temporarily unnecessary and recycling their amino acids while expending abundant photosynthetic energy (*12*).

However, the control of luciferase through its turnover may be one example of a more general strategy whereby the biological clock regulates enzymes. In modulating metabolic pathways, the clock regulates the activity of enzymes that are rate-limiting [such as *Gonyaulax* luciferase, pineal *N*-acetyltransferase, liver tyrosine aminotransferase, and β-hydroxy–β-methylglutaryl (HMG) coenzyme A (CoA) reductase]; rate-limiting enzymes are usually degraded rapidly, as with tyrosine aminotransferase and HMG CoA reductase (*13*). Therefore, since the daily clock regulates rate-limiting enzymes whose half-life is only a small fraction of the circadian period, it seems reasonable that it should exploit their characteristically rapid turnover. Indeed, our findings support the proposal that synthesis and degradation are involved in the control mechanism of pineal *N*-acetyltransferase (*14*) and hepatic HMG CoA reductase (*15*). Enzyme turnover may therefore be a common mode of circadian control over biochemical pathways.

CARL HIRSCHIE JOHNSON
JAMES F. ROEBER
J. W. HASTINGS

Biological Laboratories,
Harvard University,
Cambridge, Massachusetts 02138

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8. Relative amounts of luciferase were determined by electrophoresing and blotting various dilutions of day and night luciferase and comparing the ratio of titer to density of the luciferase band in the linear range by analogy with the messenger RNA "dot blot" method [B. A. White and F. C. Bancroft, *J. Biol. Chem.* **257**, 8569 (1982)].
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16. *Gonyaulax* cells (strain 70) were grown at 19° to 20°C (*5*). At given intervals 1 liter of cell culture (1×10^4 to 1.2×10^4 cells per milliliter) was harvested (without photoinhibition) by filtration (Whatman 541) and resuspended in 5 ml of extraction buffer (100 m*M* tris, 10 m*M* EDTA, and 5 m*M* 2-mercaptoethanol, *p*H 8.5 and 4°C). The cells were broken by passage through a Kirkland emulsifier (Brinkmann); cell debris was removed by a 10-minute centrifugation at 800*g*. This supernatant constitutes the total protein fraction (including organelles). A portion of this supernatant was then centrifuged for 20 minutes at 27,000*g*, giving the soluble protein fraction.
17. Luciferase activity was assayed (*5*) at *p*H 6.3 with a sufficient dilution of the extract for linear response (usually a 1:100 dilution).
18. Soluble and total protein samples were subjected to electrophoresis through sodium dodecyl sulfate–polyacrylamide gels and transferred to nitrocellulose paper overnight [H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979)]. This "blot" was reacted with antibody to luciferase, washed, and reacted with ^{125}I-labeled protein A (H. Towbin *et al.*, *ibid.*). After a final washing, the immunoblot was autoradiographed for 1 to 3 days with or without an intensifying screen (an intensifying screen was used for Figs. 1B and 2B, but the densitometer data in Figs. 1A and 2A are from the same blots autoradiographed without screens).
19. This report is dedicated to Colin S. Pittendrigh on the occasion of his 65th birthday. We thank L.-A. Yeh and L. Safranek for their assistance and advice and J. Dunlap for suggestions and antibody. Supported by NIH postdoctoral fellowship GM08288 (C.H.J.) and NIH grant GM19536 (J.W.H.).

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Salt-Sensitive Hypertension: Contribution of Chloride

Abstract. *The effect of the anion associated with sodium loading on the development of hypertension in the Dahl salt-sensitive rat was determined. For 5 weeks rats were fed a diet containing normal or high concentrations of sodium chloride or high concentrations of sodium provided as a mixture of sodium bicarbonate, phosphate, and amino acids. After 1 week on these diets and until the end of the study the rats receiving high concentrations of sodium chloride had higher systolic blood pressures than the rats in the other two groups. There were no statistically significant group differences in plasma volume, arterial* p*H, or plasma concentrations of* Na^+, K^+, Cl^-, Ca^{2+}, *or creatinine, or in renomedullary prostaglandin* E_2 *production. Compared to the animals receiving normal concentrations of sodium chloride, those receiving high concentrations of sodium chloride or amino acids showed decreased plasma renin activity and plasma aldosterone concentrations. Thus, the anion ingested with sodium alters the development and severity of hypertension in the Dahl salt-sensitive rat.*

We previously proposed that inhibition of renin release by sodium chloride is specifically related to a renal tubular effect of chloride (*1, 2*). In 1850, Redtenbacher and Gesell reported that urinary chloride excretion decreased with febrile illness (*3*), and in 1904 Ambard and Beaujard reported that a salt-restricted diet resulted in lowering of urinary chloride excretion which was associated with a decline of blood pressure in hypertensive patients (*4*). The prominence of chloride rather than sodium in these early reports was related to the ease with which it could be measured. However, with the advent of techniques for measuring sodium, interest in salt-dependent hypertension became focused on sodium. In the study described here, we evaluated the importance of chloride to hypertension in the Dahl salt-sensitive (Dahl S) rat—an experimental model of sodium chloride–induced hypertension.

