This is to certify that the

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EFFECTS OF DESOXYCORTICOSTERONE ACETATE (DOCA) ON SALT APPETITE

By

Erkadius

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ABSTRACT

EFFECTS OF DESOXYCORTICOSTERONE ACETATE (DOCA) ON SALT APPETITE

By

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DOCA 5 mg/kg/12hr s.c. increased salt appetite more reliably and predictably than 25 mg DOCP i.m. Increased salt appetite was preceded by increased water intake, which was in turn preceded by increased urine volume. Taste preference threshold for saline was decreased from 0.03 M to less than 0.001 M, and the highest preferred concentration was increased from 0.1 to 0.3 M. Saline intake reached 61 and 57 ml/100g at 0.1 and 0.15 M, respectively, which was six times greater than control. Voluntary sodium intake was 8.56, 8.69, and 8.20 mEq/100g at 0.15, 0.2, and 0.3 M, respectively, eight times greater than control. These increases were accompanied by increased sodium excretion and urine volume. ANP 4.0 µg/hr i.v. for 4 days did not further increase the salt intake that was caused by DOCA. We conclude that DOCA increased salt appetite by decreasing taste preference threshold and increasing the acceptance for higher concentrations, and that a higher dose of ANP might be needed to show further increase in salt appetite.

dedicated to

my wife, UPIK SURYANI

my son, HIPPOCRATES

ACKNOWLEDGEMENTS

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May God bless you all.

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INTRODUCTION

The cause and physiological significance of the increase in salt appetite that occurs during the administration of the mineralocorticoid desoxycorticosterone acetate (DOCA) or aldosterone has been widely investigated[15, 41, 76, 78, 80]. Adrenalectomized rats, whose source of mineralocorticoids has been removed, also develop an increased salt appetite, for which the explanation is a possible increase of angiotensin II in the brain[39]. This explanation is also true for other cases of salt appetite induced by sodium deficiency. But in intact rats treated with aldosterone or DOCA, renin and angiotensin concentrations are depressed and cannot be expected to participate in this model of salt appetite, which also differs from the others in that it does not result from lack of sodium.

We wished to investigate the salt appetite of DOCA treated rats by doing salt preference tests, in which a wide range of concentrations of saline were studied to determine the most preferred concentration and the concentration at which aversion begins. We also wished to examine urine volume and sodium excretion during ingestion of the respective saline concentrations. To achieve this, before starting the preference tests, we maintained the rats on DOCA injections of 5 mg/kg body weight twice daily until the effect of injection on water intake, urine volume, sodium excretion, and body weight. had been stabilized.

Recent discovery of atrial natriuretic peptide (ANP), whose synthesis and plasma level have been shown to increase during escape from the sodium retention effect of mineralocorticoids in the kidney, and to remain elevated during mineralocorticoid administration[7, 44, 49, 57], has led to the widely accepted theory that it plays an important role for initiating and maintaining the escape[45]. It is important to point out that during escape the

sodium retaining effect of mineralocorticoid is sustained[24, 45, 59]. It is only in the kidney that the retention effect is overridden by ANP[45].

The coincidence that synthesis and plasma levels of ANP increase during mineralocorticoid administration, and that salt appetite also increases during this time without any satisfactory explanation, led us to think that ANP might have a role in causing this appetite. To test this possibility, we conducted another experiment in which injection of DOCA 5 mg/kg twice daily for seven days, was followed by intravenous infusion of 4.0 μg ANP/hr. The effect on salt appetite was tested by giving the rats a choice of 0.5 M saline and distilled water to drink. Based on the assumption that ANP level had already increased during the injection of DOCA, administration of exogenous ANP would be expected to further increase its concentration, and contribute to a greater increase in salt appetite.

REVIEW OF THE LITERATURE

SALT APPETITE AS A REGULATORY MECHANISM

Sodium deficiency in nature and in the laboratory results in spontaneous increase in salt appetite. This can be seen in domesticated and wild animals in inland areas where there is lack of environmental sodium[26], and in the laboratory where sodium deficiency is produced experimentally[39].

The first procedure used to produce sodium deficiency and stimulate salt appetite in the laboratory was adrenalectomy, which was reported by Richter in 1936[67]. He showed that adrenalectomized rats, which could not survive without receiving extra sodium in their food, could select the needed salt if they were given a choice of water and a salt solution to drink. He also showed later that increased salt appetite was mediated by a change in taste function, that is, preference threshold for salt solutions was decreased and suprathreshold responses were greater than normal[68]. Administration of adrenal cortical extract or DOCA to these rats returned their salt preference and intake to normal levels, thus confirming that the increased appetite was due to lack of salt-retaining hormones[69, 70].

For Richter[70], salt appetite was seen as an expression of need. He reported that adrenalectomy also increased the appetite for sodium phosphate, sodium iodide, potassium chloride, ammonium sulphate, and calcium lactate in proportion to their concentrations in normal blood serum. Fregly[33] in rats, and Denton[27] in sheep confirmed his report that adrenalectomy increased appetite for various sodium salts (sulfate, bicarbonate, and nitrate), but they also reported that the appetite did not increase for non-sodium salts such as KCl, LiCl, $CaCl_2$, and $MgCl_2$.

Adrenalectomy as the procedure used to produce sodium deficiency and stimulate salt appetite in the laboratory was followed later by other procedures. These include parotid fistula in sheep[26], intraperitoneal dialysis[30], subcutaneous injection of formalin[81] and polyethylene glycol[73] in rats, administration of diuretics to rats[36, 38, 52] and sheep[26], and placing animals on a low sodium diet[22, 37, 61, 77]. These methods deplete the body of sodium by different routes, but they all act by decreasing preference threshold, and each can be linked to an effect on the renin-angiotensin-aldosterone system[39]. In all of these cases, salt appetite acts as an adaptive mechanism to meet the body's need for sodium.

The adaptive nature of salt appetite was further demonstrated when rats made hypertensive by encapsulating their kidneys[1, 32, 34, 76] diminished their salt intake by reducing their preference for suprathreshold concentrations.

SALT APPETITE IN THE ABSENCE OF NEED

When Rice & Richter[66] and later, others[15, 41, 76, 78, 80] showed that administration of DOCA and aldosterone to normal rats also increased their salt appetite, it was unexpected and considered paradoxical. Not only did the same drug produce opposite effects, but salt appetite under these conditions is clearly not adaptive, since the animals are retaining sodium and have no need for more.

The paradox of salt appetite in the presence of mineralocorticoid was further highlighted by the finding that the drive and motivation for sodium were the same in adrenalectomized rats and in normal rats treated with DOCA[65]. Additionally, the preference threshold for salt solution, which is the concentration at which NaCl intake is significantly greater than water intake, was also diminished in both situations. In adrenalectomized rats, preference threshold for NaCl solution decreased from 0.055% to 0.003% [68], while in normal rats treated with DOCA, threshold was reduced from 0.024% to 0.005%[51].

Wolf[79] found that when adrenalectomized rats were injected with desoxycorticosterone trimethylacetate (DOCT), they reduced their appetite for NaCl in a dose-dependent manner up to 11 mg/kg body weight. However, doses higher than this caused an increase of their appetite. He described this phenomenon as the restoration of sodium retaining ability at low doses of the mineralocorticoid, and stimulation of salt appetite at higher doses.

Fregly[35, 41] reported a similar U-shaped dose-response relationship in adrenalectomized rats treated with other mineralocorticoids, with the low point occuring with daily injection of 400 μg DOCA, 32 μg aldosterone, or 16 μg 9-α-fluorocortisol. These effects seemed to be specific for mineralocorticoids, since it did not occur with cortisone, estrone, or testosterone. The dose of aldosterone used was roughly the same as the aldosterone secretion rate measured from the left adrenal vein[39], showing that the appetite for saline is minimal at normal aldosterone concentration in the blood.

These experiments showed that in normal rats, which do not have a need for additional salt, increasing salt appetite can be invoked by increasing mineralocorticoids in their body. What causes this phenomenon, and what is the physiological meaning of it, are questions that still need to be answered.

ROLE OF RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

Aldosterone secretion is increased by administration of angiotensin II, high concentration of potassium, and low concentration of sodium in the systemic circulation as well as in the adrenal artery[13]. Decrease in plasma sodium concentration has been reported to increase plasma renin and angiotensin II concentrations[12], and subsequently, aldosterone concentration. In adrenalectomized rats, there is the possibility that increased appetite for sodium is caused by a high level of angiotensin II concentration, resulting from decreased plasma sodium concentration. When aldosterone is administered, plasma concentration of

sodium increases and angiotensin II declines until the administered dose mimics normal aldosterone secretion[39], and salt appetite returns to normal.

In rats depleted of sodium by intraperitoneal dialysis, increased salt appetite is abolished by nephrectomy, suggesting that the kidney plays a role in increasing salt appetite. In those rats, intraperitoneal administration of renin reestablished the appetite within 20 minutes[20], showing that renin and angiotensin are responsible for increasing the appetite. Intracerebroventricular administration of angiotensin I as well as angiotensin II also increased the appetite of those rats, while the effect of angiotensin I administration was blocked by concomitant administration of SQ 20881, an angiotensin converting enzyme inhibitor[20]. This observation laid the foundation for the now widely accepted theory that angiotensin II acts in the brain to increase salt appetite.

It is also important to note that in the sodium-deprived nephrectomized rats, aldosterone concentration would have been elevated, but not angiotensin II because the renal source of renin had been removed. As was pointed out by Blair-West[13], decreased sodium concentration alone in the adrenal artery can increase the secretion of aldosterone. The fact that salt appetite was abolished in these rats showed that aldosterone does not directly increase salt appetite, although in normal rats it appears to do so.

Rats fed low salt diet show a brisk appetite for saline solutions[22], and the resulting low plasma sodium and volume reduction would be expected to increase both angiotensin II and aldosterone concentrations in the plasma. It is not known whether increased angiotensin II in the plasma will lead to its increased concentration in the brain, but there is the possibility that increased angiotensin I in plasma will lead to increased angiotensin I and thus angiotensin II concentration in the brain[39].

Fregly[40] reported that captopril administration, which blocks the conversion of angiotensin I to angiotensin II and subsequently lowers aldosterone secretion, caused an increase in salt appetite. Administration of DOCA in this case reduced salt appetite in the previously described U-shaped dose-response manner, with the lowest intake occuring at

102 µg/d. This confirmed that low levels of mineralocorticoid can result in a high salt appetite, but in this study increased salt appetite could not be explained by an increase in plasma level of angiotensin II.

This confusion was resolved when Epstein and colleagues[58] reported in sodium-depleted rats that while salt appetite was increased further by intravenous infusion of captopril at 0.04 mg/hr or lower, doses higher than 5 mg/hr decreased the appetite. The explanation for this was that at lower doses, captopril increased plasma angiotensin I, which then entered the brain and was converted to angiotensin II, which stimulated salt appetite. At higher doses, captopril also entered the brain and prevented this conversion, and no stimulation of salt appetite occurred.

Angiotensin II in the brain has been reported to increase water intake when administered over short periods of time[6, 23, 28], and to increase salt appetite with chronic administration[6]. The increase in salt appetite was reported to be specific for sodium, and not secondary to increased water intake or natriuresis. Angiotensin II administered intraventricularly also has the ability to restore sodium appetite in sodium deprived, nephrectomized rats, as has been pointed out earlier[20].

While increasing mineralocorticoid concentration has been reported to increase salt appetite at the same time that it reduces plasma angiotensin II, Brooks[16] has reported that intraventricular infusion of angiotensin II can also reduce aldosterone secretion. She also reported that administration of SQ 20881, an angiotensin converting enzyme inhibitor, and administration of P-113, a competitive inhibitor to angiotensin II, had resulted in higher aldosterone secretion. This led to the conclusion that there is a tonic inhibition of aldosterone secretion by brain angiotensin, which is released when its effect is blocked by an inhibitor, or when its concentration is decreased by the converting enzyme inhibitor.

From the fact that increased brain angiotensin II and increased plasma aldosterone can individually or together work to increase salt appetite, and from the reports that they can inhibit each other, it is clear that angiotensin II and aldosterone play a complicated role

in the mechanism of salt appetite. The question that remains to be explored is how high plasma mineralocorticoid levels stimulate salt appetite in the absence of high plasma or brain angiotensin levels. It has not been possible to stimulate salt appetite by injecting aldosterone or DOCA in the brain[17].

MINERALOCORTICOID ESCAPE PHENOMENON

Prolonged administration of DOCA or aldosterone has been reported to result in transient retention of sodium in the kidney, which then 'escapes' from the mineralocorticoid effect[5, 24, 25, 59]. This phenomenon has been reported in humans[5], rats[54, 59], dogs[25], and hamsters[31].

The observation that led to the idea of escape was described by Daughaday and MacBride[24] when they reported that after restoration of sodium balance following its initial retention, plasma potassium and sweat sodium concentrations remain low, suggesting a sustained effect of the mineralocorticoid's action. August[5] several years later observed that administration of large doses of aldosterone resulted in initial sodium retention and weight gain, which then returned approximately to control levels after three days. This escape was thought to be the explanation for the absence of marked edema and sodium retention in primary aldosteronism.

An important observation made by Mohring & Mohring[59] in rats is a nyctohemeral rhythm in the escape phenomenon. They reported that during the entire period of observation, small amounts of sodium were continually retained during the day and escape occurred at night. This also showed that the mineralocorticoid effect was sustained throughout its administration, and that some regulatory mechanism overrode it during the night and resulted in the escape from its retention effect.

This escape phenomenon has been a subject of intense interest to find what causes it. One of the leading hypotheses to explain it is that volume expansion due to sodium re-

tention stimulates the release of atrial natriuretic peptide which acts in the kidney to increase sodium excretion[3, 18, 45, 55, 57, 62].

