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# Circadian-Dependent Effect of Sodium Chloride Intake on the Development of Hypertension in Rats (41034)

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*Abstract.* The possibility that circadian phase of NaCl intake may play a role in the development of hypertension was evaluated with the use of DOCA-implanted rats, receiving either saline (1%) or water as follows: Group I, saline *ad libitum;* Group II, saline from 6 PM to 6 AM; Group III, saline from 6 AM to 6 PM; Group IV, water *ad libitum.* All animals were kept on a 12:12 light schedule (lights on from 6 AM to 6 PM) and regular rat chow *ad libitum.* The mean daily fluid intake (ml/100 g body wt/24 hr) during the first 2 weeks of the experiment was: 57 ± 1.5; 59 ± 1; 45 ± 1; and 25 ± 1.6 for Groups I, II, III, and IV, respectively. On the third experimental week, the mean systolic BP (mm Hg) ± SE was as follows: Group I, 154 ± 4.1; Group II, 140 ± 3; Group III, 154 ± 5; Group IV, 132 ± 4.5. The data suggest that hypertension develops more readily among rats ingesting excessive salt loads while at rest (circadian phase of low natriuresis) than among those doing so during the active phase (circadian phase of high natriuresis) of their day.

The possible role of sodium in the pathogenesis of hypertension and the blood pressure lowering effect of low salt diets have been recognized since the turn of the century (1). In this regard, the work of Ambard and Beaujard (1) as well as those of Allen (2) and Kempner (3) can be considered as landmarks upon which currently accepted low-salt regimens for the management of hypertension are based. Subsequent investigative work done both with man and the use of laboratory animals support the existence of a direct relationship between salt and high blood pressure (4–8). Genetic factors may, in many individuals, be linked to sensitivity to salt and its adverse effect on blood pressure (9, 10). More recently, alterations in electrolyte handling mechanisms have been identified in erythrocytes of hypertensive individuals. It has been suggested that these alterations may be useful in the identification of patients with a high risk of becoming hypertensive (11–13). Yet, despite this wealth of accumulated knowledge, the mechanism(s) through which high-sodium intake leads to hypertension remains undefined.

In attempts to gain additional insight into the salt:hypertension relationship, from a chronobiological point of view, studies were carried out in which *time* of salt intake represented the main variable within the DOCA–salt hypertension model in rats. Recognizing that natriuresis occurs with a distinct circadian periodicity, we decided to investigate whether not only the total amount of daily sodium but also the time of its intake could in any way play a role in the development of hypertension. The results obtained are presented in this communication.

**Materials and Methods.** Charles River, S/D strain, male rats weighing 75 ± 1.1 g (mean ± SE) at arrival, were acclimatized to our animal quarters for 1 week prior to the beginning of the experiment. The animals were kept five per cage, with regular rat chow and water *ad libitum.* The temperature in the room was maintained at 23 ± 1° and on a programed 12:12 hr light schedule with lights on from 6 AM to 6 PM (0600 to 1800 hr).

The experiment was started with sc implantation of a pellet containing: deoxycorticosterone (DOCA) 10 mg + carbowax 6 mg + flexowax 4 mg (14). The animal's weights ranged from 100 to 134 g (mean ± SE = 119 ± 1.3) on the day of DOCA implantation. Following DOCA treatment, the rats were placed on the following fluid regimen: Group I, saline (NaCl 1%) *ad libitum;* Group II, saline available from 6



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PM to 6 AM; Group III, saline available from 6 AM to 6 PM; and Group IV, water *ad libitum*. The animals in Groups II and III had their fluid available for 12 hr a day only, to assure the intake of saline exclusively. Other conditions remained as during the acclimatization week.

Total fluid intake was calculated as milliliters per 100 g of body wt/24 hr. These mean values were obtained from daily measurements of remnant fluid from their respective containers (two containers/cage, each with 600-ml capacity). Systolic blood pressures were measured by the tail-cuff method with the use of a desk model physiograph DMP-4A and electrosphygmomanometer PE-300 (Narco Bio-Systems, Inc.). Three weekly blood pressure readings were made on each rat. The three blood pressure values were then averaged to give blood pressures at 1, 2, 3, and 4 weeks after DOCA implant. The individual weekly values were then pooled with those of animals in the same experimental group, so as to give us a weekly BP reading/group ± SE of the mean values. Hypertension was considered as established when mean systolic blood pressure was above 150 mm Hg. All animals were weighed daily, five times/week.

