Some Effects of High and Low Sodium Intakes During Pregnancy in the Rat1,2

II. ELECTROLYTE CONCENTRATIONS OF MATERNAL PLASMA, MUSCLE, BONE AND BRAIN AND OF PLACENTA, AMNIOTIC FLUID, FETAL PLASMA AND TOTAL FETUS IN NORMAL PREGNANCY

AVANELLE KIRKSEY,4* RUTH L. PIKE AND JACQUELINE A. CALLAHAN5
Department of Foods and Nutrition, The Pennsylvania State University, University Park, Pennsylvania

In a previous paper (Kirksey and Pike, '62) data was presented relative to the effects of a dietary sodium chloride deficit and surfeit on food consumption, weight gain, reproductive performance, sodium and potassium balances, urinary excretion of sodium and potassium, hematocrit, hemoglobin, plasma total protein and protein fractions during the course of normal pregnancy in the rat. The present report is concerned with the effects of salt intake by the normal pregnant rat upon sodium and potassium levels of maternal plasma, muscle, bone and brain and on fetal plasma, amniotic fluid, placenta and total fetus.

METHODS

The experimental design and the dietary treatment for the entire study have been presented previously (Kirksey and Pike, '62).

Prior to withdrawal of blood samples, animals were fasted for 1.5 hours to eliminate effects of recent food ingestion on sodium and potassium concentrations of plasma. All blood determinations were conducted on an ultramicro scale to avoid excessive bleeding of animals. Blood from the tip of the tail was collected in heparinized capillary tubes. Less than 0.2 ml of blood was taken each week of the experimental period. Blood was centrifuged immediately and the cells and plasma separated to minimize electrolyte shifts. Five- and 20-uliter samples of plasma were used for sodium and potassium analyses, respectively. Each sample was diluted with distilled demineralized water by means of a microburette to a ratio of 1:350 for sodium and 1:100 for potassium determinations. To correct for mutual interference of sodium and potassium in flame readings, standards were prepared to contain sodium and potassium ions in a 30:1 ratio, which corresponds to that found in normal plasma. Lab-Trol, an artificial serum containing known amounts of sodium and potassium was prepared similarly to plasma and analyzed as a control. Details of the flame photometry method used were described previously (Kirksey and Pike, '62).

On the twenty-second day of the gestation period, each animal was fasted for 1.5 hours, chloroformed, and the uterus exposed and removed intact. The uterine wall was split and the intact placenta and sac of each live fetus removed. The sac was suspended by the placenta and the amniotic fluid was withdrawn with a micropipette and delivered into a 55 × 5 mm test tube and stoppered. The placenta was separated from the sac, blotted and weighed.

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4 General Food Funds Fellow, 1958–60. Present address: Department of Food and Nutrition, Purdue University, West Lafayette, Indiana.
5 Presented in partial fulfillment of the requirements for the degree of Master of Science.
* Dade Reagents, Inc., Miami, Florida.
Blood was drawn from each of the fetuses by heart puncture and collected in heparinized capillary tubes. The method described by Grazer ('58) was adapted to eliminate a transfer of blood from the syringe by the insertion of a heparinized capillary tube into the system between the needle and the syringe. The blood was centrifuged and the cells and plasma separated.

Amniotic fluid and blood samples were treated in an identical manner. For sodium determinations a 4.5-ulliter sample was diluted with distilled demineralized water to a ratio of 1:400, for potassium, a 14.5-ulliter sample was diluted to a ratio of 1:100. Samples were analyzed according to the procedure described for maternal plasma.

Individual fetuses and maternal gastrocnemius muscle, tibia bones and the brain were removed, freed of fat and other extraneous materials, blotted with Whatman no. 40 filter paper and weighed immediately on a torsion balance. Each was dried to constant weight at 105°C and the percentage moisture calculated.