Recent experiments have shown that ANP synthesis and release can be induced by atrial stretch subsequent to volume expansion in rats[29, 56], pigs[49] and dogs[57, 71]. Its effects have been shown to increase glomerular filtration rate in rats[21], increase sodium excretion in humans[2] and in rats[53], inhibit renin secretion in dogs[44, 72] and rats[14], and inhibit aldosterone secretion in rats[14, 19, 74] and in humans[4]

During mineralocorticoid escape, ANP has been shown to increase in the plasma of humans[42, 82], dogs[44, 46, 57], pigs[49] and also rats[7]. The increase in the plasma was accompanied by increasing synthesis of ANP in the atria of the heart[7, 57]. Further, ANP concentration in the plasma was sustained during the time mineralocorticoid was administered[7, 44, 49, 57], and rapidly declined when the administration was stopped[44].

These observations, together with the report that in DOCA escape sodium reabsorption decreases in proximal tubules as well as in inner medullary collecting duct[54], suggest that ANP plays a critical role in this phenomenon. Gonzalez-Campoy suggested two major effects of ANP in escape, viz. to block the antinatriuretic mechanisms and to promote the natriuretic pathways[45]. ANP may not play a significant role in blocking the antinatriuretic mechanism in primary aldosteronism or in DOCA treated animals because the concentration of mineralocorticoid is already high, and plasma renin concentration is already depressed. In these cases, the ANP effect is directed more toward promoting the natriuretic pathways such as increasing sodium excretion by reducing its reabsorption from the proximal tubule and the inner medullary collecting duct.

ROLE OF ANP IN NEED-FREE SALT APPETITE

The role of ANP in mineralocorticoid escape has led to the hypothesis that ANP may also play a causal role in the gradually increasing salt appetite that follows chronic ex-

posure to DOCA. The important role of volume expansion in triggering the ANP system has also suggested the hypothesis that other forms of need-free salt appetite may utilize the same mechanism. Need-free salt intake has been found in rats placed on a high Na diet[63, 64], in spontaneously hypertensive (SHR) rats [9], and in rats [10] and rabbits [11] receiving ACTH. Since these procedures do not create Na deficiency, but may involve volume expansion to some degree, they may share the same ANP mechanism.

Mooney & Bernard[60] showed that salt appetite increased in rats injected with DOCA, and in rats treated with different concentrations of hydrochlorothiazide (HCZ), but in rats treated with DOCA and HCZ, the effect on salt appetite was not additive. This result showed that reducing volume expansion by HCZ reduced salt appetite, and that this was presumptively due to reduced plasma concentration of ANP.

In human history, salt preference, as well as the desire to consume salty tastes in the absence of any known physiological need for sodium, have made salt a status symbol, and a reason to start a war to control its source[8, 26]. But in modern life, where salt has been more easily acquired, the preference is still exhibited by humans even when they are not sodium deficient[8]. Physiological and mental stress could be responsible for increasing secretion of ACTH and subsequent increase of DOCA and corticosterone from the zona fasciculata of the adrenal cortex[43, 50]. Whether the increase in salt appetite in the absence of sodium need can be attributed to the increase in mineralocorticoids, and subsequently to ANP, still needs further investigation.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

This experiment was designed to examine the effect of long term administration of desoxycorticosterone (DOC) to rats on intakes of water and salt solutions over a wide range of concentrations. To achieve this, we first determined whether a long- or short-acting form of DOC should be used, and at what doses, during a series of preliminary experiments using DOC-pivalate (DOCP) alone, DOCP followed by DOC-acetate (DOCA), and DOCA alone.

Since the effect of DOC on salt and water intake develops gradually, the preference tests were preceded by an adaptation period, during which water alone was offered. The preference tests were begun after water intake reached a steady state level.

To determine whether administration of ANP will have an additive effect with endogenous ANP that was increased by DOCA administration, in enhancing salt appetite, another experiment was performed using ANP infusion in rats which had been injected with DOCA for several days. ANP delivery was designed to coincide with the first day of 0.5 M saline offering.

PRELIMINARY EXPERIMENTS

The experiments were conducted on adult male Sprague-Dawley rats (Sasco Inc., Omaha, NE) weighing between 300 to 450 grams on the first day of injection. Each animal was housed in a plexiglass cage (32 x 35 x 16 cm) with sawdust bedding and a detachable

metal top. Room temperature was maintained between 70 -72 °C and lights were kept on from 9 am to 9 pm. Two drinking bottles, one filled with distilled water and the other with 0.5 M saline were provided ad libitum along with a purified rat chow diet (Teklad™, Madison, WI) containing 0.19 mEq Na/gm. Position of the water and 0.5 M saline bottles was changed daily on a random schedule. An adjustment period preceded the drug injections. The forms of DOC used were DOCP (Percorten® Pivalate, 25 mg/ml, CIBA) and DOCA (Desoxycorticosterone Acetate, Sigma) which was dissolved in sesame oil 7.5 mg/ml through heating with 95% alcohol.

In the first experiment, DOCP (7.5 mg i.m.) was administered to six rats after an eight-day adjustment period, the dose was increased to 12.5 mg five days later, and a third dose of 25 mg (12.5 mg in each hindquarter) was given five days after the second dose. Four rats served as controls and received sesame oil at the same volume as the DOCP injections. This experiment was extended to 148 days because water intake took a long time to return to normal. All rats were sacrificed at the end of the experiment.

In the second experiment, DOCP (25 mg/day, i.m.) was injected in eight rats for two consecutive days, followed by another 25 mg 14 days later. Fourteen days after this, DOCA was injected subcutaneously 5 mg/kg per day for four days, followed by 5 mg/kg per 12 hours for eight days. Eight rats served as controls and received sesame oil at the same volume as DOCP or DOCA. All rats were sacrificed after the experiment ended.

The third experiment was performed on six rats which received DOCA 7.5 mg/kg every 12 hours, and six control rats which were injected with the same volume of sesame oil.

PREFERENCE EXPERIMENT

This experiment was performed on 20 male Sprague-Dawley rats divided into two equal groups. They weighed 297 to 324 grams when the injections of DOCA were begun

5 mg/kg every 12 hours. The control group were injected with a similar volume of sesame oil. Rats were housed individually in 24 by 18 cm (i.d. x height) plexiglass metabolic cages (Nalgene, cat. no. 650-0350) that had been modified to allow for attachment of a second drinking bottle. Cages were mounted in two tiers of four on stainless steel racks with locking swivel casters (Nalgene, cat. no. 350-0500). Standard rat chow (Teklad™, powdered) which contained 0.19 mEq Na /gm was offered ad libitum in a removable feed drawer.

During the adaptation period, distilled water was offered in both drinking bottles. During the preference tests, salt solutions were offered in one of the bottles in order of increasing concentration, that is 0.001, 0.003, 0.01, 0.03, 0.1, 0.15, 0.2, 0.3, 0.5, and 1.0 M each for a two-day period, except for the first concentration, which was offered for three days. Position of the bottles was adjusted daily so that the saline bottle was never on the same side of the cage for the two days in which a given concentrations of saline was being tested.

ANP EXPERIMENT

The experiment was performed on male Sprague-Dawley rats weighing between 350 to 389 grams at the start of the experiment. The rats were divided into two groups of seven each, housed individually in the same metabolic cages and given the same diet as was used for the preference experiment. The course of the experiment was divided into three phases, that is, pre-DOCA, DOCA, and DOCA-ANP. Surgery was performed in the last two days of pre-DOCA phase to implant minipumps for ANP delivery. During the DOCA phase DOCA alone was given to both groups, and ANP infusion accompanied DOCA injection in the experimental group during the DOCA-ANP phase. The salt intake test was performed in the DOCA-ANP phase.

Both groups were offered distilled water in both drinking bottles during the first two phases of the experiment. Half-molar saline was offered in one of the two bottles, on the left-hand side, in the third phase, to coincide with the scheduled beginning of ANP delivery.

ANP solution was prepared by diluting 3.5 mg ANP (Atriopeptin III, Sigma®) in 0.9% saline to make 0.4 ml total. Alzet 2002 (Alza®) minipumps were filled with 0.9% saline, and incubated in a waterbath at 37°C for at least four hours before implantation. This incubation is needed to prime the minipumps so they can begin delivery once they are put in the body and connected to the vein via PE tubing. PE 60 (Intramedic®) tubing was prepared by coiling it in 1 cm diameter by immersing it in boiled water for 30 seconds, and was filled to 194 mm length with 0.9% saline and 121 mm length with ANP solution. to give a pumping period of eight and five days, respectively. These lengths were chosen to allow pumping of saline during the second period when DOCA alone was given, to be followed by ANP infusion to coincide with presentation of 0.5 M saline. The end of the tubing containing ANP was connected to the minipump and the saline end was inserted into the jugular vein. With a pumping rate of 0.457 μL/hr, and ANP concentration of 8.75 mg/ml, ANP was calculated to be delivered at a rate of 4.0 μg/hr beginning eight days after minipumps implantation.

For the control group, PE 60 was filled with 0.9% saline, while the rest of the procedure was the same as for the experimental group.

Surgery was conducted on the experimental rats on one day, and on the control group the following day. All surgical equipment was autoclaved prior to the procedure. Rats were anesthetized using inhalation of methoxyflurane (Metofane®), they were then secured on the operating board, the neck hair was shaved, and the exposed skin was disinfected with Betadine®.

Guided by a dissecting microscope, the skin of the neck was opened with a right paramedian incision. Fat tissue, fasciae and muscle were pushed aside, and the right jugu-

lar vein was isolated, cleaned, and the cranial part was closed. The middle section was punctured to allow for insertion of PE 60 tubing, which was inserted for 1 cm toward the heart and tied to the vessel. Skin in the left abdominal area was loosened to insert the minipump, Panalog® ointment was applied to the area, and the skin was closed with surgical wound clips.

DOCA was injected 5 mg/kg twice daily in both groups beginning two days after the initial surgery. All rats were sacrificed after four days of saline testing, then PE 60 attachment to the jugular vein was examined, and the minipumps were dissected to see if they had delivered the solution.

DATA COLLECTION

For the preliminary experiments, intakes of saline and water were measured by daily weighing of the water and saline bottles using an electronic balance (Brainweigh™ B1500, Ohaus) which was connected to an Apple® II computer running the "Bottle Weigh" program developed by Andrew Bernard. The program calculated daily intakes from each bottle, and salt preference as a percentage of saline intake to the total fluid intake. Body weight was measured manually using the same balance, and the values were entered together with bottle weigh results into an Apple® Macintosh Plus computer running Microsoft® Excel.

For the preference and ANP experiments, the bottles, food containers, and individual rats were weighed daily using the same balance. Urine volume was measured in a graduated cylinder, and a 100 µl sample was used to measure urinary sodium and potassium concentrations with a flame photometer (Instrumentation Laboratory, model 943). All data were entered into a Microsoft® Excel spreadsheet running on an Apple® Macintosh II computer, which calculated the intakes from each drinking bottle and food container,

sodium intake from food and salt solutions, sodium and potassium excreted in the urine, water balance, and sodium balance.

DATA ANALYSIS

Daily intakes by control and experimental animals in the preliminary experiment were compared using Student's 't' test on an Apple® Macintosh Plus computer running Microsoft® Excel.

Data from the preference and ANP experiments were analyzed using an Apple® Macintosh II computer running Microsoft® Excel for data analysis, and Cricket Graph (Cricket® Software) for charting. Data entry consisted of each day's value for body weight, weight of food container, water and saline bottle, volume and Na & K concentrations of urine. From these data the program calculated daily weight gain, daily saline, water and total fluid intake, daily saline, food and total Na intake, and daily water and Na balance (Figure 1). Water and sodium balance was calculated based on total intake of fluid minus urine volume, and total sodium intake minus urinary sodium excretion.

For the preference tests, data from the two days on each concentration were combined to produce a daily average for each rat. For the the ANP experiment, data were also analyzed using averages of four-day periods of each rat on the period before surgery was performed, on the last four days before saline was offered, and during saline offerings.

Student's 't' test was used to compare the daily values of all experiments, and also on the daily averages in the preference test, and on the four-day averages of the ANP experiment. Data are presented in charts comprising means and standard error of the means.

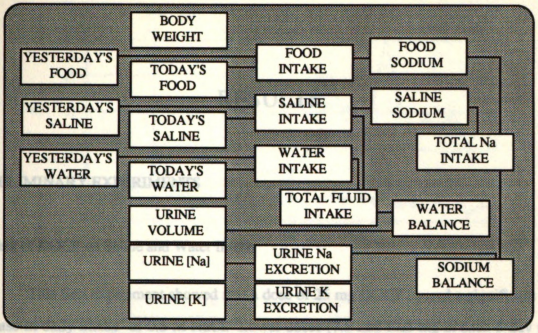

FIGURE 1. FLOW CHART FOR DATA ANALYSIS.
Yesterday's values are imported from previous day's spreadsheet, the second column is the data collected on the current day, and the result of analysis is shown on the two columns on the right.

RESULTS

PRELIMINARY EXPERIMENTS

Effect of DOCP on Saline and Water Intake

This first experiment showed that a dose of 25 mg DOCP caused a significant increase in daily intake of 0.5 M NaCl. Lower doses (7.5 and 12.5 mg in each 5-day period) did not produce a significant difference in saline intake between experimental and control groups. DOCP at 25 mg produced significant increases on days 13 - 18 (Figure 2), and then saline intake gradually decreased to the control level (Figure 3).

