the Brattleboro rat, which does not contain vasopressin or NP-VP, stains only with the NP-OT antibody [M. H. Whitnall, J. Ben-Barak, K. Ozato, S. Key, H. Gainer, in preparation].
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Dietary Chloride as a Determinant of "Sodium-Dependent" Hypertension

Abstract. *The uninephrectomized rat given desoxycorticosterone (DOC) provides a classic model of "sodium-dependent" hypertension. In such rats, the extent to which a given dietary intake of sodium induced an increase in blood pressure depended on whether or not the anionic component of the sdoium salt was chloride. With normal and high dietary intakes of sodium, sodium chloride induced increases in blood pressure much greater than that induced by approximately equimolar amounts of sodium bicarbonate, sodium ascorbate, or a combination of sodium bicarbonate and sodium ascorbate. A normal amount of dietary sodium chloride induced hypertension, whereas an equimolar amount of sodium bicarbonate did not increase blood pressure. This difference could not be attributed to differences in sodium or potassium balances, weight gain, or caloric intake. The DOC model of "sodium-dependent" hypertension might better be considered sodium chloride–dependent.*

In many clinical settings, restriction of dietary sodium chloride (NaCl) can mitigate, and its supplementation can exacerbate, hypertension, an abnormally increased blood pressure (*1*). Although Ambard and Beaujard suggested in 1904 that increased retention of chloride might be important in the pathogenesis of hypertension (*2*), Dahl, in 1954, stated the now prevalent view that "the sodium ion alone is important" [in the pathogenesis of hypertension] (*3*). This view, however, is based almost entirely on studies in which dietary sodium has been varied mainly by varying the dietary intake of NaCl (*4*). It is now recognized that the chloride ion (*5*), like the bicarbonate ion (*6, 7*), can be specifically transported by a variety of tissues and exert its own biological effects. The pathogenesis of NaCl-dependent hypertension might therefore depend on the dietary intake of chloride. We now report a positive test of this hypothesis in a well-characterized model of "sodium-dependent" hypertension, the uninephrectomized rat given desoxycorticosterone and dietary sodium (*8*). This mineralocorticoid hormone stimulates the kidney to reclaim both sodium and chloride and can induce continued retention of both ions when administered over time in pharmacological amounts (*9*).

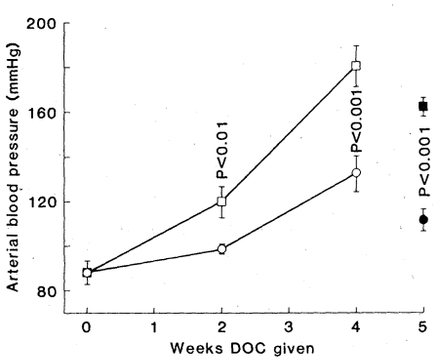

Fig. 1. Squares and circles represent the measured blood pressure of rats that consumed an average of 40.4 mmole of sodium chloride per week (N = 8) or 40.9 mmole of a combination of sodium bicarbonate and sodium ascorbate per week (N = 8), respectively. Open symbols denote systolic pressures measured with the tail plethysmography method. Closed symbols denote mean arterial pressures measured directly through femoral artery catheters placed 3 hours beforehand. Symbols and their brackets indicate means ± standard errors. P values indicate statistical differences (one-tailed Student's t-test) between the group receiving sodium chloride and the group receiving sodium bicarbonate and sodium ascorbate at the times indicated. In a similar experiment (results not depicted), the blood pressures in six rats given DOC and NaCl (38.5 mmole per week) were significantly greater than those in six rats given DOC and $NaHCO_3$ (38.8 mmole per week).