Dried gastrocnemius muscle and fetuses were digested in hot concentrated HNO₃. The digested samples were diluted with distilled demineralized water for sodium and potassium analyses.

Dried tibia bones and brain were ashed at 525°C for 48 hours and placenta for 36 hours. The ash was dissolved in 2 N HCl and diluted with distilled demineralized water for sodium and potassium analyses. Since calcium ions may interfere with the accuracy of sodium determinations made with the flame photometer (Bauer, '54), Ca₃(PO₄)₂ was added to sodium standards used in bone analyses to produce a sodium-to-calcium ion ratio of 1:50, which is the approximate ratio found in rat bone (Cheek et al., '57).

RESULTS AND COMMENT

Plasma sodium and potassium. Sodium and potassium concentrations in maternal plasma are shown in table 1. The slight decreases in plasma sodium concentrations (P = 0.05) observed for pregnant animals late in the gestation period in this study are similar to observations reported for normal pregnant humans (Goodland et al., '54; Newman, '57; Venning et al., '59). A decrease of approximately 5 mEq/liter of plasma total electrolytes in normal pregnant humans was attributed primarily to a decrease in concentration of the sodium ion (Newman, '57). In this investigation, control pregnant animals had an over-all decrease of 4.3 mEq/liter of plasma sodium with no demonstrable change in plasma potassium level. Decreases in plasma electrolytes appear to be associated with hemodilution which normally accompanies pregnancy. According to Goodland et al. ('54) blood pH changes, which could occur in conjunction with the decreased plasma cation concentration in normal pregnancy, are prevented by compensatory mechanism.

The most significant change in plasma sodium (P = 0.001) and potassium levels (P = 0.05) occurred for pregnant animals receiving low sodium. During the gestation period, plasma sodium concentration decreased from 146.6 to 126.5 mEq/liter and plasma potassium increased from 5.1 to 6.7 mEq/liter. The fetuses of mothers fed low sodium had normal concentrations of sodium and potassium in plasma and tissues. These observations suggested that when dietary sodium was inadequate for maternal and fetal needs, the fetus sequestered sodium from maternal stores. Hyponatremia and hyperkalemia observed in pregnant animals fed low sodium are typical manifestations of sodium depletion (Marriott, '47; Black, '60). Although hyponatremia may be directly associated with sodium depletion, the cause of hyperkalemia is conjectural. Berliner ('54) suggested that it was the result of a failure of kaliuresis. In the present study, however, kaliuresis was not reduced as a result of sodium depletion (Kirksey and Pike, '62). This observation confirms that of Anderson and Laragh ('58) for sodium-depleted dogs. These investigators suggested that available sodium in the tubule was not the sole factor governing potassium excretion in the sodium-retaining state. Welt et al. ('60) theorized that potassium was in a competitive position with hydrogen for sodium exchange and that elevated plasma hydrogen ion concentration, associated with hyponatremia in so-
### Table 1

<table>
<thead>
<tr>
<th>Dietary Treatment</th>
<th>Na, mEq/100 gm</th>
<th>K, mEq/100 gm</th>
<th>Na, mEq/liter</th>
<th>K, mEq/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant</td>
<td>146.8 ± 2.9</td>
<td>53.8 ± 0.3</td>
<td>146.1 ± 1.8</td>
<td>51.0 ± 0.3</td>
</tr>
<tr>
<td>Day 1</td>
<td>147.0 ± 2.4</td>
<td>51.0 ± 0.4</td>
<td>146.3 ± 2.3</td>
<td>51.0 ± 0.3</td>
</tr>
<tr>
<td>Day 21</td>
<td>145.8 ± 1.7</td>
<td>52.0 ± 0.2</td>
<td>144.5 ± 2.2</td>
<td>52.0 ± 0.4</td>
</tr>
</tbody>
</table>

*Standard error of the mean.