Water intake was increased on the first day after injection of 7.5 mg DOCP, and four days after injection of a 12.5 mg dose. The most significant increase in water intake was produced by the dose of 25 mg, beginning on day 12 and reaching the peak on day 18 (Figure 4). There was a gradual decline thereafter, but intake remained significantly different from control until day 137 (Figure 5), and the experiment was concluded on day 148.

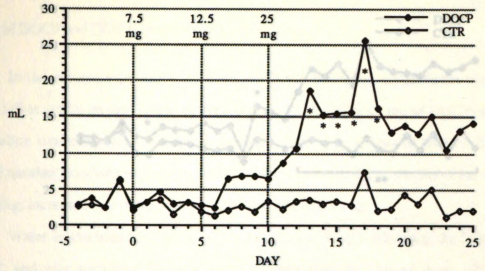

FIGURE 2. EFFECT OF THREE DOSES OF DOCP ON SALINE INTAKE. Significant difference was found only on days 13 - 18 (* P<0.05). DOCP = Desoxycorticosterone pivalate group, CTR = Control group. Vertical lines denotes the days when DOCP was injected intramuscularly.

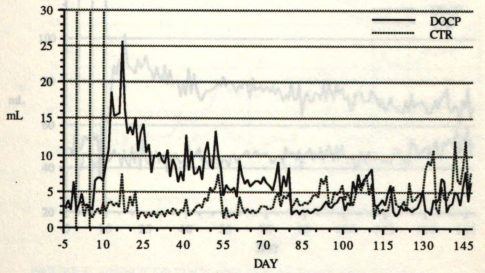

FIGURE 3. LONG TERM EFFECT OF DOCP ON SALINE INTAKE. DOCP was given on day 0, 5, and 10 at doses of 7.5, 12.5, and 25 mg i.m, respectively.

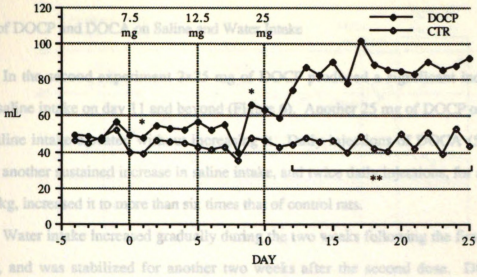

FIGURE 4. EFFECT OF THREE DOSES OF DOCP ON WATER INTAKE.
Significant difference was found on days 1 and 9 (* P<0.05), and remained significant (** P<0.01) from day 12 and beyond.

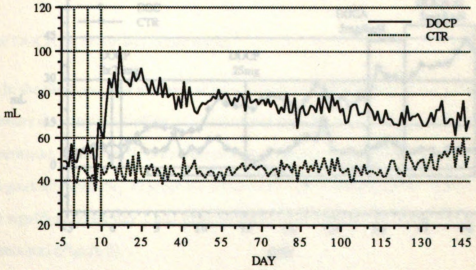

FIGURE 5. LONG TERM EFFECT OF DOCP ON WATER INTAKE.
DOCP was given on day 0, 5, and 10 at doses of 7.5, 12.5, and 25 mg i.m, respectively, and significant difference was seen between day 12 and 137 (P<0.01).

Effect of DOCP and DOCA on Saline and Water Intake

In the second experiment 2x25 mg of DOCP produced a significant increase of 0.5 M saline intake on day 11 and beyond (Figure 6). Another 25 mg of DOCP on day 15 kept saline intake elevated without increasing it. Daily injections of DOCA (5 mg/kg) caused another sustained increase in saline intake, and twice daily injections, for a total of 10 mg/kg, increased it to more than six times that of control rats.

Water intake increased gradually during the two weeks following the first dose of DOCP, and was stabilized for another two weeks after the second dose. DOCA, at 5 mg/kg/d did not change this intake significantly, but at 10 mg/kg/d increased it gradually to more than 2.5 times that of control rats (Figure 7).

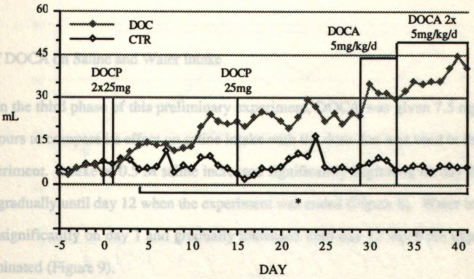

FIGURE 6. EFFECT OF DOCP AND DOCA ON SALINE INTAKE.
Two doses of 25 mg DOCP were injected i.m. on days 0 and 1, and one dose on day 15, followed by daily doses of DOCA s.c. 5 mg/kg on day 29-32, and 5 mg/kg/12hr on day 33-41. Saline intake increased significantly beginning on day 4 (* $P<0.05$). No further increase in 0.5 M saline intake was observed after the second dose of DOCP, but the first and second doses of DOCA increased it even more. DOC = DOCP- and DOCA- treated group.

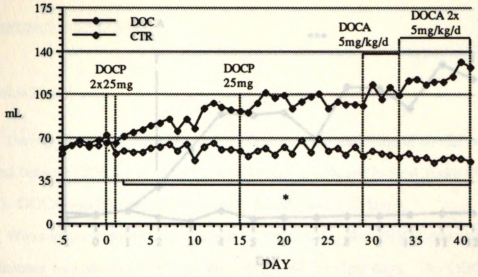

FIGURE 7. EFFECT OF DOCP AND DOCA ON WATER INTAKE.
Water intake began to increase significantly on day 2 (* P<0.05). The second dose of DOCP did not produce a further increase in water intake, but the second dose of DOCA did.

Effect of DOCA on Saline and Water Intake

In the third phase of this preliminary experiment, DOCA was given 7.5 mg/kg every 12 hours to compare its effect on saline intake with the dose that was used in the previous experiment. Intake of 0.5 M saline increased significantly beginning on day 2 and increased gradually until day 12 when the experiment was ended (Figure 8). Water intake increased significantly on day 1 and gradually increased until day 12 when the experiment was terminated (Figure 9).

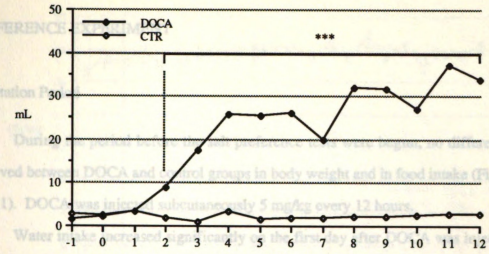

FIGURE 8. EFFECT OF DOCA ON SALINE INTAKE.
Intake of 0.5 M saline increased significantly two days after injection was begun (*** P<0.001). DOCA was injected 7.5 mg/kg/12 hr beginning on day 0.

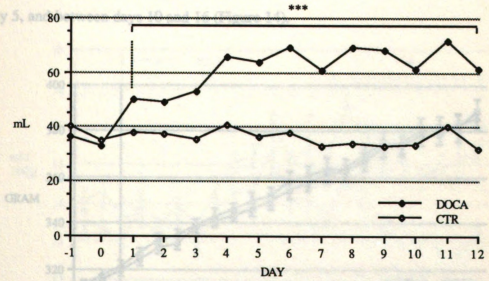

FIGURE 9. EFFECT OF DOCA ON WATER INTAKE.
Water intake increased significantly beginning one day after injection was begun (*** P<0.001).

PREFERENCE EXPERIMENT

Adaptation Period

During the period before the salt preference tests were begun, no difference was observed between DOCA and control groups in body weight and in food intake (Figures 10 and 11). DOCA was injected subcutaneously 5 mg/kg every 12 hours.

Water intake increased significantly on the first day after DOCA was injected, but no difference was observed between the two groups for eight days. The DOCA group drank significantly more water than the controls beginning on day 9 ($P<0.05$) and thereafter ($P<0.001$) (Figure 12). Urine volume of the DOCA group was significantly higher than control on day 2 ($P<0.05$), and strongly differed from them starting on day 3 ($P<0.001$) (Figure 13). Water balance of the DOCA group was lower than that of controls on day 5, and between days 10 and 16 (Figure 14).

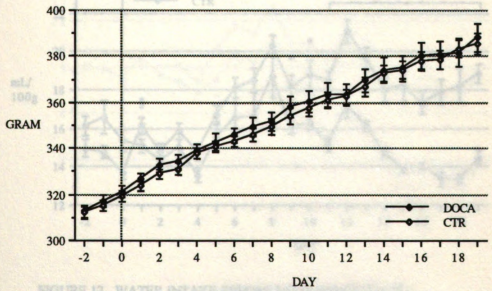

FIGURE 10. BODY WEIGHT BEFORE PREFERENCE TESTS.
DOCA was injected s.c. 5 mg/kg twice daily beginning on day 0. No significant difference was found between these groups ($P>0.05$). Values are means ± SEM.

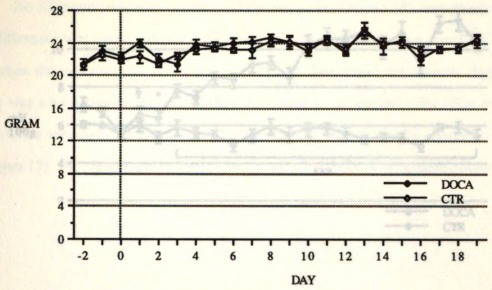

FIGURE 11. FOOD INTAKE BEFORE PREFERENCE TESTS.
No significant difference was found between these groups (P>0.05) in day-by-day comparison.

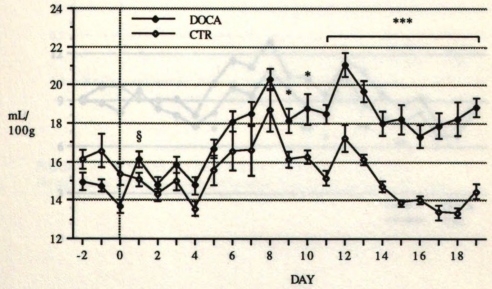

FIGURE 12. WATER INTAKE BEFORE PREFERENCE TESTS.
Significant difference between these groups first appeared on day 9 of DOCA injection (* P<0.05), and became more significant from day 11 (*** P<0.001). Water intake of the DOCA group on day 1 was significantly greater than on day 0 (§ P<0.001).

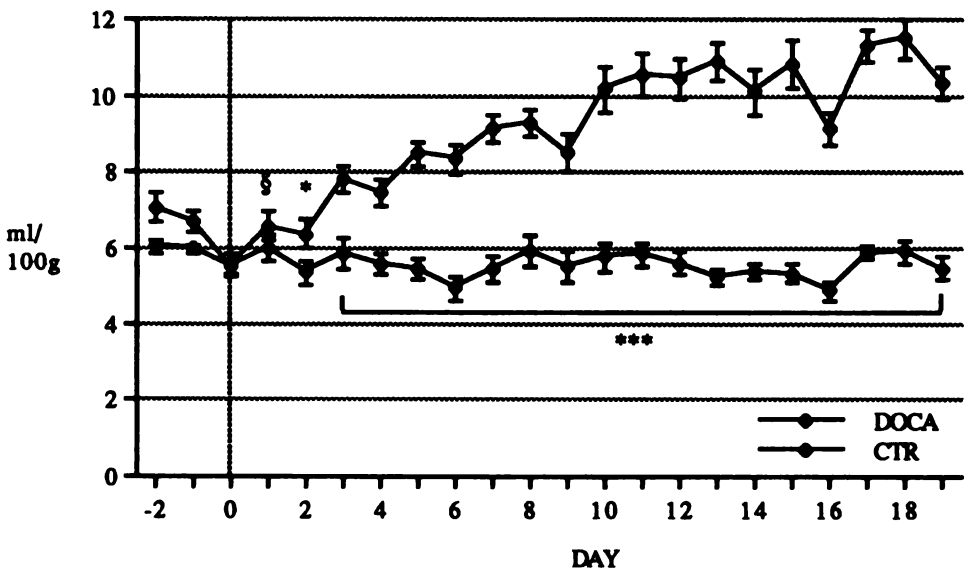

FIGURE 13. URINE VOLUME BEFORE PREFERENCE TESTS.
On day 2, the DOCA group began excreting urine in significantly higher volume than the control group (* P<0.05), and maintained significantly higher thereafter (*** P<0.001). Urine volume of the DOCA group increased significantly (§ P<0.05) on the first day after injection.

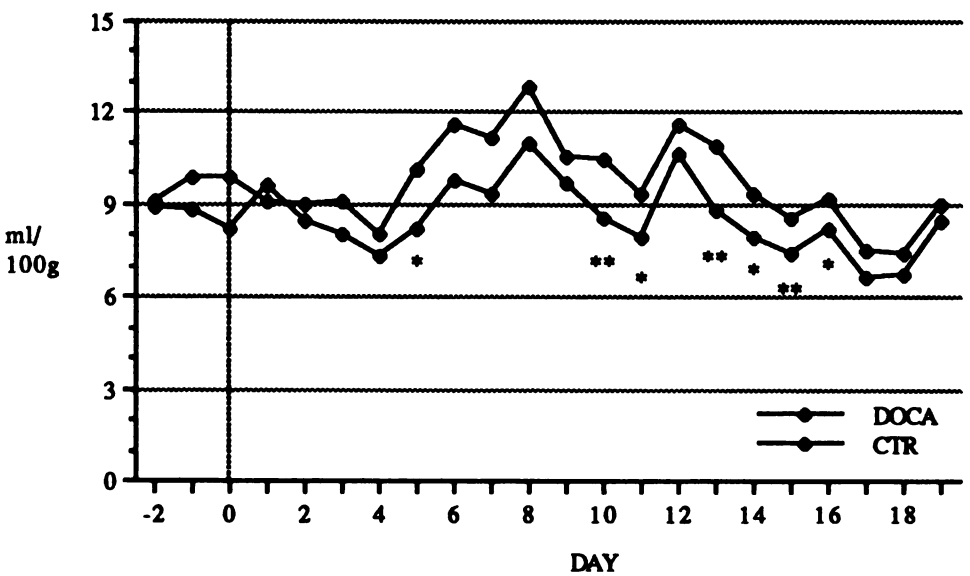

FIGURE 14. WATER BALANCE BEFORE PREFERENCE TESTS.
Water balance of the DOCA group was significantly lower than that of controls on day 5, and between days 10 and 16 (* P<0.05, ** P<0.01).