**Results and Discussion.** The therapeutic success of low-sodium diets and of natriuretic agents in the management of hypertension strongly suggest that high-sodium intake may play a key role in the pathogenesis of essential hypertension. This suggestion is supported by a wealth of clinical and experimental evidence accumulated over the past 8 decades (1–13, 15). Yet, the mechanism(s) through which high-sodium intake causes hypertension remains undefined.

The recognition that natriuresis occurs with distinct circadian periodicity led us to look for additional information on salt and hypertension, from a chronobiological point of view (16–22). In the human, natriuresis is known to be significantly higher during the morning and midafternoon than during the evening or night (sleep) hours of the day (18–26). Within this framework our leading question was: Could cultural preferences and social habits (23–24) in the human condition dictate the use of physiologically unsound meal schedules (e.g., main daily meal and snacks in the evening) and thereby add to the overall "salt load" problem (ingestion of >10 g NaCl/day) with a "time of loading" strain on renal handling mechanisms for sodium excretion? We felt that it could and, with the use of the DOCA–salt hypertension model, an attempt was made to look into the possible relationship between time of salt intake and the development of hypertension. Considerations were given to two possible approaches to the experimental work:

1. Circadian manipulation of sodium chloride intake in DOCA-implanted rats.
2. Administration of single daily doses of deoxycorticosterone, at different circadian phases.

The first approach was chosen for our initial experiment, as it would allow us to examine—in the presence of steady blood and tissue levels of DOCA—the influence of schedules of sodium intake upon onset, progress, and final levels of hypertension. Thus, for the purpose of the experiment, 38 DOCA-implanted rats were separated into four groups and each placed on a different fluid intake regimen as described under Materials and methods. The rises in systolic blood pressure, as observed through the 4 weeks of the experiment, are shown in Fig. 1.

As seen in Fig. 1, those animals on either saline *ad libitum* or on saline from 0600 to 1800 hr sustained a more rapid increase in blood pressure than those rats ingesting saline from 1800 to 0600 hr.

The rise in systolic blood pressures among the rats on DOCA–water (Group IV), while lagging behind those of all three saline groups, was not significantly ($P > 0.05$) different from the rise observed among those rats in Group II (DOCA–saline from 1800 to 0600 hr). Differences in total fluid intake, total body weight, and numbers of hypertensive animals (BP > 150 mm Hg), as observed at the end of the experiment, are shown in Table I.

Rats in the DOCA + water *ad libitum* (Group IV, Table I) gained more weight and

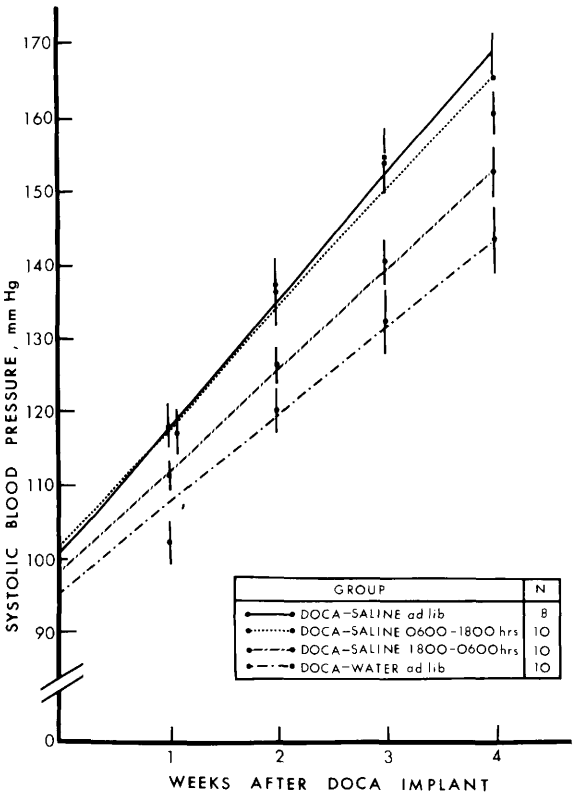


FIG. 1. Systolic blood pressures (mean ± SEM) among DOCA-implanted rats (10 mg, sc) on regimens of either water or saline (NaCl, 1%) as indicated in the figure.