Male Sprague-Dawley rats (100 to 150 g) were uninephrectomized and individually housed in metabolic cages (Nalge Company, No. 650-0100) so that food and fluid intake could be controlled and sodium and potassium balances cumulatively measured. Desoxycorticosterone (DOC) was injected intramuscularly as Percorten pivalate (Ciba-Geigy), 50 mg/kg initially and 25 mg/kg each week thereafter. All animals were fed a NaCl-deficient, natural ingredient diet (Teklad) (sodium content less than 0.03 mmole/g). Dietary sodium was thus provided mainly by adding sodium to the drinking water as near equimolar amounts of NaCl, sodium bicarbonate ($NaHCO_3$), sodium ascorbate (Na-ascorbate), or a combination of sodium bicarbonate and sodium ascorbate ($NaHCO_3$/Na-ascorbate). The sodium concentrations of the solutions ranged from 70 to 172 mmole/liter depending on the weekly dietary sodium intake being studied. The daily amount of food and fluid provided to each animal drinking the solution of NaCl was equal to that amount consumed by a partner drinking the solution of $NaHCO_3$ or Na-ascorbate or both. The animals drinking the solution of NaCl usually consumed all of the food and fluid provided to them.

Systolic blood pressures and body weights were similar for all rats before DOC was administered. Afterward, at dietary intakes of sodium ranging from approximately 17 to 40 mmole per week, the systolic or mean blood pressures of rats given NaCl were significantly greater than those of rats given $NaHCO_3$ or Na-ascorbate or both (Figs. 1 and 2). At the lowest dietary intake of sodium employed, approximately 17 mmole per week, the blood pressure of rats given $NaHCO_3$ with DOC was not significantly different from that of rats given NaCl without DOC (Fig. 2). With DOC, however, this normal amount of NaCl (*10*,

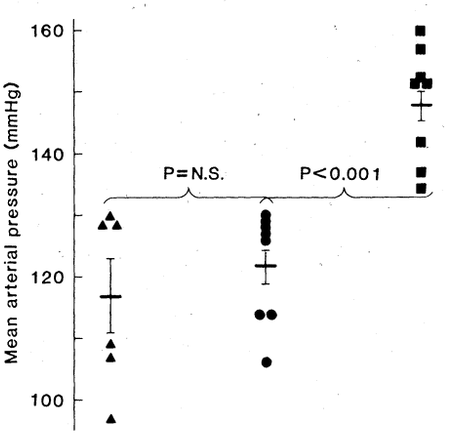

Fig. 2. Mean arterial pressures (measured through femoral artery catheters implanted 24 hours beforehand) in conscious, unrestrained, uninephrectomized rats given sodium chloride without desoxycorticosterone pivalate (DOC) (▲, N = 6), sodium bicarbonate with DOC (●, N = 8), or sodium chloride with DOC (■, N = 8), in amounts of 16.1, 17.9, and 16.6 mmole of sodium per week, respectively, for 5 weeks. Horizontal bars and their brackets indicate group means ± standard errors. P values indicate statistical differences between group means by Student's one-tailed t-test with the Bonferonni correction for multiple comparisons. Metabolic data from these studies are presented in Table 1. In a similar experiment (results not depicted), the mean arterial pressures of eight rats given NaCl, 22.8 mmole per week for 5 weeks, with DOC were significantly greater than those of five rats given sodium ascorbate, 24.7 mmole per week for 5 weeks, with DOC (mean = 169 ± 7 versus 134 ± 3 mmHg, respectively; $P < 0.01$).

Table 1. Physiologic measurements in uninephrectomized rats given NaCl without desoxycorticosterone pivalate (DOC) (group 1), $NaHCO_3$ with DOC (group 2), or NaCl with DOC (group 3) in amounts of 16.1, 17.9, and 16.6 mmole per week, respectively, for 5 weeks. External electrolyte balances are calculated by subtracting total sodium and potassium output (from urine and feces) from total sodium and potassium input (from food and fluid). All balances of Na^+ and K^+ are positive. Values are means ± standard errors. Statistical analysis was performed using Student's one-tailed *t*-test with the Bonferonni correction for multiple comparisons. Statistical significance is defined as $P < 0.05$. Similar results (not shown) were obtained in other studies that compared rats given DOC and NaCl to those given: (1) DOC and $NaHCO_3$ (38.8 mmole per week), (2) DOC and Na-ascorbate (24.7 mmole per week), or (3) DOC and the combination of $NaHCO_3$ and Na-ascorbate (40.9 mmole per week), 41 rats being studied in total.

