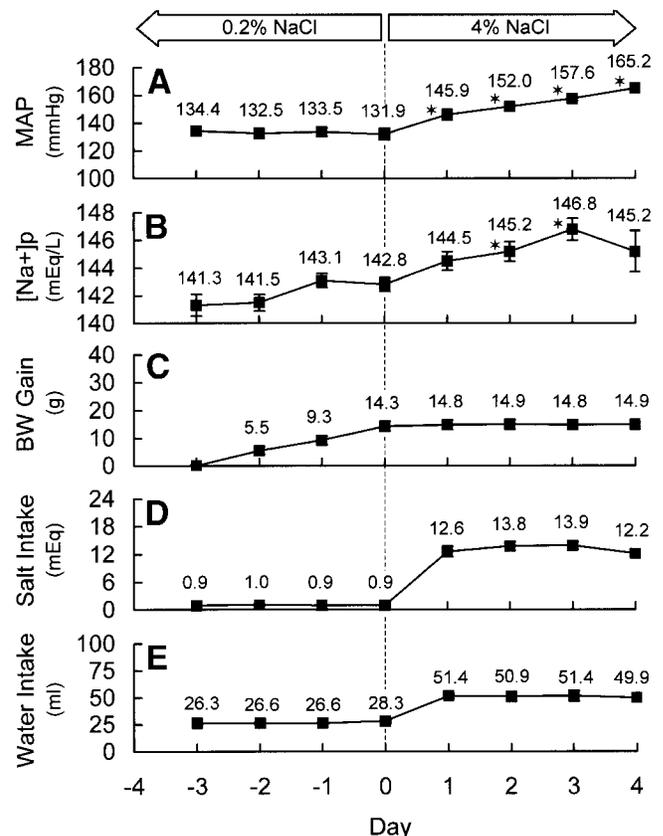


Fig. 3. Changes in MAP,  $[Na^+]_p$ , body weight gain, salt and water intake in SS/Jr rats that were on high-salt (4% NaCl) diet while their body weight was controlled constant during 4 days of experiments. \*Significantly higher than average of control measurements ( $P < 0.05$ ).

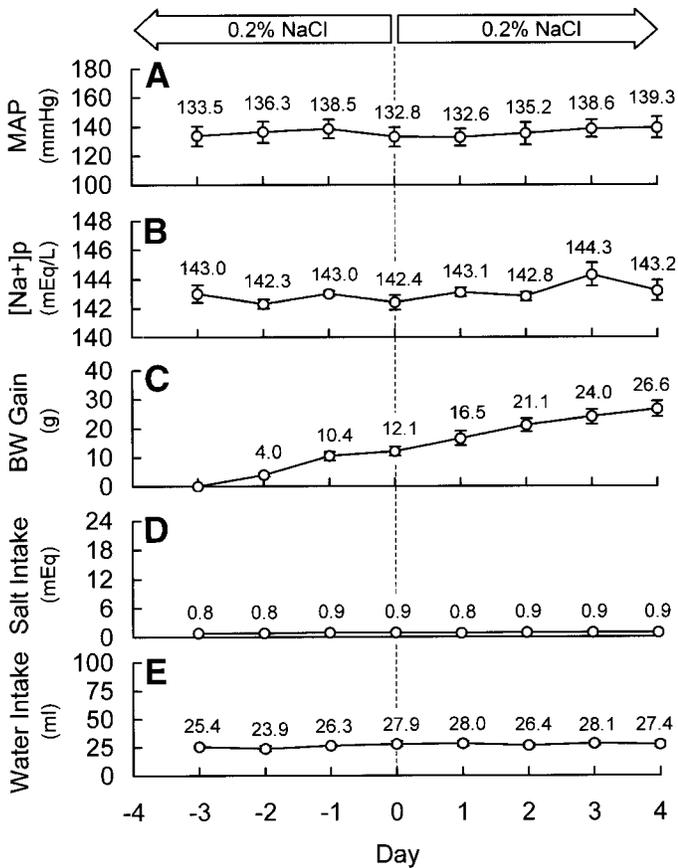


Fig. 4. Changes in MAP, [Na<sup>+</sup>]<sub>p</sub>, body weight gain, salt and water intake in SS/Jr rats that were on low-salt (0.2%) diet while their body weight was not controlled during 4 days of experiments.

lower than that in the SS/Jr rats when on high salt with their body weight controlled.