No difference was observed in food sodium intake (Figure 15), and also no significant difference was seen in urinary sodium output except on the first day after DOCA injection when the experimental group was significantly lower than the controls (Figure 16). There was also a significant, but smaller difference in the opposite direction on day 6. There was a very significant increase in sodium balance on day 1 and a smaller one on day 4 (Figure 17). No significant difference was observed in urinary potassium output (Figure 18).

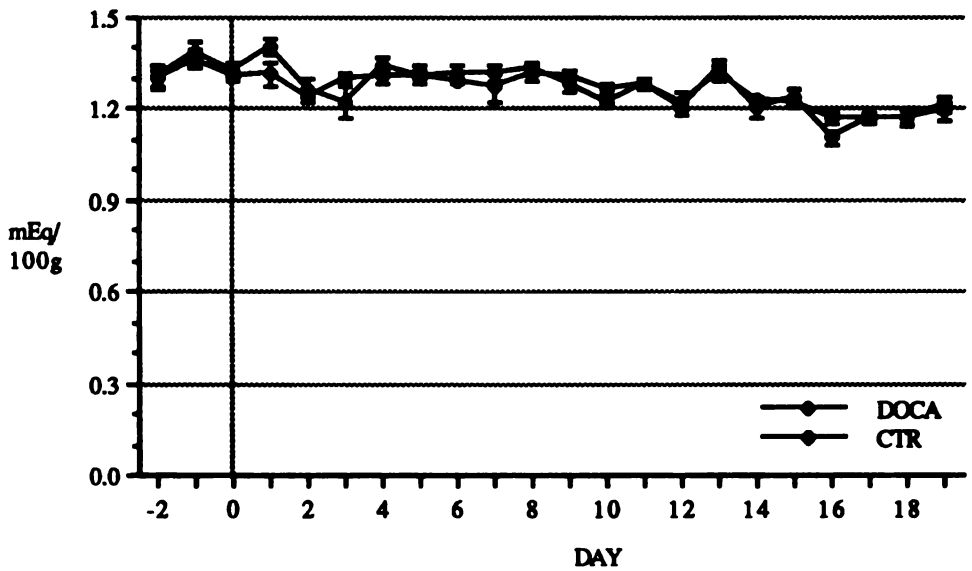

FIGURE 15. SODIUM INTAKE BEFORE PREFERENCE TESTS.
No significant difference was found between these groups on day-to-day basis.

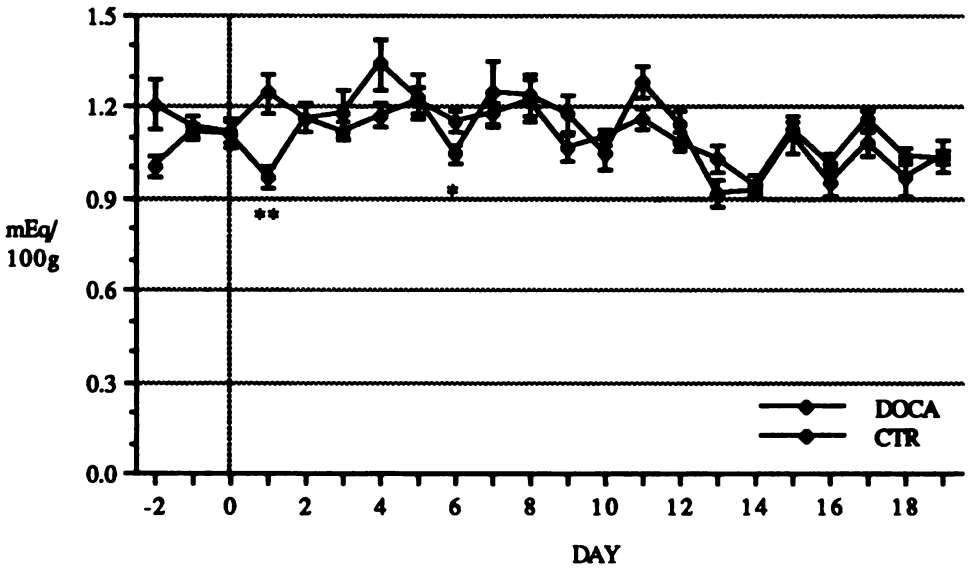

FIGURE 16. URINARY SODIUM BEFORE PREFERENCE TESTS.
Sodium retention was observed on day 1, on which the DOCA group excreted less sodium than control group (** P<0.01). Sodium escape from retention began on day 2.

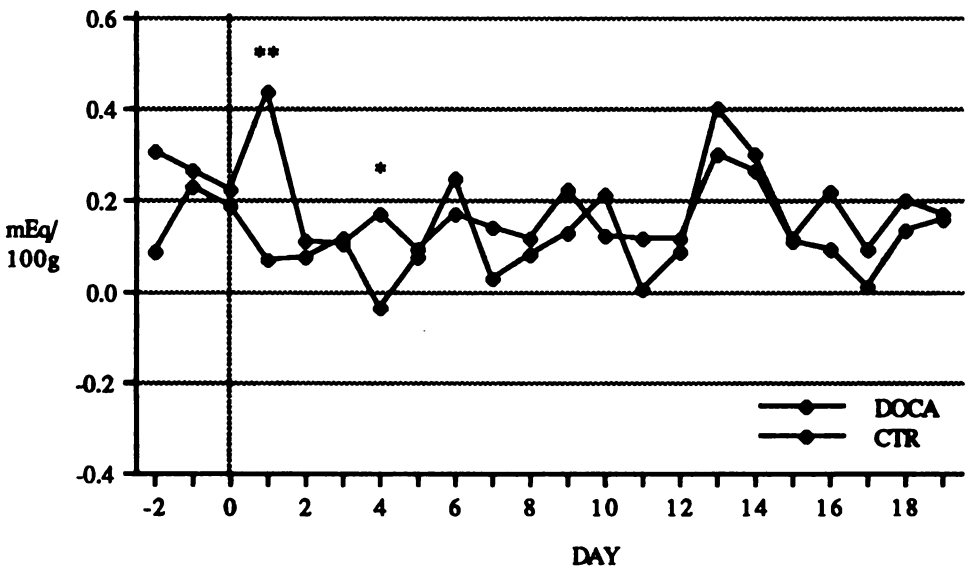

FIGURE 17. SODIUM BALANCE BEFORE PREFERENCE TESTS.
Significant difference was observed on day 1 (** P<0.01) and on day 4 (* P<0.05), and escape began on day 2.

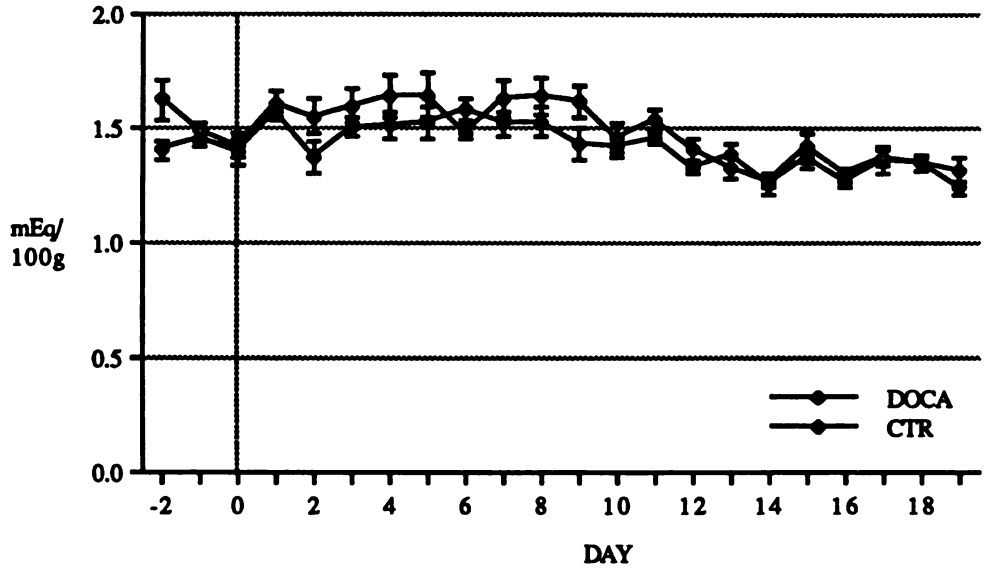

FIGURE 18. URINARY POTASSIUM BEFORE PREFERENCE TESTS.
No significant difference was noted between both groups (P>0.05).

Preference Tests

No significant difference was seen in body weight and food intake between the two groups (Figs. 19 & 20). The DOCA group drank significantly more saline than controls at all concentrations tested, reaching a peak at 0.1 M and declining thereafter, although the difference between 0.1 M and 0.15 M was not significant (Figure 21). Control rats drank significantly more water than the DOCA group at saline concentrations between 0.001 and 0.15 M, with no significant difference at 0.2 M, and significantly less above this level (Figure 22). Total fluid intake of the DOCA group was significantly higher than the controls at all concentrations of saline offered (Figure 23).

Saline intake was significantly greater than water intake in the DOCA group from 0.001 M to 0.3 M (Figure 24). In the control group, saline intake was higher than water intake at 0.03 and 0.1 M, although it was also significantly higher at 0.003 M (Figure 25).

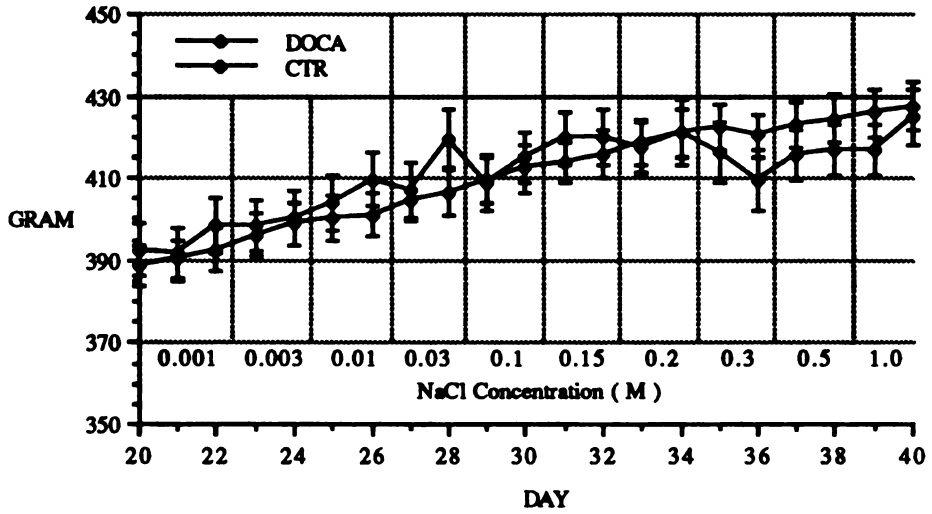

FIGURE 19. BODY WEIGHT DURING PREFERENCE TESTS.
No significant difference was noted between the DOCA and control groups (P>0.05).

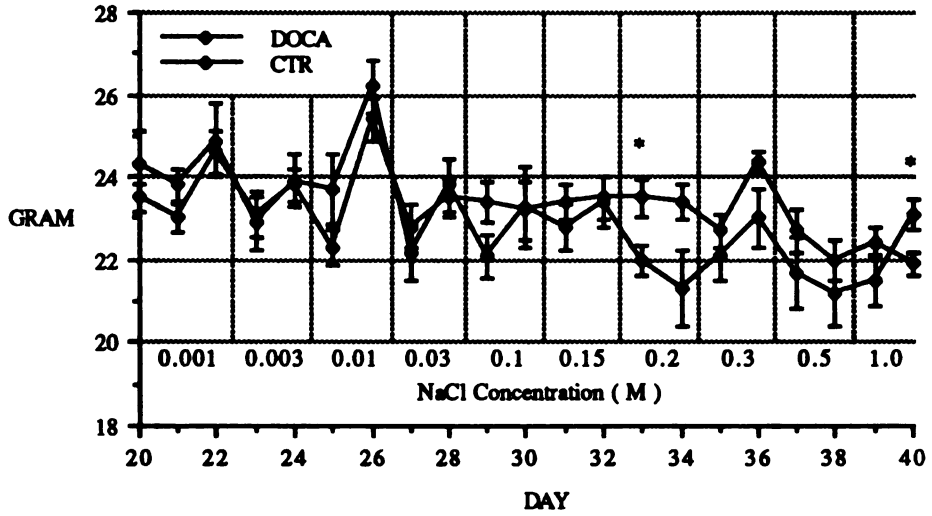

FIGURE 20. FOOD INTAKE DURING PREFERENCE TESTS.
Significant difference between DOCA and control groups occurred only on days 33 and 40 (* P<0.05).