Group	*N*	Body weight (g)	External balances		Arterial blood			Plasma electrolytes (mmole/liter)		
			Na^+ (mmole/100g body weight)	K^+	*p*H	PCO_2 (mmHg)	HCO_3^- (mmole/liter)	Na^+	K^+	Cl^-
1 NaCl without DOC	6	308 ± 10			7.47 ± 0.01	37.8 ± 1.1	26.3 ± 0.9	141 ± 3	4.3 ± 0.3	101 ± 2
2 $NaHCO_3$ with DOC	8	295 ± 6	13.2 ± 0.2	3.8 ± 0.4	7.58 ± 0.01*	47.3 ± 1.2*	43.1 ± 1.4*	143 ± 2	2.1 ± 0.1*	82 ± 2*
3 NaCl with DOC	8	300 ± 12	11.9 ± 0.7	4.2 ± 0.8	7.51 ± 0.01†	38.8 ± 1.1	30.1 ± 0.9†	141 ± 2	3.3 ± 0.1†	93 ± 2†

*Significant difference when comparing group 2 versus group 1 and group 2 versus group 3. †Significant difference when comparing group 1 versus group 3.

11) induced a clear-cut increase in blood pressure (Fig. 2), as expected (*11, 12*).

In patients with chronic renal failure, Husted *et al.* found that orally administered $NaHCO_3$ (200 mmole/day for 4 days) did not induce an increase in blood pressure, whereas an equimolar amount of NaCl did (*13*). Although the urinary excretion of sodium in patients given $NaHCO_3$ was greater than that of patients given NaCl, a significant degree of sodium retention occurred in both groups. In preliminary communications of studies in patients with essential hypertension and in the Dahl salt-sensitive rat, chronic oral loading with $NaHCO_3$ was reported to have induced a lesser increase in blood pressure than that induced by oral loading with NaCl (*14*).

In the currently studied rats given DOC and NaCl, the finding that blood pressure was greater than that in rats given DOC, together with $NaHCO_3$ or Na-ascorbate or both, could not be attributed to a greater caloric intake or weight gain, a greater positive balance of sodium or water, or a lesser positive balance of potassium (Table 1). However, in the rats given NaCl, a greater urinary excretion of calcium than that occurring in rats given $NaHCO_3$ (*7, 15*) might have resulted in a lesser positive external balance of calcium and thereby a greater blood pressure (*16*).

Alterations in blood *p*H and electrolyte concentrations can affect local control of vascular resistance (*17*): hypokalemic alkalosis would be expected to increase vascular resistance and it has been proposed that hypokalemia and metabolic alkalosis contribute to vasoconstriction in mineralocorticoid hypertension (*17*). In the present study, however, the blood pressure was lower when the severity of the hypokalemia and alkalosis was greater (Table 1). The finding that hypokalemia was more severe in rats given $NaHCO_3$ or Na-ascorbate, or both, than that in rats given NaCl could reflect more severe potassium depletion but also only a greater shift of potassium into cells resulting from more severe metabolic alkalosis (*18*). Under certain circumstances, potassium depletion may reduce blood pressure (*19*). In a study of two sodium-restricted patients with apparently essential hypertension, restriction of dietary potassium was inferred to have prevented blood pressure from increasing further when DOC was administered (*20*). No evidence was presented, however, that administration of DOC would have exacerbated hypertension in these patients even had their dietary intake of potassium been maintained normal. In a study in rats in which potassium deficiency is said to have prevented the hypertension otherwise predictably induced by DOC (*21*), sodium intake, caloric intake, and growth were not controlled.

An "autoregulatory" increase in total peripheral resistance that follows a blood volume–mediated increase in cardiac output is thought by some investigators (*22*) to be the central pathogenetic mechanism of mineralocorticoid hypertension, although there is evidence to the contrary (*23*). However, in both man and rat, oral administration of $NaHCO_3$ is said to expand plasma volume to the same extent as does NaCl (*24*). It has been suggested that blood volume is redistributed centrally in patients with metabolic acidosis (*25*) and in patients and animals with some forms of hypertension (*26*). In the current study, $NaHCO_3$ or Na-ascorbate, or both, might have induced a lesser increase in blood pressure than that induced by NaCl because of their having induced a greater severity of metabolic alkalosis and, as a consequence, a lesser increase in central blood volume and hence in cardiac output as well.