Lewis K. Dahl bred Sprague-Dawley rats on the basis of their predisposition to develop hypertension on a high sodium chloride diet (*5*). We first compared the effects on blood pressure of adding 8 percent sodium chloride or equimolar sodium bicarbonate to the diets of 7-week-old Dahl S rats (*6*). Despite a massive positive sodium balance, blood pressure did not increase in the sodium

Table 1. Body weight, net electrolyte balance, and muscle electrolyte content in Dahl S rats on three different diets. Results shown are mean ± standard error of the mean.

Diet	Body weight (g)		Cumulative electrolyte balance (mEq/100 g body weight for 5 weeks)			Muscle electrolyte content (μEq/g dry weight)		
	Start of study	End of study	Na^+	K^+	Cl^-	Na^+	K^-	Cl^-
Normal NaCl	227.8	394.3*	12.74*	31.38*	11.97*	84.4	457.0	90.9
(N = 8)	±7.7	±10.2	±0.44	±1.57	±0.61	±3.3	±24.6	±7.9
High NaCl	220.4	355.6	70.95*	23.16	53.33*	86.7	457.8	90.7
(N = 8)	±6.2	±6.3	±2.33	±0.45	±1.95	±3.9	±13.9	±6.8
High NaAA	212.6	358.1	80.81	20.97	7.53	99.1*	470.3	89.9
(N = 8)	±5.4	±7.3	±2.92	±0.91	±0.53	±5.0	±13.5	±4.0

*$P < 0.05$ (or less) compared to the other two groups (Newman-Keuls test).

Table 2. Plasma values for Dahl S rats at the end of the study. Results shown are mean ± standard error of the mean.

Diet	Concentration (mEq/liter)				Protein (g/dl)	Creatinine (mg/dl)	Hematocrit (%)	*p*H	PCO_2 (mmHg)
	Na^+	K^+	Cl^-	Ca^{2+}					
Normal NaCl	140.9	4.8	101.1	2.33	4.9	1.3	45.0	7.42	46.4
(N = 8)	±0.7	±0.1	±1.3	±0.09	±0.1	±0.2	±0.7	±0.01	±0.6
High NaCl	139.8	5.0	103.3	2.35	4.6	1.2	46.8	7.43	46.1
(N = 8)	±0.6	±0.1	±1.2	±0.09	±0.1	±0.2	±0.4	±0.02	±1.1
High NaAA	140.8	4.7	101.1	2.33	4.4	1.2	45.0	7.45	45.5
(N = 8)	±0.7	±0.3	±1.3	±0.10	±0.2	±0.2	±1.0	±0.02	±0.4

bicarbonate–fed animals, whereas the sodium chloride–fed animals became markedly hypertensive. However, in contrast to the sodium chloride–fed animals, the sodium bicarbonate–fed animals failed to gain weight and were alkalotic, hypokalemic, and markedly hypochloremic. Kurtz and Morris subsequently reported that sodium bicarbonate loading in contrast to sodium chloride loading, did not produce hypertension in uninephrectomized rats given desoxycorticosterone (*7*) but did result in hypokalemia and alkalosis. In both our study and that of Kurtz and Morris, the sodium bicarbonate–fed animals had received a low sodium chloride diet. A similar syndrome of failure to thrive, hypochloremia, and hypokalemic metabolic alkalosis has also been observed in human infants fed a chloride-deficient diet (*8*).

In the present study with the Dahl S rat, we compared blood pressure responses to sodium chloride and equimolar sodium loading provided as a mixture of nonchloride-containing sodium salts. To prevent chloride depletion, we added both sodium chloride and the nonchloride-sodium salt mixture to a "normal" sodium chloride diet (1 percent NaCl; 0.17 mEq of Na^+ per gram, and 0.19 mEq of Cl^- per gram).

Twenty-four male Dahl S rats (from Brookhaven National Laboratory) were weaned at 4 weeks and then fed a normal (1 percent) NaCl diet (Ralston Purina) for 3 weeks. We then began the study by dividing the animals into three groups of eight animals each. The first group remained on the 1 percent NaCl diet; the second group was fed a 7 percent NaCl diet (1.20 mEq of Na^+ per gram); and the third group received a diet equimolar in sodium, provided as sodium phosphate, bicarbonate, and amino acids (NaAA). To construct the NaAA diet, we added the following to 100 g of the 1 percent NaCl diet: 5 mEq of sodium phosphate, 15 mEq of sodium bicarbonate, 20 mEq of monosodium aspartate, 20 mEq of monosodium glutamate, and 40 mEq of monosodium glycinate. We had previously determined that animals eating this NaAA diet gained weight at a normal rate.