TABLE I. DEVELOPMENT OF HYPERTENSION, GROWTH, AND FLUID INTAKE IN DOCA-TREATED RATS

| Group (*N*) | No. hypertensive rats[a] (systolic BP >150 mm Hg) | | Weight gain[b] (g) | | Mean fluid intake/24 hr[b] (ml/100 g body wt) | |
|---|---|---|---|---|---|---|
| I (8) | 7/8 | | 159 ± 5.5 | | 51.6 ± 1.9 | |
| II (10) | 5/10 | | 157 ± 6.9 | | 51.7 ± 2.1 | |
| III (10) | 9/10 | | 163 ± 4.4 | | 45.5 ± 1.0 | |
| IV (10) | 4/10 | | 184 ± 3.5 | | 21.3 ± 1.3 | |
| | $\chi^{2c}$ | *P* | $t^c$ | *P* | *t* | *P* |
| IV × I | 4.2 | <0.025 | 3.84 | <0.005 | 12.6 | <0.001 |
| IV × II | 0.2 | >0.05 | 3.49 | <0.005 | 12.3 | <0.001 |
| IV × III | 5.4 | <0.0125 | 4.46 | <0.001 | 14.7 | <0.001 |
| II × III | 3.8 | <0.05 | 0.74 | >0.05 | 2.67 | <0.02 |

*Note.* The experiment started with the subcutaneous, implantation (Day 0) of pellets containing DOCA 10 mg + carbowax 6 mg + flexowax 4 mg and the administration (drinking) of either NaCl (1% solution) or water according to the following schedule: Group I, saline *ad libitum;* Group II, saline available from 1800 to 0600 hr; Group III, saline available from 0600 to 1800 hr; Group IV, water *ad libitum*. The animals' mean body weight ($N$ = 38), at the beginning of the experiment, was 119 ± 1.33 g.

[a] Rats with BP >150 mm Hg are considered to have developed hypertension.

[b] Mean values ± SE as determined over a 4-week period.

[c] One-tailed $\chi^2$ and two-tailed Student's *t* test.

drank substantially smaller amounts of fluid than the animals in any of the DOCA–saline groups (Groups I, II, and III, Table I). Most interesting, from our point of view, was the lower fluid intake observed among the rats on saline from 0600 to 1800 hr (Group III), when compared to those on either saline *ad libitum* or on saline from 1800 to 0600 hr (Groups I and II). At the practical level these differences (Fig. 2) meant that animals in Group III while ingesting less salt than those in Group II (both groups on 12-hr schedule of saline intake), sustained a more accelerated rise in blood pressure.

Thus, within the first 2 weeks of the experiment, the total NaCl ingestion was 8.3 g/rat (mg/100 g body wt/day × 14 days) in Group II and 6.3 g/rat in Group III.

Future studies will have to be done in attempts to determine whether the lower-salt intake observed in Group III is accompanied by a greater degree of sodium retention. We believe that this will prove to be the case as well as explain both the steeper blood pressure rises and the greater number of hypertensive rats found in Group III as compared to Group II.

In conclusion, the data suggest that hypertension develops more readily among rats ingesting excessive salt loads while at rest (low natriuresis) than among those doing so during their circadian phase of high natriuresis. Conversely, the data also suggest that circadian phase restrictions of sodium intake could be more effective than net reductions of salt intake in the management of hypertension.

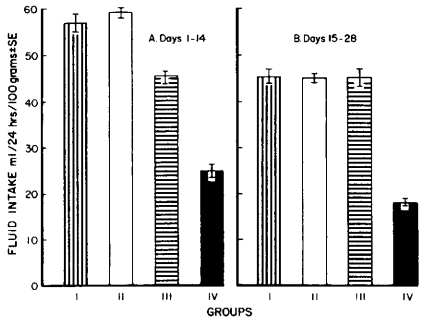


FIG. 2. Fluid intake among DOCA (10 mg)-implanted rats during the first and last 2 weeks of the experiment. The schedule and regimen (water or NaCl, 1%) of fluid intake was as follows: Group I, saline *ad libitum;* Group II, saline from 1800 to 0600 hr; Group III saline from 0600 to 1800 hr; Group IV, water *ad libitum.*

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