It has recently been suggested that an active chloride pump may be present in smooth muscle (*27*). Accordingly, dietary chloride might be a pathogenetic determinant of DOC hypertension through a mechanism involving altered vascular reactivity. Whatever the mechanism for the chloride dependence of the DOC model of hypertension, it seems clear that in this model of "sodium-dependent" hypertension, the anionic component of the sodium salt consumed can determine the occurrence of hypertension. It seems possible that in other forms of "sodium-dependent" hypertension, the anionic component of the sodium salt consumed might also be a critical pathogenetic determinant. In any case, given the sketchy understanding of nutritional factors as pathogenetic determinants of hypertension, it seems prudent to speak of sodium chloride–dependent hypertension rather than "sodium-dependent" hypertension.

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X-ray–Induced Breakage and Rejoining of Human Interphase Chromosomes

Abstract. *A method was developed for the high-resolution measurement of breaks in prematurely condensed chromosomes at the G_1 phase of the cell cycle. The dose response for fragments (breaks) produced immediately after x-irradiation of confluent cultures of normal human cells was linear down to 10.9 rad (0.109 Gy) and extrapolated to zero effect at zero dose. The curve had a slope of 0.063 breaks per cell per rad, which is at least an order of magnitude greater than that for breaks scored in the same cells after they have progressed to mitosis following subculture. When incubated at 37°C half of the breaks disappeared in 2 hours. A slower, perhaps nonrejoining component was apparent at later incubation times. The initial rate of break rejoining was similar to the rate of increase in survival after incubation because of the repair of potentially lethal damage and is also in close agreement with recently reported values for the rejoining of double-strand breakage in DNA.*

The issue of risk assessment from exposure to ionizing radiation centers around oncogenic, mutagenic, and teratogenic effects in humans exposed to low doses of radiation. Since precise measurement of the effects of low doses in humans or mammals is not possible, we must rely on extrapolations of mathematical models that describe dose-effect data for much higher doses. Unless such models are based on sound biophysical principles the issue cannot begin to approach a satisfactory settlement. It is possible that a dose-response relation fits the data well at higher doses, but deviates appreciably from extrapolated values at low doses. For example, at high doses the effects may be governed by the response of a resistant subpopulation of cells, whereas at low doses they may be governed by a relatively small sensitive subpopulation. Since chromosomal breaks and rearrangements may play a crucial role in both mutagenesis and oncogenesis following exposure to ionizing radiation (*1*, *2*), studies of their dose-effect and rejoining kinetics are germane to the biophysical principles we seek.

In 1970 Johnson and Rao (*3*) discovered that the fusion of mitotic and interphase cells resulted in the premature condensation of interphase chromosomes. Shortly thereafter, Waldren and Johnson (*4*), and Hittleman and Rao (*5*), and others, used this technique to study the breakage of chromosomes immediately after they occurred, and the rejoining of these breaks during interphase. However, several problems prevented the adoption of this approach for high-resolution studies at low doses. Since the prematurely condensed interphase chromosomes (PCC's) and their fragments often overlap and intermingle with the mitotic chromosomes, some are easily obscured. For low-dose studies the uncertainty associated with the detection of

A B

Fig. 1. (A) Prematurely condensed G_1 chromosomes (dark magenta) of an AG1522 human cell fused with a mitotic HeLa cell (light pink chromosomes) which had been grown for several generations in F12 medium supplemented with 10 percent fetal bovine serum and containing 5×10^{-6} mole of BrdU per liter. The cell was stained with Giemsa after treatment with Hoechst 33258 dye and exposure to near-ultraviolet light. (B) Prematurely condensed G_1 chromosomes of an AG1522 cell fused with a mitotic HeLa cell and stained with Giemsa in the conventional way. In some cases (not shown) the PCC's were more segregated toward one edge of the cell, but it is still not possible to tell whether a fragment is lost among the mitotic chromosomes.