For the duration of the study, the animals were housed in individual metabolic cages and allowed free access to deionized water. Weight and systolic blood pressure (SBP) were measured twice weekly in unanesthetized rats. Blood pressure was measured by tail plethysmography after placing the animals in an incubator at 37°C for 15 minutes. Electrolyte balances were calculated as dietary intake minus urinary excretion. Although fecal electrolytes were not measured, in our earlier study of sodium bicarbonate loading in the Dahl S rat (*6*) we found that daily fecal sodium excretion was less than 1 percent of urinary sodium excretion and did not differ in sodium chloride– and sodium bicarbonate–fed animals. In the current study, after remaining on the various diets for 5 weeks, all animals were anesthetized with sodium pentobarbital (40 mg/kg body weight). Direct intra-arterial pressure measurements were recorded from a femoral artery and plasma volume was determined with the use of radioiodinated serum albumin. The animals were quickly killed and thigh muscles were removed for determination of tissue electrolytes by means of nitric acid digestion. Plasma renin activity was measured in quadruplicate with the radioimmunoassay of Haber *et al.* (*9*). Plasma aldosterone levels and prostaglandin E_2 (PGE_2) production in vitro from slices of renal medulla-papilla were measured by radioimmunoassay (*10, 11*). Analysis of variance and the Newman-Keuls' multiple range test were used to test statistical significance. Data are expressed as mean and standard error of the mean.

Systolic blood pressure increased progressively in the animals fed 7 percent NaCl (Fig. 1). From week 2 until the end of the study the SBP of animals eating 7 percent NaCl was higher than that of animals on 1 percent NaCl or NaAA. At no time were there statistically significant blood pressure differences between the 1 percent NaCl and NaAA groups. Similarly, at the time the rats were killed, the direct intra-arterial mean pressures of the animals fed 7 percent NaCl (148 ± 10 mmHg) were higher ($P < 0.05$) than respective values in either the NaAA-fed animals (114 ± 10 mmHg) or the animals on the 1 percent NaCl diet (113 ± 7 mmHg).

Before starting the separate diets, the rats showed no statistically significant differences in body weight (Table 1). At the time they were killed, the animals eating 7 percent NaCl and those eating the NaAA diet showed no statistically significant differences in their body

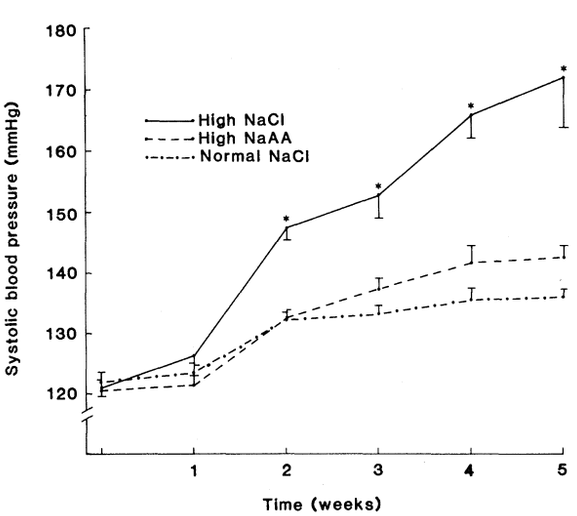

Fig. 1. Effect of the three diets on systolic blood pressure of Dahl S rats. A two-way analysis of variance with repeated measures on the second factor and the Newman-Keuls' multiple range test were used to test statistical significance; $^*P < 0.01$ compared to the other two groups. Animals were 7 weeks old at the start of the diets; $N = 8$ in each group. Error bars show standard error of the means.

Table 3. Plasma volume, plasma renin activity, aldosterone concentration, and in vitro PGE_2 production. Results shown are mean ± standard error of the mean.

Diet	Plasma volume (ml/100 g)	Plasma renin activity (ng ml^{-1} $hour^{-1}$)	Aldosterone (ng/dl)	PGE_2 (ng mg^{-1} 60 min^{-1})
Normal NaCl (N = 8)	4.13 ± 0.16	7.1 ± 1.0*	72.3 ± 4.5*	0.58 ± 0.07
High NaCl (N = 8)	4.46 ± 0.29	1.2 ± 0.2	45.1 ± 3.1	0.61 ± 0.07
High NaAA (N = 8)	4.27 ± 0.11	1.4 ± 0.3	41.4 ± 2.1	0.60 ± 0.11

*$P < 0.01$ compared to the other two groups (Newman-Keuls test).

weights, but the animals eating 1 percent NaCl were heavier than the rats in the other two groups. Compared to that in animals on 1 percent NaCl, the net 5-week sodium balance was more positive and potassium balance less positive in both of the sodium-loaded groups. The sodium balance of the NaAA group was actually greater than that of the 7 percent NaCl group. The net chloride balance of animals on 7 percent NaCl was greater than that of the other two groups, and the chloride balance of the animals on 1 percent NaCl was slightly greater than that of the animals fed NaAA. The muscle sodium content of the group on NaAA was greater than that of the other two groups, but muscle potassium and chloride contents did not differ among groups.