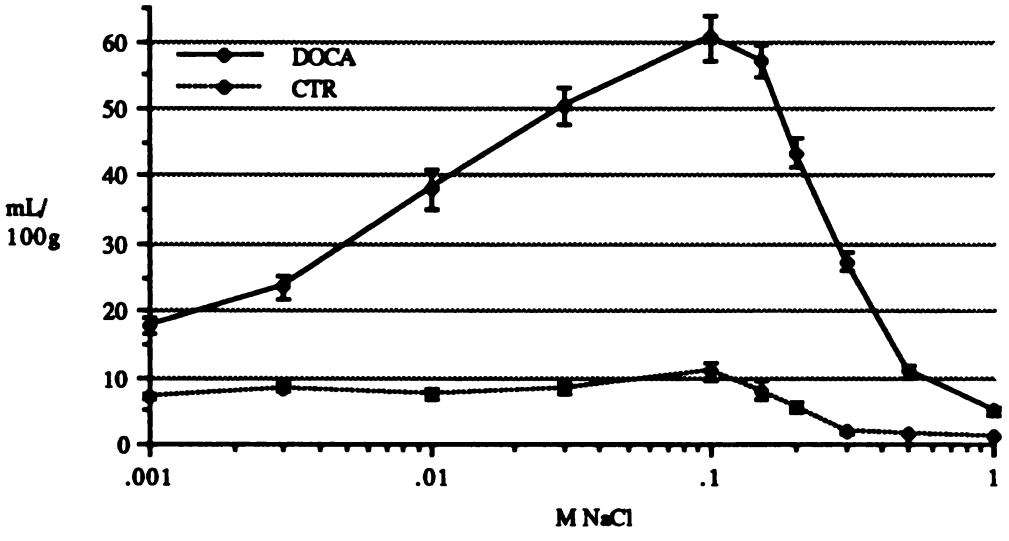

FIGURE 21. SALINE INTAKE DURING PREFERENCE TESTS.
The DOCA group drank much more saline than the control group at all concentrations offered ($P<0.001$). Peak intake was observed at 0.1 M saline, although the difference between this intake and the intake at 0.15 M was not significant ($P>0.05$).

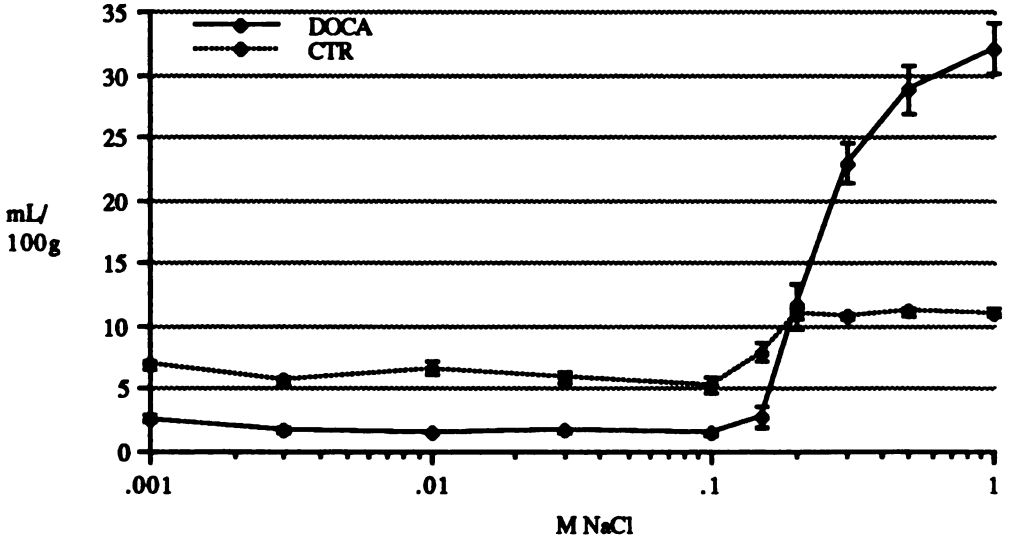

FIGURE 22. WATER INTAKE DURING PREFERENCE TESTS.
The DOCA group drank less water than the control group before saline concentration reached 0.2 M ($P<0.001$), at which point the difference was negligible, but drank significantly more water beyond this point ($P<0.001$)

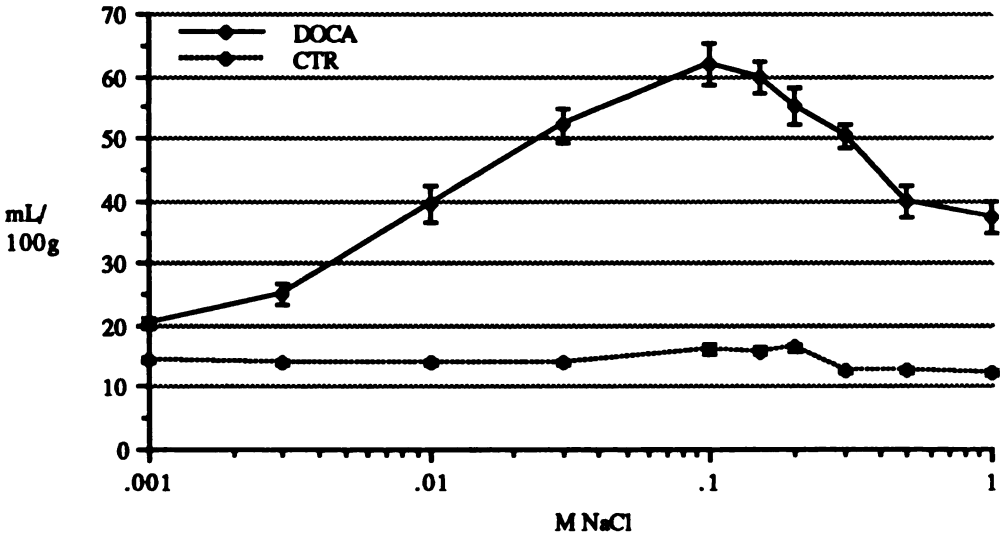

FIGURE 23. TOTAL FLUID INTAKE DURING PREFERENCE TESTS.
The DOCA group drank significantly more total fluid than control at every concentration of saline ($P<0.001$). They drank maximally when saline concentration was 0.1 M although it didn't differ significantly from 0.15 M ($P>0.05$).

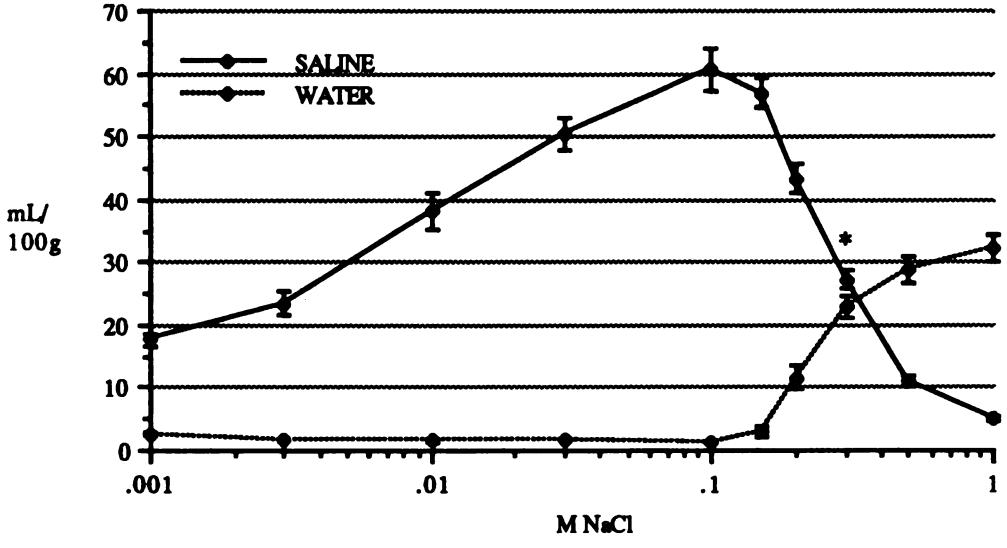

FIGURE 24. SALINE AND WATER INTAKES OF DOCA GROUP.
Saline intake was significantly greater than water from 0.001 M to 0.2 M ($P<0.001$), and 0.3 M (* $P<0.05$). Beyond 0.3 M, water intake far exceeded that of saline ($P<0.001$).

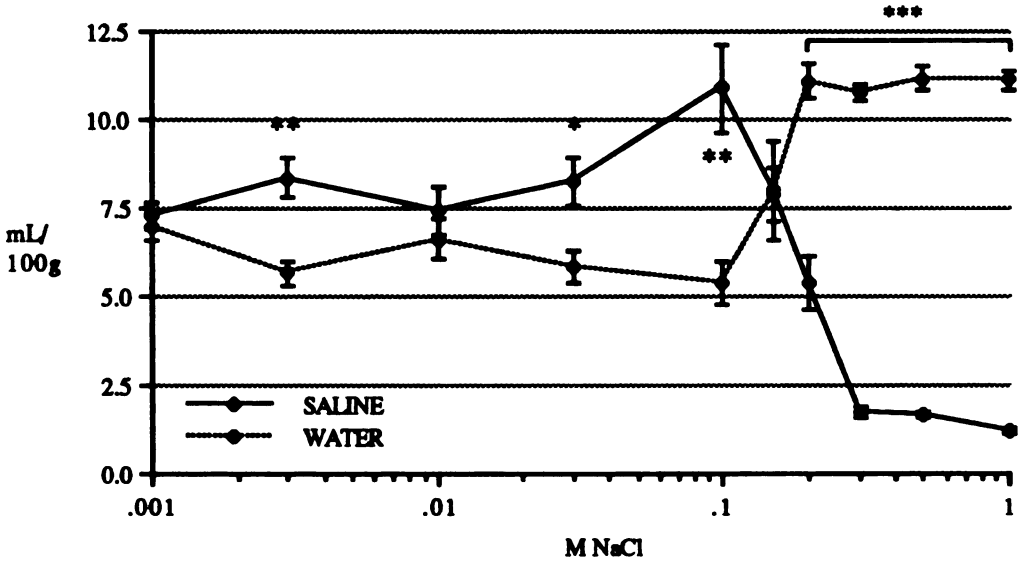

FIGURE 25. SALINE AND WATER INTAKES OF CONTROL GROUP.
Saline intake was significantly higher at 0.003 M (* P<0.01) but no difference was observed at 0.01 M. Significantly higher preference was observed at 0.03 M (* P<0.05) and 0.1 M (** P<0.01). No difference in saline and water intake was observed at 0.15 M, and beyond this concentration saline intake was significantly less than water intake (*** P<0.001).

Urine volumes of the DOCA group were greater than control at every concentration of saline offered. The maximum output was achieved at 0.15 M saline, which was not significantly different from 0.1 M (Figure 26). Water balance of the DOCA group was significantly higher than controls at 0.01, 0.03, and 1.0 M (Figure 27).

Sodium intake from the salt solutions was higher in the DOCA group at all concentrations offered (Figs. 28, 29), with the maximum intake occuring at 0.15, 0.2, and 0.3 M. Total sodium intake of the DOCA group was higher beginning at 0.01 M, and the maximum was again reached at 0.15, 0.2, and 0.3 M (Figure 30, 31).

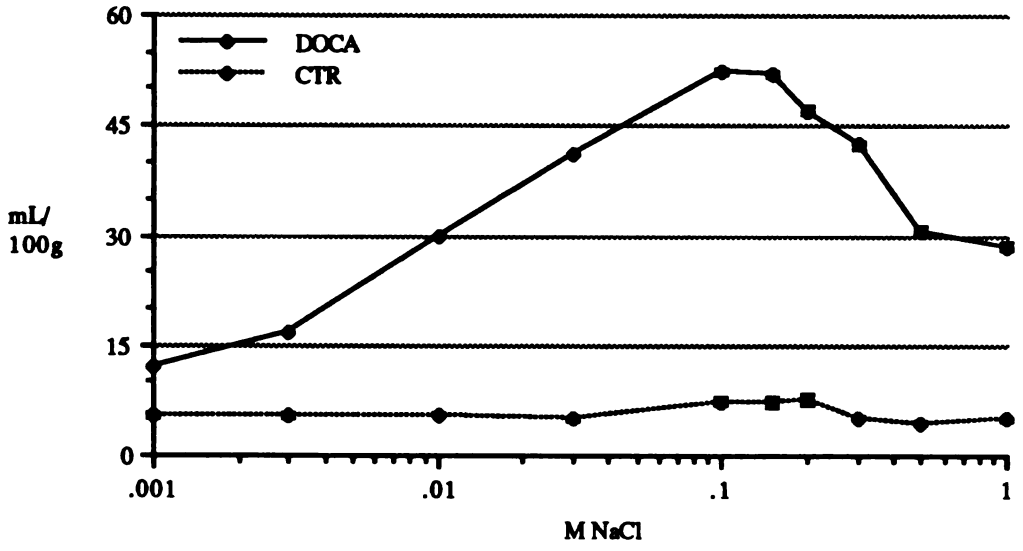

FIGURE 26. URINE VOLUME DURING PREFERENCE TESTS.
The DOCA group excreted far more urine than control in every saline concentration offered ($P<0.001$). Urine volume of DOCA group reached maximum at 0.15 M, although it didn't differ significantly from 0.1 M ($P>0.05$).