The measurements of hematocrit, plasma volume, central venous pressure, and plasma potassium concentration are shown in Tables 2 and 3. None of the values for hematocrit, plasma volume, or central venous pressure differed significantly in any of the conditions, despite the marked changes in salt intake and water consumption. There were no differences in plasma potassium concentration in the SR/Jr rats or the SS/Jr rats that were on low salt (Table 2). Compared with the 4 control days, however, the plasma potassium concentration was decreased in all of the SS/Jr rats that were on high salt for 4 days (Table 3).

Figure 7 shows the relationship between mean arterial pressure and plasma sodium concentration over the 4 days of low salt and the 4 days of high salt for the combined data shown in both Figs. 2 and 3 (SS/Jr rats with and without body weight control). Each 1-meq/l increase in plasma sodium concentration was associated with an increase of arterial pressure of 6.8 mmHg. Approximately 73% of the variation in plasma sodium concentration was associated positively with variation in arterial pressure ( $r = 0.85$ ,  $P < 0.001$ ).

## DISCUSSION

In 1967 Arthur Guyton and Thomas Coleman (13) created a computer model of arterial blood pressure

regulation based on experimentally derived characteristics of the circulation. Surprisingly, the model predicted that primary increases in cardiac output or total peripheral resistance did not result in permanent increases in arterial pressure, so long as renal function was not altered. Their model predicted that permanent, steady-state increases in arterial pressure can occur only as the result of 1) a change in renal function that operates via pressure diuresis and/or 2) increases in salt and water intake. Pressure diuresis refers to the direct effect of arterial pressure on the urinary excretion of salt and water. That is, as the pressure increases, the renal output of salt and water increases. When the output increases above the net intake of salt and water, negative body fluid balance occurs, causing the extracellular fluid volume (ECFV) to decrease. The decrease in body fluids results in decreases in central venous pressure, cardiac output, and thus arterial pressure, which declines until the input and output of salt and water are equal once again. Conversely, if the pressure declines below the level for balance, the intake will be greater than output, the ECFV will expand, and the arterial pressure will rise until input and output are equal. Another way to state the central principle of the Guyton-Coleman model is that an animal always has just enough arterial pressure to keep the input of salt and water equal to output in the steady state (3, 12).

In 1990, Greene et al. (11) published a critical and thoughtful study that provided the specific idea for