At the time the rats were killed there were no statistically significant differences among groups in plasma concentrations of Na^+, K^+, Cl^-, creatinine, total protein, ionized calcium, arterial pH, CO_2 pressure, or hematocrit (Table 2). Similarly, plasma volume did not differ among groups (Table 3). Compared to the respective values in the 1 percent NaCl group, plasma renin activity and plasma aldosterone were lower in the 7 percent NaCl and NaAA groups. Renomedullary PGE_2 production did not differ among the three groups.

It has been suggested that hypertension in the Dahl S rat is related to an inability of the kidney to excrete sodium, since the kidney of this rat requires a higher renal perfusion pressure than the kidney of the salt-resistant rat to excrete a comparable sodium load (*12*). However, our observations suggest that hypertension development in the Dahl S rat may be more closely related to dietary chloride consumption than to sodium consumption. It is unlikely that the absence of hypertension in the rats consuming a high dietary intake of sodium is related to a nonspecific effect of the amino acids, since the sodium bicarbonate–fed animals also did not develop hypertension. Furthermore, in a separate experiment we have found that glycine loading does not lower blood pressure in a "one-kidney, one-clip" model of hypertension (one kidney removed and partial occlusion of the renal artery to the remaining kidney).

Our results support Dahl's original observation that hypertension in the Dahl S rat is not due to an increase in whole body sodium content when compared to the normotensive Dahl salt-resistant rat (*13*). Tobian *et al.* suggested that hypertension may be related to increased sodium content in the walls of arterioles (*14*). Although we found that the sodium content of skeletal muscle was greater in animals fed nonchloride-containing sodium salts than in sodium chloride–fed animals, we did not examine the sodium content of vascular smooth muscle. Hypertension in the Dahl S rat has not been found to be renin dependent (*15*). In the present study, plasma renin activity and plasma aldosterone did not differ in the two sodium-loaded groups. It has also been suggested that decreased synthesis of PGE_2 may be important in the genesis of hypertension, since PGE_2 acts as a vasodilator (*16*). PGE_2 also inhibits absorptive chloride transport in the thick ascending limb in the loop of Henle and hence may also be natriuretic (*17*). Limas *et al.* reported that renomedullary PGE_2 synthesis in the Dahl S rat is decreased compared to that of both the Dahl salt-resistant rat and the normal Sprague-Dawley rat (*18*). However, in the present study we found that renomedullary PGE_2 production did not differ among groups of Dahl S rats consuming different sodium and chloride diets.

In the Sprague-Dawley rat on a low sodium chloride diet, we previously reported that plasma renin activity is not inhibited by dietary supplementation or acute intravenous infusion of sodium salts other than sodium chloride, and we suggested that inhibition of renin release by sodium chloride is related to increased absorptive chloride transport in the thick ascending limb of the loop of Henle (*1, 2, 6*). In the present study, the Dahl S rats were on a "normal" rather than low sodium chloride diet, and plasma renin activity and aldosterone were suppressed comparably by dietary supplementation with either NaCl or NaAA. If one assumes comparable responsiveness of the Dahl S rat and the Sprague-Dawley rat, these results are consistent with our hypothesis that the renin response to sodium loading is dependent on chloride intake. Recent evidence suggests that sodium and chloride are cotransported in the thick ascending limb of the loop of Henle and that this active transport process is dependent on both tubular fluid chloride and sodium (*19*). Consequently, in the rat, increasing the chloride intake from a low to a normal level may result in sufficient chloride uptake in the thick ascending limb of the loop of Henle such that large increases in sodium intake inhibit renin release, regardless of its accompanying anion.

In summary, the development of hypertension in the Dahl S rat is dependent on the provision of sodium as sodium chloride. This observation has implications for future studies of mechanisms by which sodium chloride produces hypertension in a susceptible host.

SHIRLEY A. WHITESCARVER
COBERN E. OTT
BRIAN A. JACKSON
Department of Physiology, University of Kentucky College of Medicine, Lexington 40536
GORDON P. GUTHRIE, JR.
THEODORE A. KOTCHEN
Department of Medicine, University of Kentucky College of Medicine

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