### Table 2

<table>
<thead>
<tr>
<th>Dietary Treatment</th>
<th>Muscle, Bone, and Fluid Electrolytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na, mEq/liter</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>109.2 ± 2.5</td>
</tr>
<tr>
<td>Muscle</td>
<td>154.3 ± 3.0</td>
</tr>
<tr>
<td>Bone</td>
<td>20.1 ± 1.5</td>
</tr>
</tbody>
</table>

*Standard error of the mean.

### Control Groups

<table>
<thead>
<tr>
<th>Dietary Treatment</th>
<th>Na, mEq/liter</th>
<th>K, mEq/liter</th>
<th>%</th>
<th>Na, mEq/liter</th>
<th>K, mEq/liter</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant</td>
<td>107.4 ± 2.5</td>
<td>51.0 ± 0.3</td>
<td>75.8 ± 0.9</td>
<td>51.0 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>154.2 ± 3.0</td>
<td>51.7 ± 1.3</td>
<td>102.1 ± 1.0</td>
<td>51.7 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>20.1 ± 1.5</td>
<td>40.5 ± 3.5</td>
<td>79.9 ± 0.3</td>
<td>40.5 ± 3.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Standard error of the mean.
dioium depletion, favored a sodium for hydrogen ion exchange in the tubule resulting in plasma potassium accumulation. It is possible that renal ion-exchange mechanisms promoting potassium accumulation counterbalanced the potassium-excretory effects of the increased level of aldosterone associated with dietary sodium restriction (Eisenstein, '57).

Muscle sodium, potassium and moisture. According to current theory, ion concentration gradients of muscle cells may be maintained not only by physiochemical processes such as the Gibbs-Donnan equilibrium, but also by hormonal or enzymatically controlled intracellular metabolic processes, or by both (Manery, '54; Conway, '57).

The slight decreases in muscle sodium content (P = 0.05) observed for pregnant animals in this study are similar to observations of Tatum ('56) and Kumar et al. ('59) for normal pregnant humans (table 2). However, these observations are in direct contrast with those of Dieckmann and Pottinger ('56) who reported significant increases in muscle sodium in normal pregnant subjects. The factors governing muscle sodium changes in pregnancy are conjectural. Kumar et al. ('59) postulated a sodium-lowering factor in muscle cells operating antagonistically to the increasing levels of aldosterone observed in normal pregnancy. In this study, the slight decreases in muscle sodium in pregnancy, without concomitant changes in potassium, offer some support for Manery's theory ('54) that sodium and potassium movements in cells are not coupled, but are controlled by separate mechanisms.

Woodbury ('56) reported that decreased muscle sodium was associated with hyponatremia produced by intraperitoneal injection of isosmolar glucose in rats. In addition, Yannet and Darrow ('40) suggested that the muscle sodium content in rats varied directly with the concentration of serum sodium. The slight decreases in muscle sodium content of pregnant groups in this study paralleled the decrease in plasma sodium concentration. A decrease of approximately 35% in muscle sodium (P = 0.01) and 13% in plasma sodium (P = 0.001) was observed in pregnant animals receiving low sodium intakes as compared with values for those with higher intakes. However, sodium and potassium concentrations of their fetuses were within the normal range. It is suggested that sodium from maternal muscle, as well as plasma, was utilized by the fetus. This is in agreement with Hammond's theory (‘44) that fetal tissue has priority for nutrients over maternal tissue in a nutrient shortage.

Muscle potassium concentration was higher (P = 0.05) for pregnant animals receiving the low sodium intake than for those with higher intakes. This increase paralleled a 24% increase in plasma potassium concentration. Meyer et al. ('50) observed increased muscle potassium in sodium-depleted rats and theorized that this increase was a reflection of the hyperkalemia observed in these animals. Rogers and Wachenfeld ('58) postulated that physiological acids, associated with severe sodium depletion, penetrated cells and thereby lowered the intracellular buffering capacity which resulted in the replacement of hydrogen ions in the cell by potassium.