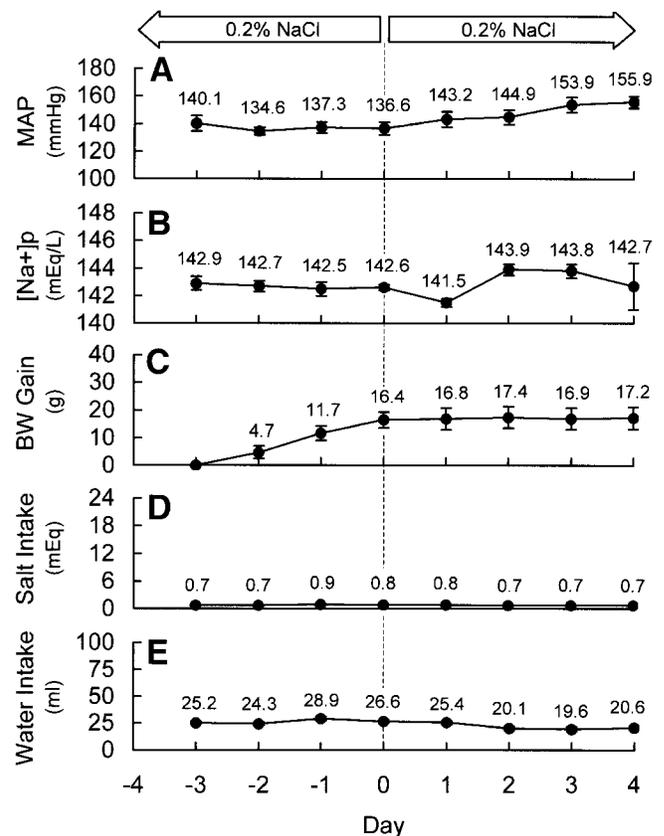


Fig. 5. Changes in MAP, [Na<sup>+</sup>]<sub>p</sub>, body weight gain, salt and water intake in SS/Jr rats that were on low-salt (0.2%) diet while their body weight was controlled constant during 4 days of experiments.

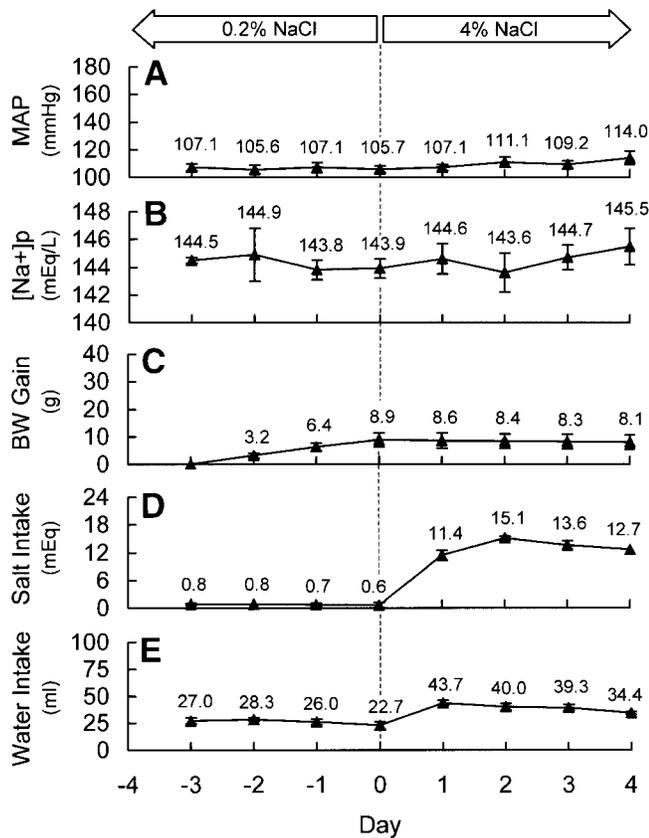


Fig. 6. Changes in MAP, [Na<sup>+</sup>]<sub>p</sub>, body weight gain, salt and water intake in SR/Jr rats that were on high-salt (4% NaCl) diet while their body weight was controlled constant during 4 days of experiments.

performing the present study. The objective of their study was to evaluate the Guyton-Coleman hypothesis regarding the volume dependence of salt-induced hypertension. Their studies were conducted in outbred Dahl S and R rats obtained from Brookhaven National Laboratories, Upton, NY. They instrumented rats

with chronic arterial and venous catheters and servo-controlled body weight constant during intravenous administration of a low-salt input (1 meq/day) and a high-salt input (20 meq/day) for 4 days. Body weight was servo-controlled at its initial value by continuously weighing the rat on an electronic balance and infusing water and salt intravenously at the rate of body weight decline. With body weight not controlled, the intravenous administration of the high salt for 4 days caused the body weight, plasma volume, and cardiac output of both the Dahl S and Dahl R rats to increase, but only the S rats exhibited increased arterial pressure on the high salt (27 mmHg). Arterial pressure did not increase in the Dahl R rats because total peripheral resistance decreased. When body weight was servo-controlled at a constant value (thus avoiding body fluid volume expansion), the intravenous administration of the high salt load did not cause the arterial pressure of the Dahl S rats to increase. From these results, Greene et al. (11) concluded that "fluid retention is required to trigger the rise of pressure in Dahl S rats." It is apparent that this result is explicitly consistent with the Guyton-Coleman model. That is, the blood pressure response to salt appears to be volume dependent in Dahl S rats.