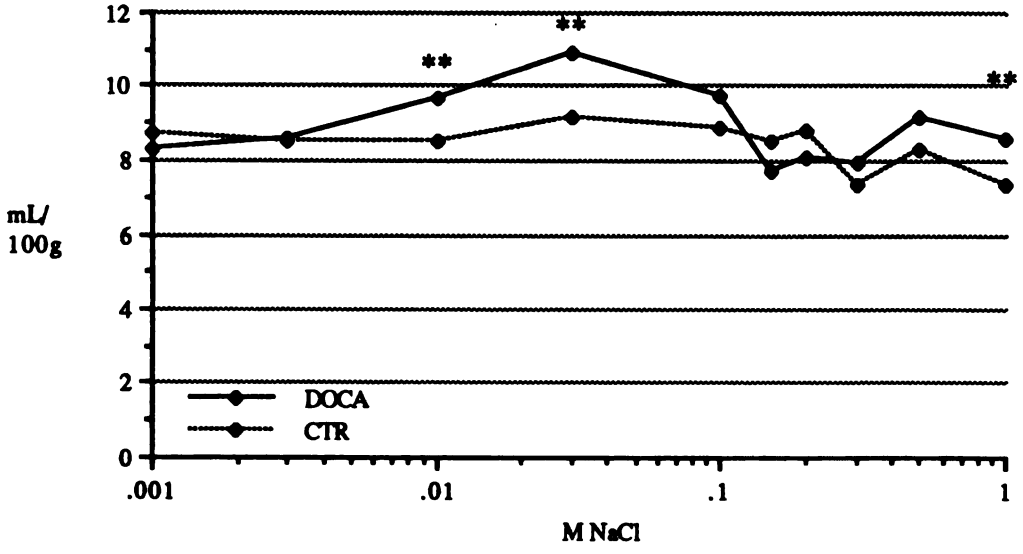

FIGURE 27. WATER BALANCE DURING PREFERENCE TESTS.
The DOCA group retained significantly more water than control when saline was offered at 0.01 M, 0.03 M, and 1.0 M (* $P<0.05$).

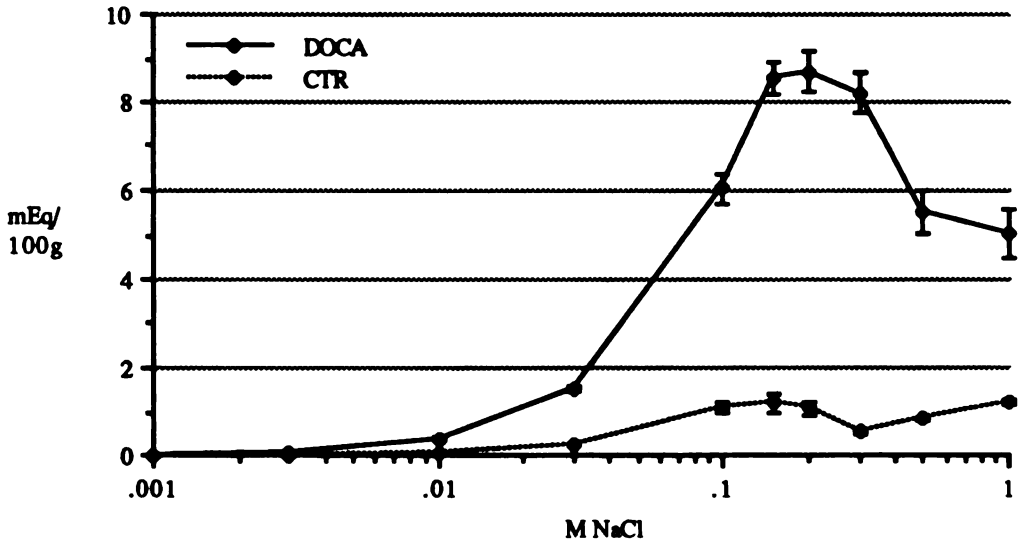

FIGURE 28. SODIUM INTAKE FROM SALINE SOLUTIONS.
Sodium intake of the DOCA group was significantly higher than that of control group ($P<0.001$) at all concentrations. The difference in sodium intake of the DOCA group from 0.15 M to 0.3 M saline was not significant ($P>0.05$).

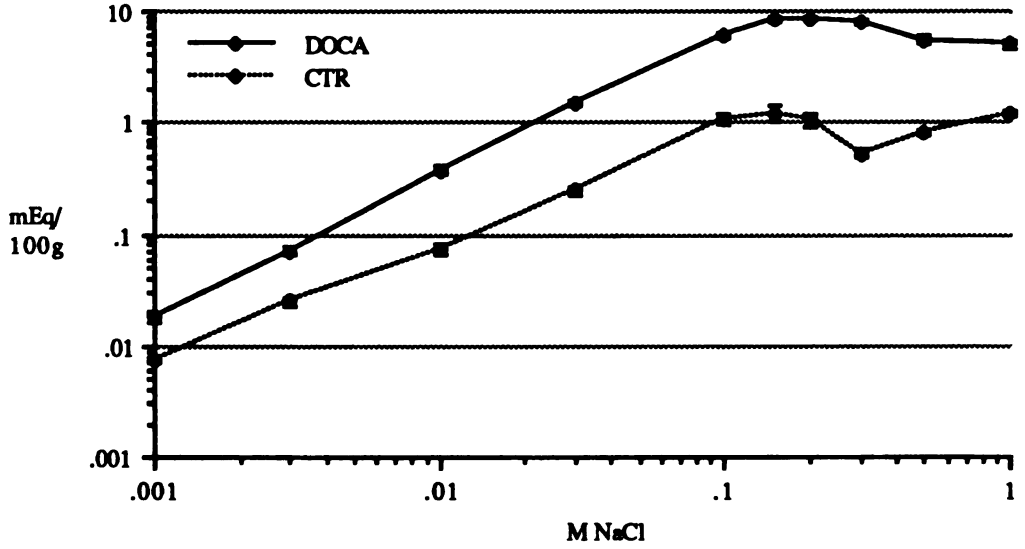

FIGURE 29. SODIUM INTAKE FROM SALINE, PLOTTED LOGARITHMICALLY.

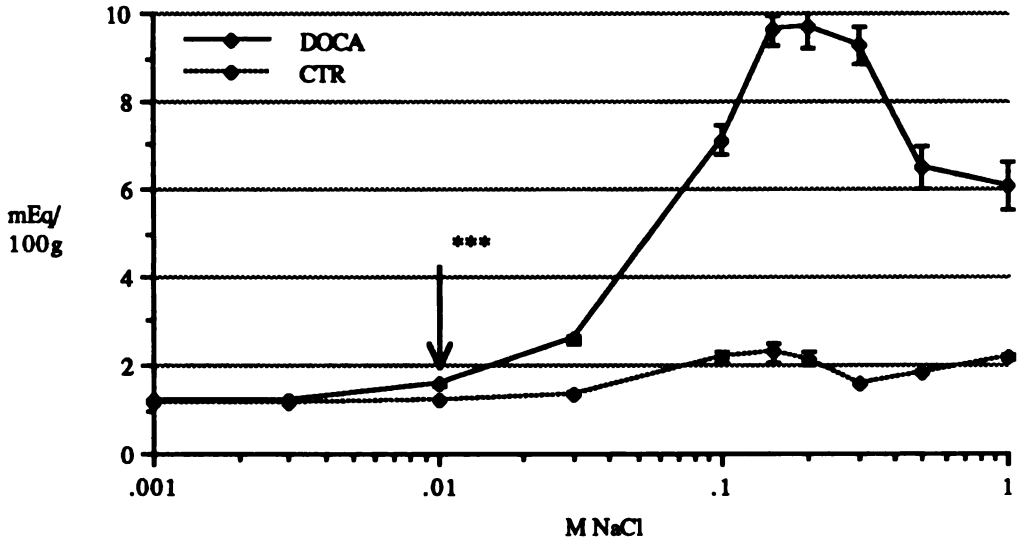

FIGURE 30. TOTAL SODIUM INTAKE DURING PREFERENCE TESTS.
The DOCA group had significantly higher sodium intake at all concentrations of saline between 0.01 and 1 M (*** $P<0.001$). The DOCA group's total sodium intake reached maximum at 0.2 M, although this was not significantly different from 0.15 M or 0.3 M ($P>0.05$). Arrow denotes the beginning of significant difference.

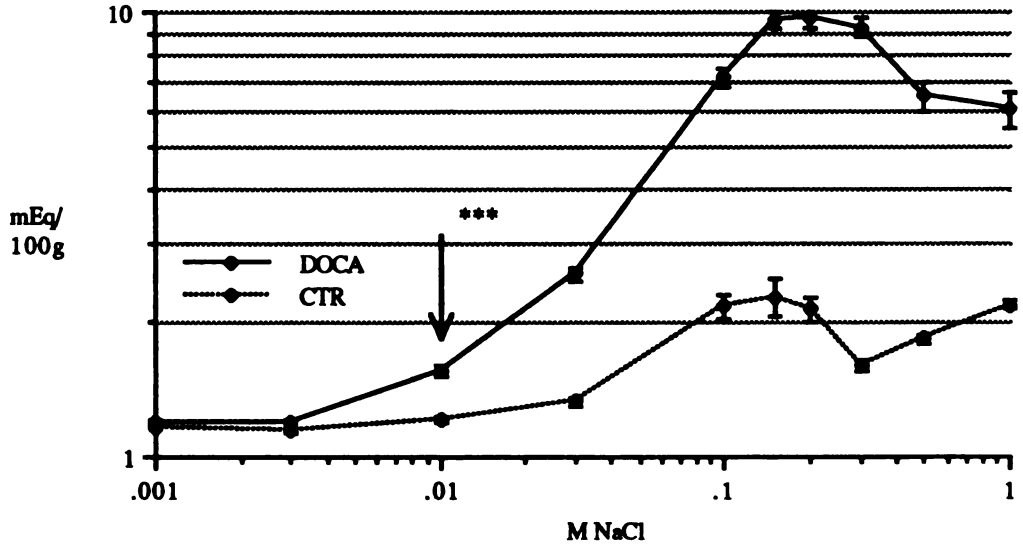

FIGURE 31. TOTAL SODIUM INTAKE, PLOTTED LOGARITHMICALLY.

Urinary sodium output of the DOCA group was significantly higher ($P<0.001$) than that of the control group at all concentrations offered except 0.001 M. The peak was achieved at 0.3 M, which was not significantly different from excretion at 0.15 and 0.2 M (Figure 32). The DOCA group had significantly higher sodium balance at saline concentrations of 0.003 M and 0.03 M (but very much lower at 0.3 M (Figure 33). The DOCA group excreted significantly less potassium than the control group at saline concentrations of 0.001 to 0.03 M and at 0.5 M (Figure 34).

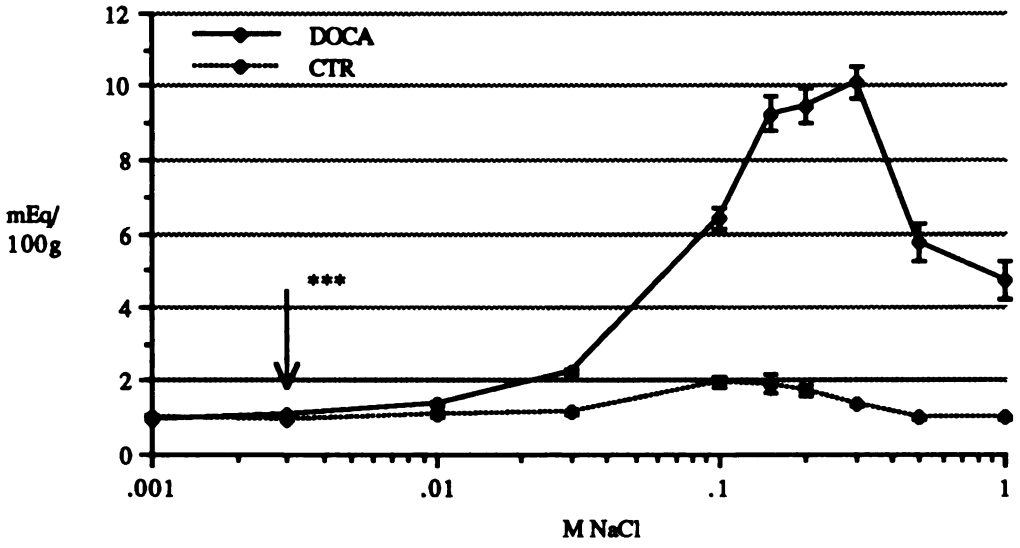

FIGURE 32. URINARY SODIUM DURING PREFERENCE TESTS.
The DOCA group excreted significantly greater amounts of sodium than control at all concentrations except 0.001 M (*** $P<0.001$). Sodium excretion reached maximum at 0.3 M, but this did not differ significantly from 0.15 M or 0.2 M ($P>0.05$).

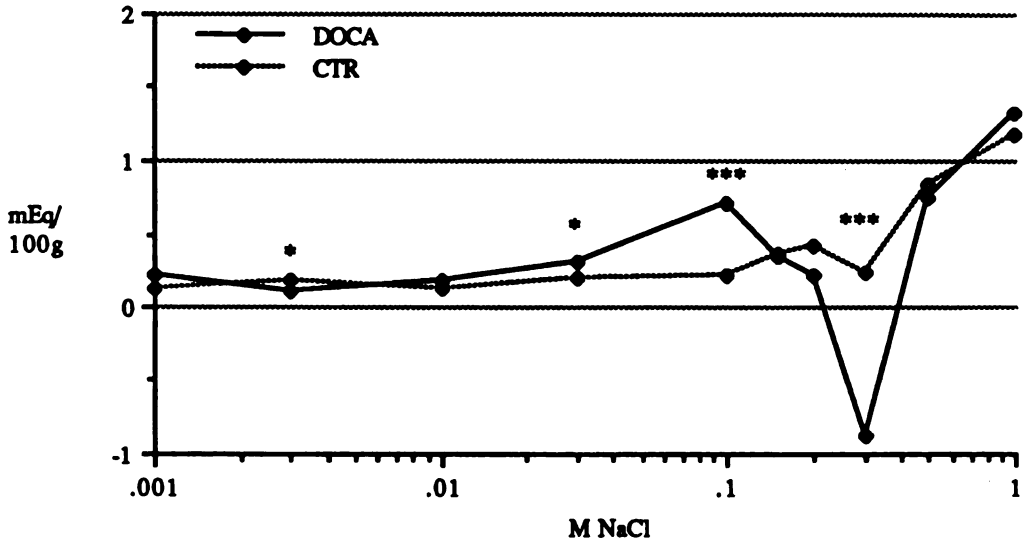

FIGURE 33. SODIUM BALANCE DURING PREFERENCE TESTS.
The DOCA group had significantly higher balance at 0.003 M and 0.03 M (* P<0.05), but very much lower at 0.3 M (*** P<0.001).