Small, but significant increases (P = 0.01) in muscle moisture were observed for the pregnant group receiving the low sodium intake (table 2). Since intracellular hydration occurs in sodium depletion in order to maintain osmotic relations between intracellular and extracellular environments (Black, '60; Danowski et al., '55; Burnell et al., '60), these physiological alterations may account for the observed increases in muscle moisture in this study.

Bone sodium, potassium and moisture. Bone, as compared with other body tissues, has the highest content of sodium. It contains approximately 20% of the total sodium in the rat (Cheek et al., '57), most of which is in the crystalline lattice and only a small fraction in the extracellular phase (Bergstrom, '55). According to Bergstrom ('56), the large surface area of bone provides a site which sodium may occupy by adsorption or ionic substitution without interference with the internal crystalline structure. Therefore, he suggests that an ideal situation exists for dynamic equilibrium between bone sodium and surrounding extracellular fluid. By means of isotopes, it has been demon-
strated that 30 to 40\% of the sodium in bone is exchangeable with extracellular sodium; the remaining sodium appears to be less accessible (Bergstrom, '55; Bauer, '54).

Bone sodium decreased 22 mEq, or approximately 10\% in pregnant rats fed low sodium as compared with those with higher intakes (table 2). This loss paralleled a 13\% decrease in plasma sodium concentration. This confirms the work of Forbes ('58) who reported that rats fed low sodium diets lost approximately 8 to 10\% of bone sodium which was associated with a comparable decrease in blood sodium. However, White et al. ('59) observed that bone sodium was not altered by hypotension in rats.

It is possible that other physiological changes caused the bone sodium losses observed in this study. Forbes et al. ('59) demonstrated losses as a result of alterations in blood pH, plasma sodium concentration or total extracellular sodium and suggested that bone was responsive to a wide variety of stimuli, or that some common physiologic mechanism controlling bone sodium levels was affected by these alterations. Losses, as great as 28\%, have been noted following the production of acute acidosis by intraperitoneal dialysis against ammonium chloride (Bergstrom and Wallace, '54; Bergstrom and Ruva, '60). Since severe sodium depletion is accompanied by acidosis (Marriott, '47; Black, '60), and since many signs of sodium depletion were exhibited by the pregnant animals receiving a low sodium intake, it is possible that these animals were acidic and that bone sodium losses occurred as a consequence.

Owen ('52) suggested that soft tissues take priority over bone for supply of mineral in a nutrient shortage but that rapidly metabolizing tissues can successfully compete with bone for mineral. If this postulation is true, then it may be assumed that fetal tissue can compete with bone for sodium. This phenomenon appeared to have occurred in the pregnant animals receiving the low sodium intake. These animals had low levels of sodium in plasma, muscle and bone but their fetuses had total sodium and potassium contents comparable to fetuses of control animals.

Bone potassium concentrations and percentages of moisture were not significantly different for any of the experimental groups (table 2). Because marked electrolyte changes were observed in muscle, it appears that electrolytes in muscle may be more labile than those in bone.

**Brain sodium, potassium and moisture.** Sodium and potassium maintain osmotic relations and electroneutrality in the cell and are also associated with enzyme activity and nerve impulse conduction (Manery, '54). The larger amounts of sodium in brain as contrasted with muscle may be associated with the latter functions.

The lowest concentration of brain sodium observed in any experimental group was in pregnant animals receiving the low sodium intake (table 2). According to Hammond’s theory ('44), in a nutrient shortage the brain and central nervous system have a priority rate equal to that of the placenta and fetus. However, the data in this study suggest that fetal tissue may even exert priority for sodium over maternal brain tissue, since the former showed no change in the pregnant animals receiving the low sodium intake, whereas in the latter tissue, a decrease was evident.