The purpose of the present study was to once again evaluate the volume dependence of salt-induced hypertension in Dahl S rats. This time, however, the salt and water challenges were delivered orally while body weight was either allowed to increase or while body weight was held constant. Our view is that, in the physiological sense, oral presentation of salt and water represents a more meaningful route of delivery compared with the intravenous route because no reflex paths that regulate salt and water homeostasis have been bypassed. Numerous studies have demonstrated the potential for hepatic and gastrointestinal mechanisms to influence salt and water excretion (20, 23), although the role of the hepatic receptors in regulating

Table 2. Values for PV, CVP, Hct, and [K<sup>+</sup>]<sub>p</sub> for all groups of rats during 4 consecutive days (–3 to 0) on low-salt diet

Day		S, 4% NaCl, no body wt control	S, 4% NaCl, +body wt control	S, 0.2% NaCl, no body wt control	S, 0.2% NaCl, +body wt control	R, 4% NaCl, +body wt control
–3	PV	—	—	—	—	—
	CVP	–1.80 ± 0.6	–2.36 ± 0.6	–1.94 ± 0.4	–1.12 ± 0.3	–1.98 ± 0.5
	Hct	36.9 ± 1.3	38.9 ± 1.3	43.1 ± 2.0	38.5 ± 0.5	40.4 ± 0.7
	[K <sup>+</sup> ] <sub>p</sub>	4.63 ± 0.09	4.72 ± 0.21	4.65 ± 0.10	5.11 ± 0.07	4.47 ± 0.12
–2	PV	6.3 ± 0.4	6.3 ± 0.5	6.2 ± 0.2	7.6 ± 0.3	5.2 ± 0.1
	CVP	–1.70 ± 0.4	–0.80 ± 0.8	–1.59 ± 0.4	–0.86 ± 0.4	–2.57 ± 0.5
	Hct	37.8 ± 1.6	37.8 ± 1.7	41.2 ± 1.2	36.2 ± 0.4	39.2 ± 0.4
	[K <sup>+</sup> ] <sub>p</sub>	4.56 ± 0.06	4.38 ± 0.06	4.36 ± 0.11	4.55 ± 0.12	4.14 ± 0.09
–1	PV	—	—	—	—	—
	CVP	–1.40 ± 0.2	–0.67 ± 0.7	–2.43 ± 0.3	–1.39 ± 0.3	–1.68 ± 0.6
	Hct	37.9 ± 1.3	38.1 ± 1.7	42.0 ± 1.3	36.8 ± 0.4	40.8 ± 0.4
	[K <sup>+</sup> ] <sub>p</sub>	4.69 ± 0.17	4.59 ± 0.08	4.81 ± 0.07	4.84 ± 0.10	4.33 ± 0.15
0	PV	—	—	—	—	—
	CVP	–1.00 ± 0.5	–1.24 ± 0.7	–1.98 ± 0.5	–1.41 ± 0.2	–1.97 ± 0.3
	Hct	37.9 ± 1.1	38.5 ± 2.1	40.6 ± 1.6	37.3 ± 0.5	39.5 ± 0.5
	[K <sup>+</sup> ] <sub>p</sub>	4.79 ± 0.13	4.62 ± 0.05	4.64 ± 0.07	4.99 ± 0.08	4.22 ± 0.15

Values are means ± SE in ml/100 g body wt for plasma volume (PV), cmH<sub>2</sub>O for central venous pressure (CVP), % for hematocrit (Hct), and meq/l for plasma potassium concentration ([K<sup>+</sup>]<sub>p</sub>). Body weight was not controlled in any of the groups, but headings indicate which groups had body wt controlled, or not controlled, on subsequent 4 days (see Table 3).