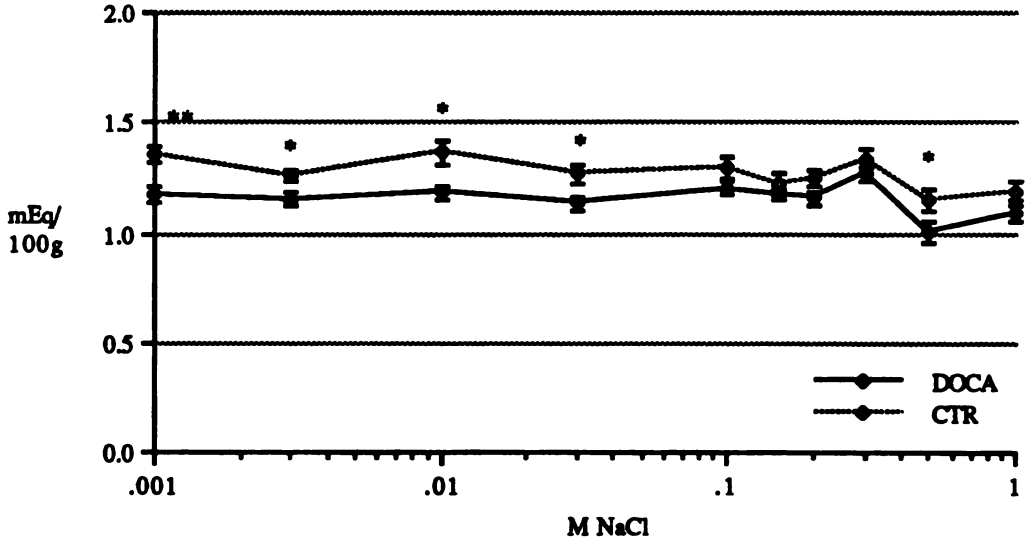

FIGURE 34. URINARY POTASSIUM DURING PREFERENCE TESTS.
The DOCA group excreted significantly less potassium than control group at many different concentrations of saline (* P<0.05, ** P<0.01).

ANP EXPERIMENT

After the rats were sacrificed, examination showed that the PE 60 attachment to the jugular veins was in good condition, and that minipumps had worked as expected. This confirmed the calculations that ANP was delivered at the rate of 4.0 μg/hr beginning on the day that 0.5 M saline choice was initiated.

No difference was noted in body weights between experimental (EXP) and control (CTR) group before surgery was performed, that is, on day -2 for EXP and day -1 for CTR. However, it was significantly different shortly thereafter and during the rest of the experiment (Figure 35). There were no differences either between the two groups in water and 0.5 M saline intake (Figure 36), urine volume (Figure 37), total sodium intake or urinary sodium excretion (Figure 38).

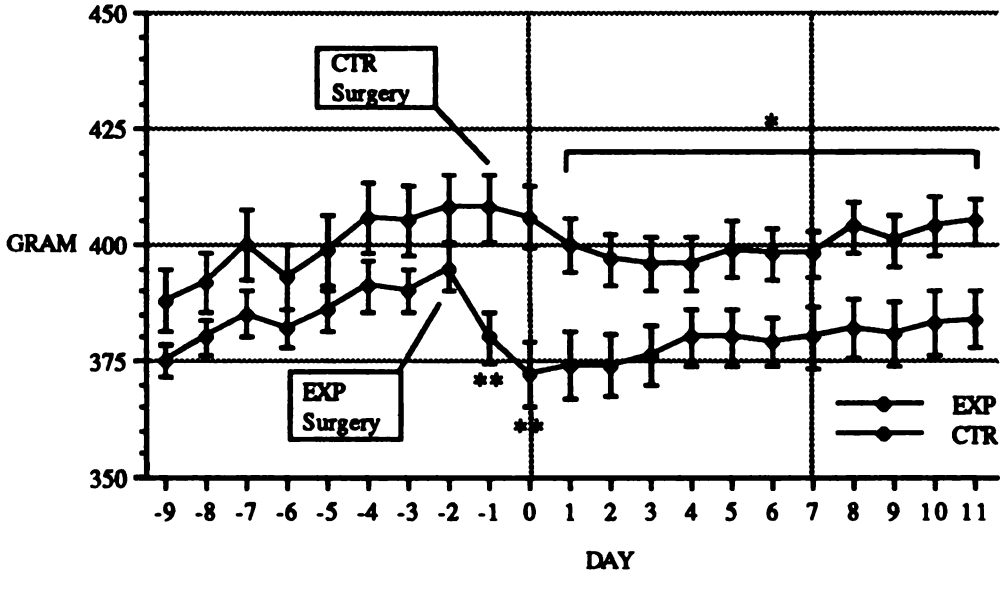

FIGURE 35. BODY WEIGHT CHANGES IN ANP EXPERIMENT.
No difference was observed between experimental (EXP) and control (CTR) groups before surgery (day -2), but significant difference was noted shortly after surgery and for the rest of the experiment (** P<0.01, * P<0.05).

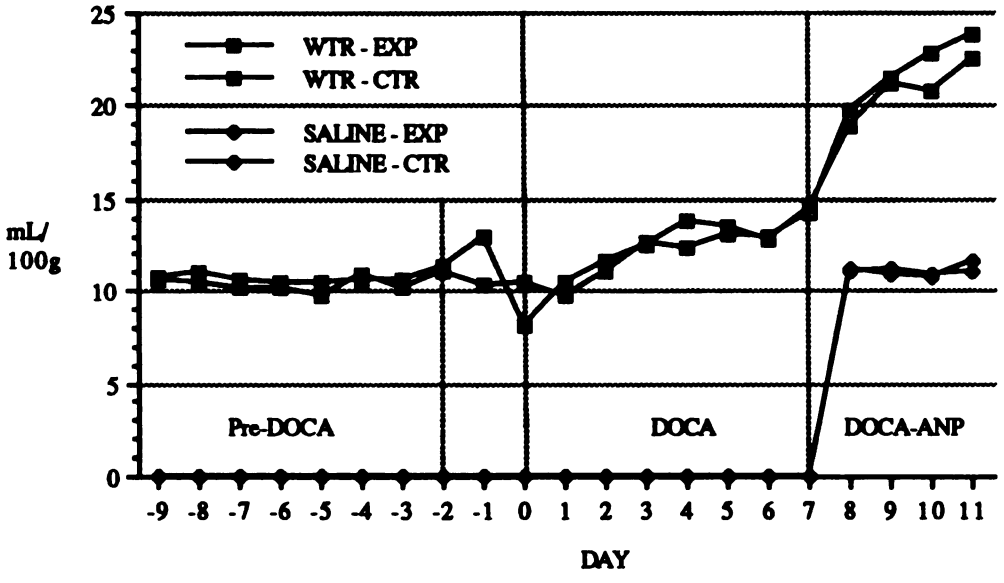

FIGURE 36. WATER AND SALINE INTAKE OF ANP EXPERIMENT.
No difference was noted between control and experimental group on any day.
WTR = water intake, SALINE = 0.5 M saline intake.

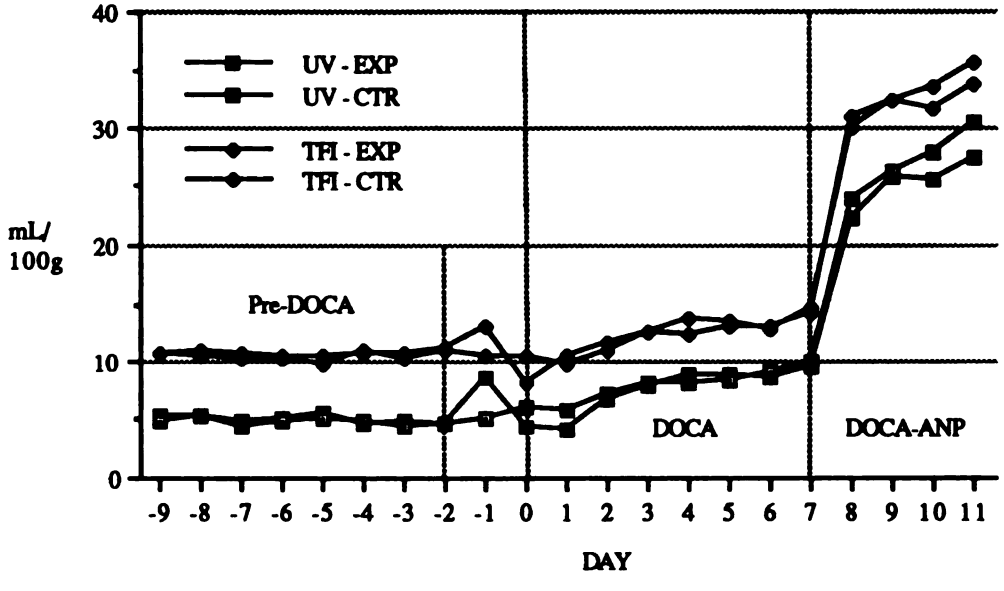

FIGURE 37. TOTAL FLUID INTAKE AND URINE VOLUME.
No difference was noted between experimental and control groups on any day.
TFI = total fluid intake, UV = urine volume.

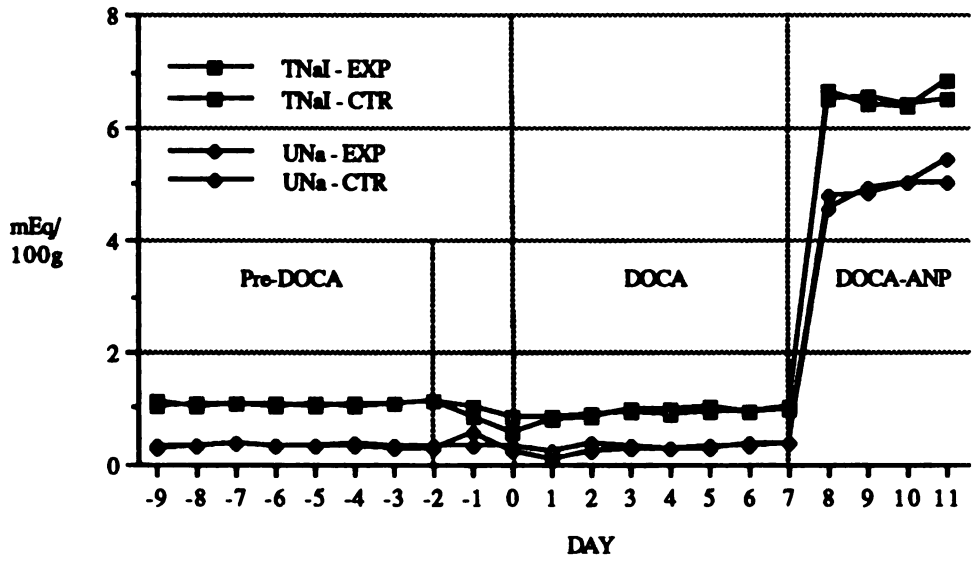

FIGURE 38. TOTAL SODIUM INTAKE AND URINARY SODIUM EXCRETION
No difference was noted between experimental and control groups on any day.
TNaI = total sodium intake, UNa = urinary sodium excretion.

Comparisons of four-day averages before surgery (Pre-DOCA), before (DOCA), and during the 0.5 M saline test (DOCA-ANP) are presented in the following figures: no difference in body weight was found before surgery, but a significant difference was found during DOCA injection and during DOCA and ANP administrations and the saline test (Figure 39); there were no differences in water and saline intakes (Figure 40), in sodium intakes from food and saline solution (Figure 41), in urine volume (Figure 42), in urinary sodium and potassium excretion (Figure 43 and 44, respectively), in water balance (Figure 45), and in sodium balance (Figure 46). ANP was designed to be delivered on day 7 in the experimental group, the day on which the saline test was begun.

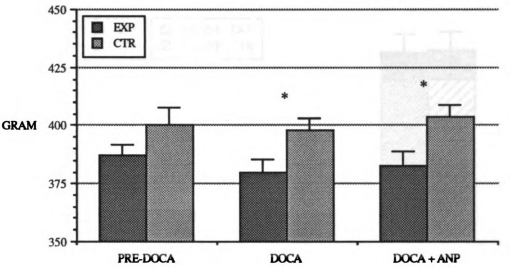

FIGURE 39. BODY WEIGHT DURING EXPERIMENTAL PERIODS. Significant difference was observed during DOCA and DOCA-ANP phases (* $P<0.05$). Pre-DOCA = before surgery was started, DOCA = during injections of DOCA 5 mg/kg twice daily, DOCA + ANP = during injection of DOCA and offering of saline to both groups.

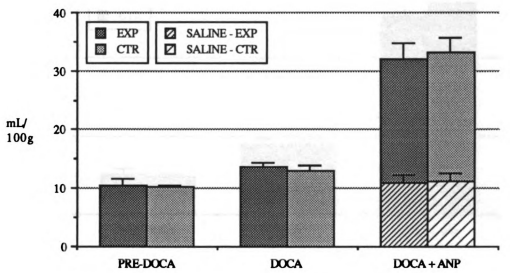

FIGURE 40. TOTAL FLUID INTAKE DURING EXPERIMENTAL PERIODS. No difference was noted between experimental and control groups in any period. SALINE = saline intake in the period when saline was offered.