The potassium content of brain was significantly decreased (P = 0.001) in all pregnant animals (table 2). This decrease may have been the result of a decrease in osmotic pressure of extracellular fluid associated with the reduced concentration of plasma sodium observed in the pregnant animals. Yamnet ('40) noted that a decrease of 1 mEq/liter of plasma sodium resulted in a brain potassium decrease of approximately 0.5 mEq/kg. A similar relationship was observed for pregnant groups in this study.

Pregnant animals receiving the low sodium intake had a significantly lower concentration of brain potassium (P = 0.05) than those with higher intakes. This group also had a significantly lower plasma sodium concentration (P = 0.001), adding support to the suggestion that decreased brain potassium is associated with reduced plasma sodium levels. It is also possible that decreases in brain potassium and sodium in pregnant animals receiving the
low sodium were intensified by acidosis accompanying sodium depletion. Woodbury and Koch (ʼ57) demonstrated that acidosis produced in rats by inhalation of carbon dioxide led to decreased brain electrolytes. Although metabolic acidosis may produce slower changes in brain than direct inhalation of carbon dioxide (Leusen, ʼ54), the possibility exists that this derangement could alter brain electrolytes. Electrolyte movements in brain cells may be affected by hormonal activity (Woodbury and Koch, ʼ57; Zeigler et al., ʼ44). Hoagland and Stone (ʼ48) reported that rats subjected to the stress of prolonged swimming had significant reductions in brain potassium which were augmented by deoxycorticosterone. Since pregnancy is a stress condition attended by significant increases in adrenal hormone levels (Smith et al., ʼ59) the brain electrolyte changes observed in this study may have been associated with hormonal activities. Small, but significant (P = 0.001), increases in brain moisture were observed as a result of pregnancy (table 2). Since hyponatremia in rats has been associated with increased intracellular water in brain (Elliott and Jasper, ʼ49), the tendency toward hyponatremia exhibited by pregnant groups in this study may, therefore, account for the small increase in brain moisture.

**Fetal plasma.** Although the average sodium concentration of fetal plasma was decreased in the low sodium group and slightly increased in the high sodium group when compared with the control value, the differences were not statistically significant (table 3). The potassium concentrations showed little variation. In the low sodium group fetal plasma sodium concentration was 10 mEq greater and potassium was 1.4 mEq less than that in the maternal plasma for that group. Despite significant changes in maternal sodium and potassium concentrations as a result of the low sodium intake, the fetus was able to maintain relatively normal plasma concentrations of these electrolytes. The mechanism by which the sodium ion can operate against a concentration gradient has not been defined.

**Amniotic fluid.** Although average values for sodium concentration in amniotic fluid were slightly decreased in the low sodium group and slightly increased in the high sodium group when compared with the average control value, the differences were not statistically significant (table 3). This trend was similar to that noted for fetal plasma. If some of the sodium present in the amniotic fluid is derived from the sodium contained in fetal urine, it appears that the lower concentration observed in the group fed low sodium may be due to decreased fetal excretion. If this is so, it is postulated that the reduced fetal excretion of sodium may be associated with the mechanism by which these young can maintain a plasma sodium level higher than that observed in the maternal plasma.

**Placenta.** The average sodium and potassium concentration of placentae was not affected by the maternal sodium intake (table 4). There was little group variation in placental moisture content; however, a slight decrease was observed in animals receiving the low sodium diet. Villee et al. (ʼ53) noted a decrease in the moisture content of placental tissue with increased fetal age. However, Hard et al. (ʼ44) observed a constant water content in guinea pig placenta throughout preg-

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TABLE 3

<table>
<thead>
<tr>
<th>Maternal diet group</th>
<th>Fetal plasma</th>
<th>Amniotic fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na mEq/liter</td>
<td>K mEq/liter</td>
</tr>
<tr>
<td>Na, mEq/100 gm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>137.0 ± 10.41</td>
<td>5.3 ± 1.1</td>
</tr>
<tr>
<td>6.3*</td>
<td>141.3 ± 4.9</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>54.8</td>
<td>143.9 ± 8.2</td>
<td>5.2 ± 0.5</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
* Control groups.
Tissue and fluid electrolytes in pregnancy. From this study, it appears that factors other than placental age may affect the moisture content.