Table 3. Values for PV, CVP, Hct, and  $[K^+]_p$  in 5 groups of rats

Day		S, 4% NaCl, no body wt control	S, 4% NaCl, +body wt control	S, 0.2% NaCl, no body wt control	S, 0.2% NaCl, +body wt control	R, 4% NaCl, +body wt control
1	PV	6.2 ± 0.4	6.1 ± 0.5	6.6 ± 0.2	7.3 ± 0.3	5.3 ± 0.1
	CVP	-1.31 ± 0.3	-0.62 ± 0.6	-1.98 ± 0.5	-1.74 ± 0.3	-2.17 ± 0.6
	Hct	37.7 ± 1.2	37.2 ± 2.0	39.8 ± 1.8	36.7 ± 0.7	38.9 ± 0.5
	$[K^+]_p$	4.02 ± 0.12*	3.94 ± 0.08*	4.47 ± 0.09	4.72 ± 0.10	3.89 ± 0.10
2	PV	—	—	—	—	—
	CVP	-1.67 ± 0.4	-1.84 ± 0.6	-1.88 ± 0.4	-1.60 ± 0.4	-1.72 ± 0.3
	Hct	38.9 ± 1.3	37.5 ± 1.9	40.1 ± 1.8	38.1 ± 0.5	39.1 ± 1.0
	$[K^+]_p$	4.07 ± 0.14*	3.91 ± 0.09*	4.54 ± 0.08	4.95 ± 0.08	4.09 ± 0.11
3	PV	—	—	—	—	—
	CVP	-1.45 ± 0.3	-1.22 ± 0.4	-1.66 ± 0.6	-1.38 ± 0.4	-2.15 ± 0.6
	Hct	39.5 ± 1.1	37.7 ± 1.6	39.8 ± 1.8	38.6 ± 0.6	39.1 ± 1.2
	$[K^+]_p$	4.10 ± 0.14*	3.88 ± 0.06*	4.55 ± 0.17	4.86 ± 0.13	4.04 ± 0.08
4	PV	5.9 ± 0.5	6.5 ± 0.8	6.7 ± 0.4	7.2 ± 0.3	5.5 ± 0.4
	CVP	-2.38 ± 0.5	-1.48 ± 0.4	-2.4 ± 0.3	-1.56 ± 0.3	-2.08 ± 0.4
	Hct	39.4 ± 1.1	37.4 ± 1.5	39.0 ± 1.8	38.4 ± 0.5	38.4 ± 1.3
	$[K^+]_p$	4.03 ± 0.12*	4.07 ± 0.11*	4.35 ± 0.10	4.73 ± 0.07	4.16 ± 0.08

Values are means ± SE. Units of measure are same as for Table 2. \*Significantly different from values of control ( $P < 0.05$ ). Three of the groups were switched to a high-salt diet with body weight either controlled or not controlled.

arterial pressure in response to changes in dietary NaCl has been questioned (2).

As expected, 4 days of high-salt diet increased arterial pressure 31.4 mmHg with no body weight control in S rats (Fig. 2). Similarly, when body weight was controlled at zero change, 4 days of salt diet produced an increase of arterial pressure of 33.3 mmHg in S rats (Fig. 3). These data support the hypothesis that salt-induced hypertension in S rats is not volume dependent. Several other findings of our study are consonant with volume not being an obligatory step for salt-mediated hypertension in S rats. First, body weight was controlled very close to zero change from day to day (Figs. 3, 5, and 6). The absolute daily changes averaged only 0.283 g, with a range of -0.5 to 0.6 g. Because these S rats were, on average, gaining ~4.6 g/day with body weight not controlled, the control of body weight at essentially zero change represents an over correction and a decline in total body fluid volume. Second, as shown in Table 3, plasma volume, hematocrit, and