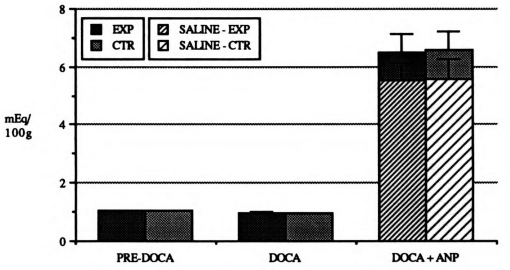

FIGURE 41. TOTAL SODIUM INTAKE DURING EXPERIMENTAL PERIODS.
No difference was noted between experimental and control groups in any period.
SALINE = sodium intake from saline in the period when saline was offered.

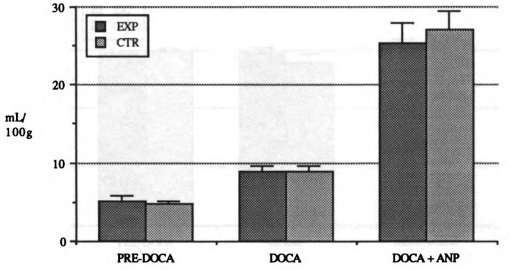

FIGURE 42. URINE VOLUME DURING EXPERIMENTAL PERIODS.
No difference was noted between experimental and control group at any period.

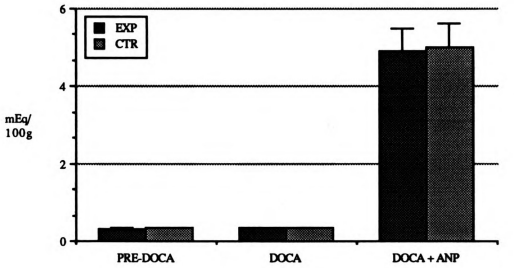

FIGURE 43. URINARY SODIUM DURING EXPERIMENTAL PERIODS.
No difference was noted between experimental and control group at any period.

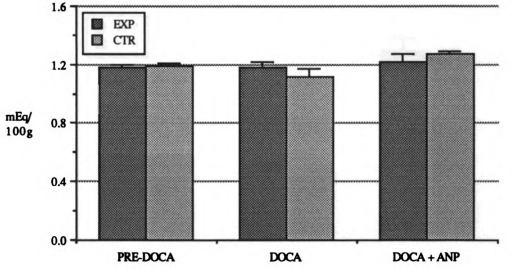

FIGURE 44. URINARY POTASSIUM DURING EXPERIMENTAL PERIODS.
No difference was noted between experimental and control group at any period.

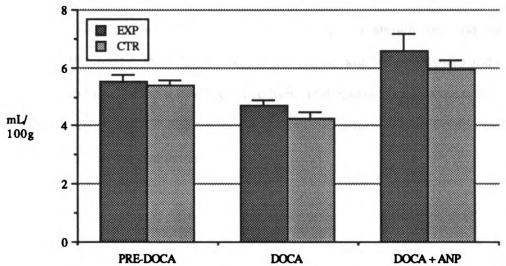

FIGURE 45. WATER BALANCE DURING EXPERIMENTAL PERIODS.
No difference was noted between experimental and control group at any period.

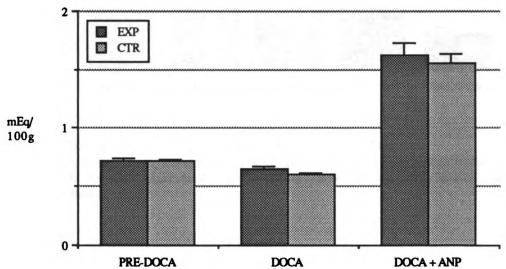

FIGURE 46. SODIUM BALANCE DURING EXPERIMENTAL PERIODS.
No difference was noted between experimental and control group at any period.

Comparisons between experimental and control groups during the saline test, when ANP was also being infused in the experimental group, are shown for fluid variables (water intake, saline intake, total fluid intake, urine volume, and water balance) and sodium variables (food sodium, saline sodium, total sodium intake, urinary sodium excretion, and sodium balance) in figures 47 and 48, respectively. No difference was noted in any of those variable between the two groups.

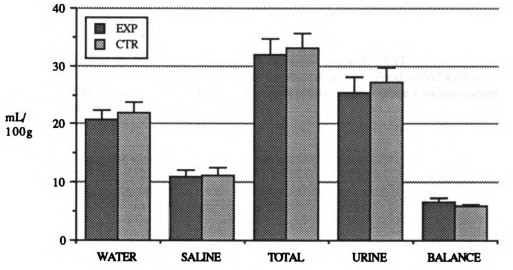

FIGURE 47. FLUID VARIABLES DURING SALINE TEST.
No difference was found in any of the variables measured. Water = water intake, saline = saline intake, total = total fluid intake, urine = urine volume, balance = water balance.

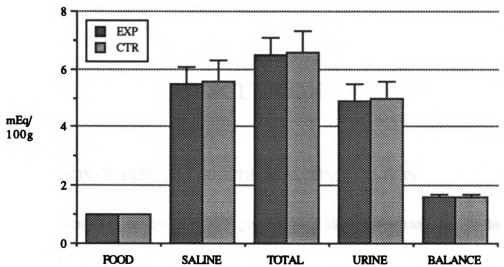

FIGURE 48. SODIUM VARIABLES DURING SALINE TEST.
No difference was found in any of the variables measured. Food = food sodium, saline = saline sodium, total = total sodium intake, urine = urinary sodium output, balance = sodium balance.

DISCUSSION

DETERMINATION OF DOSE AND ROUTE OF ADMINISTRATION

Intramuscular injection of DOCP gives a very slow absorption rate, in humans, with an estimated 1.5 - 3.5% of the injected dose being absorbed daily[75]. In the preliminary experiments we found that intramuscular DOCP increased 0.5 M saline intake up to 30 ml/day, while subcutaneous DOCA increased it to 45 ml/day when given two weeks after DOCP injection, or to 37 ml/day when given to fresh rats. Water intake reached the maximum of 108 ml/day with DOCP while DOCA increased it to 133 ml/day. Daily injections of DOCA at 10 mg/kg divided into two doses caused a larger increase in both water and saline intakes than 15 mg/kg, but this was probably due to the residual effect of previously injected DOCP.

We decided to use 10 mg/kg DOCA daily given in two doses, because it gave us a more predictable result and higher intake than DOCP, and this smaller dose gave the same or better result than 15 mg/kg/d.

EFFECTS ON SALINE AND WATER INTAKE

In the preliminary experiments (Figures 2 to 9), we observed that increased saline intake was always preceded by increased water intake. DOCP at 7.5 mg and 12.5 mg increased water intake only on one day and four days after each injection, respectively, but no difference was noted in saline intake. DOCP at 25 mg resulted in increased water intake beginning two days after injection and lasting for more than 100 days, while saline intake

became significantly elevated three days after injection and remained elevated for six days. Injection of 50 mg DOCP in two days resulted in increased water intake beginning two days after the first injection, and saline intake began to increase after four days. Also, injection of DOCA 15 mg/kg/d increased water intake on day 1, while saline intake began to increase on day 2.

During the adaptation period preceding the preference tests, before any saline was given, water intake began to increase on day 9 (Figure 12), but urine volume increased beginning on day 2 (Figure 13). Both water intake and urine volume increased significantly on the first day following the initiation of DOCA injections. Sodium retention was noted on day 1 and escape the next day, and there was no difference in sodium excretion noted between the DOCA and control groups in subsequent days (Figure 16 and 17).

These results agree with Green, who had suggested that polydipsia in DOCA administration is caused by a rise in tissue sodium content due to sodium retention[47], and that an augmented salt appetite was discernible somewhat later usually during the first week, together with a correlated increase in sodium output[48]. Davis[25] reported that, in dogs, the onset of polyuria preceded the development of polydipsia. He also found that DOCA increased glomerular filtration rate and renal plasma flow, and decreased filtration fraction.

From the temporal relation in these experiments, we found that in our experiment DOCA administration first reduced sodium excretion, which was followed by escape on the second day, and increased urinary output on the same day. This was later followed by increased water intake and later by increased saline intake.

PREFERENCE TESTS

Saline solutions were offered after water intake had stabilized for several days, indicating that the effect of DOCA had reached a plateau. The treated rats had greater saline in-

take than the control rats at all concentrations offered, from 0.001 M to 1.0 M. At 0.001 M, intake was twice as much as the controls, at 0.1 and 0.15 M, it was six times greater, and even at 1.0 M, when both group showed an aversion, the DOCA group still drank more than twice that of the controls (Figure 21). At 0.1 and 0.15 M, saline intake of the experimental was 61 and 57 ml/100g, respectively, compared to control intakes of 11 and 8 ml/100g; total fluid intakes were 62 and 60 ml/100 g for the DOCA group, and 16 ml/100g for the controls.

Preference threshold, defined as the concentration at which significantly more saline than water is drunk could not be determined in this experiment because at the lowest concentration offered saline intake was already eight times higher than water intake. This indicates that preference threshold was considerably below 0.001 M. The highest concentration at which they still drink more saline than water was 0.3 M. Compared to the controls, whose preference ranged from 0.03 M to 0.1 M (Figure 25), the DOCA group clearly expanded their preference, which ranged from less than 0.001 M to 0.3 M (Figure 24).

Richter showed that the point of divergence of saline and water intake curves of normal rats occurred at 0.009 M[68], while Herxheimer[51], using the excess intake of saline greater than 1/8 of total intake as a significant value, found a preference threshold at 0.004 M, which was reduced to 0.001 M by a 15 mg DOCA implant. The present result suggests that rats treated with DOCA not only will increase their intake of salt at lower concentrations, but they will also be more tolerant of higher concentrations.

Total sodium intake of the DOCA group reached maximum when saline was offered at 0.15, 0.2, and 0.3 M, without any significant difference between them (Figure 30). It is interesting to note, however, that saline intake at these concentrations, combined with water intake, would result in the total molarity of fluid ingested of 0.143, 0.158, and 0.163 M, concentrations that are very close to isotonicity. Voluntary sodium intakes of the DOCA group from the salt solutions at 0.15, 0.2, an 0.3 M were 8.56, 8.69, and 8.20 mEq/100g, respectively (Figure 28 and 29), about eight times greater than the control

group's or the sodium contained in the food. The control group's voluntary intakes were 1.09, 1.20, and 1.20 mEq/100g at saline concentrations of 0.1, 0.15, and 0.2 M. Sodium intake from food ranged from 1.10 to 1.40 mEq/100g in both groups before saline was offered (Figure 15), and 0.92 to 1.22 mEq/100g during preference tests, without any significant difference between them.

Urine volume increased steadily together with increasing fluid intakes, and reached maximum at 0.1 and 0.15 M (Figure 26), which were the concentrations when total fluid intakes reached maximum (Figure 23). Urinary sodium output reached maximum at 0.15, 0.2, and 0.3 M (Figure 32), also at the same concentrations when total sodium intake reached maximum. These results show that increasing appetite for saline solutions, with the resulting increase in water and sodium intakes, was dealt with appropriately by the kidneys. The negative sodium balance which occurred at 0.3 M (Figure 33) may be the result of the kidney's effort to excrete the accumulated sodium in the body.

During the adaptation period preceding the preference tests, no difference was found in urinary potassium excretion between the DOCA and control group (Figure 18), which is consistent with the return of sodium excretion during escape to control values. However, during the preference tests, the DOCA group excreted significantly less potassium than control when saline was offered at concentrations of 0.001, 0.003, 0.01, 0.03, and 0.5 M (Figure 34). The reason for this is not clear.

ANP EXPERIMENT

Daily administration of 10 mg/kg DOCA during the period before the 0.5 M saline test resulted in greater water intake and urine volume than in the period before surgery was performed, and no difference between control and experimental groups (Figures 40 & 42). The saline test, together with infusion of 4 μg ANP/hr, increased total fluid intake and urine volumes compared to the period when DOCA was given alone, but again, no differ-

ence was found between experimental and control groups. No difference was noted either between the two groups in saline intake (Figures 36 & 48), urinary sodium and potassium excretion (Figures 43 & 44), or total sodium intake (Figures 41 & 48).

These results showed that no further increase of natriuresis, diuresis, and saline intake was caused by this dose of ANP. The hypothesis that ANP increase occurring during DOCA escape was responsible for the increased salt intake cannot be proven wrong, however, because there is a possibility that plasma ANP was not sufficiently elevated by the infused dose to cause a greater effect, or that salt intake was at a maximum. These possibilities can be tested by giving a higher dose of ANP to the experimental group along with a second booster dose of DOCA to the controls.

CONCLUSION

Administration of DOCA subcutaneously at 5 mg/kg every 12 hours gave a reliable and predictable increase in salt appetite, water intake, and urine volume, compared to 5 mg/kg/d, or to single doses of DOCP intramuscularly. Increasing the dose to 7.5 mg/kg/12hr also gave good and predictable results.

Saline intake was increased to the maximum at concentrations of 0.1 M and 0.15 M, which was up to eight times greater than basal level. Preference threshold was reduced to below 0.001 M, and salt aversion did not occur until concentration of saline offered exceeded 0.3 M. Voluntary sodium intake was up to eight times greater than that of the control during the preference tests or the basal intake preceding the preference tests.

While increased salt appetite in response to sodium need can be attributed to higher angiotensin II concentration in the brain, but not directly to increased mineralocorticoid levels, the release of ANP during mineralocorticoid escape offers a possible explanation for increasing salt appetite during DOCA administration. Infusion of 4.0 μg ANP/hr for four days was not sufficient to increase ANP level enough to increase salt appetite further.

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