Fetus. The level of sodium in the maternal diet had no effect on either sodium or potassium levels in the total fetus (table 4). The influence of the level of maternal sodium intake on fetal moisture was statistically significant (P = 0.01). Fetal moisture was increased for animals receiving the low sodium intake. Because increased moisture has been associated with fetal immaturity (Moulton, '23) and because fetuses of animals fed the low sodium diet were smaller than those of animals receiving larger quantities of sodium (Kirksey and Pike, '62), the increased fetal moisture for the low sodium group may indicate fetal immaturity.

SUMMARY

Maternal plasma sodium concentrations decreased slightly as a result of pregnancy, whereas potassium concentrations were unaltered. A significant decrease in sodium and an increase in potassium levels of plasma occurred in pregnant animals fed the low sodium diet.

Muscle sodium concentrations were slightly lower in pregnant than nonpregnant groups but potassium concentrations were similar. With the low sodium intake, pregnant animals had an approximate 35% decrease in sodium and a 4% increase in potassium concentrations of muscle. These animals also had significant increases in muscle moisture.

Bone sodium was significantly decreased in the pregnant groups receiving the low sodium intake. However, potassium concentrations and moisture were not affected by the level of dietary sodium or by pregnancy.

Brain sodium and potassium concentrations were significantly lower for pregnant groups receiving the low sodium intake than for any other experimental group. Small, but significant decreases in potassium concentrations and increases in moisture content of brain were observed as a result of pregnancy.

Fetal plasma levels of sodium and potassium were not significantly influenced by the level of sodium in the maternal diet. Despite a marked decrease in sodium and an increase in potassium concentrations of plasma in pregnant animals fed the low sodium diet, the fetuses of these animals appeared to be able to maintain normal sodium levels. The mechanism by which the sodium ion can operate against a concentration gradient has not been defined.

The concentrations of sodium and potassium in amniotic fluid did not appear to be statistically related to the maternal sodium intake. It is suggested that a reduction in fetal urinary excretion of sodium may be associated with a mechanism by which the young of animals fed low sodium diets are able to maintain normal plasma sodium levels.

The sodium or potassium levels of fetuses were not significantly influenced by the maternal sodium intake. The slight increase in fetal moisture in animals receiving the low sodium diet may be associated with fetal immaturity.

Placental sodium, potassium or moisture contents were not significantly affected by the maternal sodium intake.

The data indicated that nonpregnant animals can handle wide ranges of dietary sodium without observable effects on sodium or potassium concentrations of tissues and fluids. Also, the levels of

<table>
<thead>
<tr>
<th>Maternal diet group</th>
<th>Placenta</th>
<th>Fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
<td>K</td>
</tr>
<tr>
<td></td>
<td>mEq/kg</td>
<td>mEq/kg</td>
</tr>
<tr>
<td>Na, mEq/100 gm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>81 ± 10'</td>
<td>36 ± 7</td>
</tr>
<tr>
<td>6.3*</td>
<td>82 ± 7</td>
<td>32 ± 6</td>
</tr>
<tr>
<td>54.8</td>
<td>86 ± 8</td>
<td>32 ± 6</td>
</tr>
</tbody>
</table>

1 Standard error of the mean.

* Control groups.
these electrolytes were not affected by feeding excessively high levels of dietary sodium during pregnancy. However, restriction of sodium intake during pregnancy led to marked electrolyte changes in plasma, muscle, bone and brain of the maternal organism in an attempt to maintain the normalcy of the fetus. In fact, pregnant animals receiving the restricted sodium intake exhibited changes in water and electrolyte metabolism that have been associated with complications of pregnancy in humans.

LITERATURE CITED


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