central venous pressure did not change in response to 4 days of high-salt diet in any of the groups. If volume is an important, obligatory intermediate in the blood pressure response to the high salt in SS/Jr rats, one would predict a significant increase in plasma volume and central venous pressure and a decrease in hematocrit (or at least a tendency for change in one or more of these variables). Thus these data support the hypothesis that, at least in SS/Jr rats, volume retention is not an obligatory step in the pressure increase subsequent to an oral increase in salt intake. Dobešová et al. (4) also concluded that hypertension produced by a 4% salt diet can occur in SS/Jr rats (obtained from the colony of John Rapp at the Medical College of Ohio, Toledo, OH) without expansion of intravascular or interstitial volumes. Because we studied inbred Dahl rat strains and Greene et al. (11) used outbred Brookhaven Dahl rats, the difference in results between the studies might also be contributed to by genetic differences between the animals used.

In contrast, the data support a role for a more direct effect of salt that influences blood pressure independent of the influence of salt on volume. As shown in Figs. 2 and 3, blood pressure increased in response to 4 days of high-salt diet only in the rats that had a concomitant increase in the concentration of sodium in the plasma (SS/Jr rats with body weight controlled and SS/Jr rats with no body weight control). Figure 7 shows the positive association between arterial pressure and plasma sodium concentration in the SS/Jr rats that were on high salt for 4 days both with and without body weight control. Approximately 73% ( $r = 0.85$ ) of the variation in plasma sodium concentration and arterial pressure was associated positively. Other studies have shown that directly mediated increases in plasma sodium can increase arterial blood pressure in rats by mechanisms that are apparently not related to fluid volume changes (4, 8, 9).

The daily changes in body weight in the SS/Jr rats on 4 days of low-salt diet and then 4 days of high-salt diet

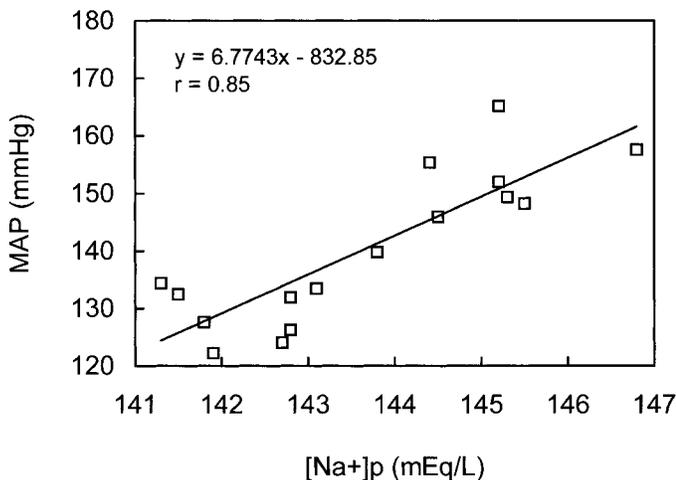


Fig. 7. Linear regression of relationship between MAP and  $[Na^+]_p$  in SS/Jr rats that were on high-salt diet while their body weight was either controlled or not controlled ( $r = 0.85$ ,  $P < 0.001$ ).

are shown in Fig. 2C. Each day the rats gained on average ~5 g. It is interesting that there was no increase in the rate of change in body weight subsequent to the start of the high-salt diet, even though the salt intake increased ~18.7-fold and the water intake increased almost threefold. Presumably, this means that there was no major change in total body fluid and that the increased water intake was excreted rapidly with no change in water balance from day to day (17). This result is consistent with a lack of significant change in plasma volume, hematocrit, and central venous pressure. Presumably, the 15.8-mmHg increase in arterial pressure that occurred on the first day of high-salt diet (Fig. 2) was one of the mechanisms that allowed a rapid excretion of water such that we recorded no change in the rate of body weight gain (28).

The role of volume expansion in the development of hypertension was originally predicted from a computer model of the circulation (13, 14). Guyton's laboratory produced evidence for the role of volume expansion in hypertension by demonstrating that body fluid volumes and cardiac output increased along with blood pressure during the development of hypertension (for review, see Ref. 15). However, other laboratories that examined the sequence of changes in blood pressure, cardiac output, and blood volume during the development of hypertension have observed contradictory results. For example, Fletcher et al. (6) and Kaneko et al. (18) found no relationship between the development of renal hypertension and changes in cardiac output. Ferrario et al. (5) and Freeman et al. (7) observed that renal hypertension developed in dogs without changes in plasma volume or mean circulatory filling pressure. Korner (19) has reviewed other evidence that is not consistent with the concept that hypertension develops as a result of a volume-dependent increase in cardiac output.

Other than our study, we are aware of only two other studies in which body fluid volumes were manipulated directly to test the volume dependence of hypertension in conscious animals. One study is the work of Greene et al. (11) cited previously. The other study, from Guyton's laboratory (25), manipulated body fluid volumes and plasma sodium concentration in sheep by dialysis, nephrectomy, and mineralocorticoid administration. Although both these studies concluded that the development of hypertension is volume dependent, they were both more invasive than the present work. Our data, obtained from intact, conscious animals ingesting salt-containing chow, appear to refute the role of volume expansion in the Guyton-Coleman model.

### *Perspectives*

If volume expansion is not an obligatory step in the development of hypertension, how can we account for the effect of NaCl ingestion on blood pressure in SS/Jr rats with body weight servo-controlled? A model that can explain our observations and suggests directions for future experiments follows.

First, from our data, an increase in NaCl intake in SS/Jr rats with body weight servo-controlled results in

an increase in plasma Na<sup>+</sup> concentration that is closely correlated to the increase in arterial blood pressure (Fig. 7).

Second, we hypothesize that this increase in plasma Na<sup>+</sup> concentration acts, likely via a central nervous system effect, to produce a widespread increase in sympathetic activity. In addition, the increased plasma Na<sup>+</sup> concentration is predicted to increase plasma vasopressin concentration via osmoreceptor stimulation. Furthermore, to the extent that blood volume may be decreased by the over correction induced by the servo-control of body weight, renin and angiotensin II concentrations in plasma will likely increase.

Third, the increase in sympathetic activity increases blood pressure by increasing either cardiac output or total peripheral resistance (TPR) or both. Increased secretion of vasopressin and angiotensin II will contribute to the increase in TPR.

Fourth, the increase in sympathetic activity to the kidney along with increases in vasopressin and angiotensin levels all act to shift the pressure-diuresis curve to the right (toward higher arterial pressures), preventing the increase in arterial pressure from being dissipated by pressure diuresis.

The fourth step in the model is essential, because if the pressure diuresis curve did not change, the increase in arterial pressure due to the increases in cardiac output and/or TPR will act directly on the kidney to induce a pressure diuresis. The pressure diuresis would then decrease plasma volume and blood pressure, counteracting the effects of cardiac output and TPR on blood pressure.

This model differs from Guyton and Coleman's original formulation in that an increase in body fluid volumes is not included as an intermediate step in the development of hypertension. However, the role of pressure diuresis is preserved in the model. There is a large body of evidence demonstrating that the effect of arterial pressure on renal excretory function is intrinsic to the kidney (for example, see Refs. 10, 27). Thus pressure diuresis may participate in arterial pressure regulation, independent of changes in body fluid volumes. Furthermore, in a series of elegant studies, John Hall and colleagues (for review, see Ref. 16) have demonstrated that increases in TPR produced by chronic infusion of vasoconstrictor hormones produce hypertension only if the renal perfusion pressure is prevented from increasing during vasoconstrictor infusion, i.e., only if a pressure diuresis is prevented. Hall et al.'s work provides the basis for the fourth step of our model, including pressure diuresis in a schema for the development of hypertension that is due at least in part to increased TPR.

In conclusion, our data provide no evidence for volume increases operating as obligatory factors in the arterial blood pressure response to a high-salt diet in SS/Jr rats. A model has been proposed that preserves the role of pressure diuresis in regulating arterial blood pressure but does not depend on volume changes acting as intermediates in adjusting pressure so that an

animal has just enough pressure to make salt and water output equal to input.

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