Salt saving in the pregnant rat

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LICHTON, I. J. Salt saving in the pregnant rat. Am. J. Physiol. 201(5): 765-768. 1961.—Pregnant rats of two strains showed average net accumulation of approximately 83 mEq of sodium/kg of weight gain throughout gestation. Of the total sodium retention, 15, 23, and 62% occurred in each successive third of gestation. Analysis of postpartum sodium balance and of fetal sodium content at term indicated that there was no net accumulation of sodium in the tissues of the dams. Near term, rats given isotonic saline solution showed diminished ability to excrete the administered water in the urine, but showed no impairment in sodium excretion. Serum sodium concentrations were slightly decreased and serum osmolalities were significantly decreased in comparison to values for nonpregnant rats. At term the pregnant rats had greater extracellular fluid volumes than did nonpregnant controls.

Since water accounts for most of the weight of the products of conception (1) it is to be expected that during gestation as weight is gained, ingested fluids with their contained sodium will be retained concurrently. It was of interest to examine the timing of weight gain and sodium retention during gestation, the ratio of sodium saved to weight gained, and the gross disposition of sodium in the tissues of dam and fetuses. Observations were made in normal pregnant rats and in pregnant rats subjected to experimental interference with circulation to the gravid uterus.

METHODS

Thirty-two young adult virgin female rats (initial weight 190-220 g) of Long-Evans and Wistar strains were housed in individual metabolism cages of a design which permitted collection of urine uncontaminated with food or feces. Distilled water and ground Purina laboratory chow were given freely. The rats were allowed a week to become used to the cages and handling. Daily measurements of body weight, drinking, food consumption, urine volume, and urine sodium concentration were then started; vaginal smears were prepared and examined daily for a premating period of 10-14 days.

After this period, rats were placed with one or more males of the same strain overnight on a day of proestrus. Successful mating took place within an average of 11 days after the end of the initial premating period. Success of mating was confirmed by presence of sperm in the vaginal smear or by persistence of a copulation plug. Measurements of body weight and of intake and urinary excretion of sodium were continued to term (usually the 21st day after conception). Vaginal smears were continued, as a check on the state of pregnancy, until the 14th day of gestation.

Intake of sodium was computed by multiplying food consumption by the sodium content of the diet. The food contained 0.17 mEq sodium/g, as determined by wet-ashing duplicate 1-g samples of ground food and performing quantitative analysis for sodium. Urinary excretion of sodium was obtained by multiplying voided urine volume by urine sodium concentration. When urinary losses of sodium were subtracted from sodium intake during pregnancy positive figures appeared which were misleading, owing to extrarenal loss of 6-10% of the ingested amount (2). To avoid such errors, apparent positive balances of sodium during pregnancy have been corrected by subtracting from them the extrarenal losses, as estimated from the apparent positive balances found for each rat in the premating period. In making the correction it was assumed that each rat was in zero sodium balance when not pregnant and that extrarenal losses did not change as pregnancy advanced. All data for accumulation of sodium by dam plus fetuses have been so corrected.

Once before mating, and on days 6, 13, and 18 or 20 of gestation, 21 rats were tested for ability to dispose of a dose of isotonic saline solution. Saline containing 150-162 mEq sodium/liter was given by stomach tube in the morning in a dose equal to 5% of body weight. Water was withheld for 6 hr. Volumes and sodium concentrations of urine voided in the first 6 hr and in the following 18 hr were measured and recorded.

The volume of distribution of sodium thiocyanate was measured in 16 rats at term and in 6 rats on the 14th day of gestation. As a first step, 1 ml of tail blood was obtained and saved for determination of the preinjection
concentration of sodium thiocyanate in the blood serum (thiocyanate blank). Then exactly 0.2 ml of a 5% solution of sodium thiocyanate containing 10 mg was injected intraperitoneally with the use of a calibrated Krogh-Keys syringe pipette, and the time noted. Rats were awake and allowed to move freely in their cages during the time necessary for equilibration of thiocyanate (SCN) with the blood serum.

Previous experiments demonstrated that mixing of injected SCN with the blood serum was complete within 2 hr after injection in nonpregnant rats and within 3 hr in pregnant rats at term. The longer equilibration time in pregnant rats may reflect the fact that SCN traverses the placenta (3) and mixes with extracellular fluids of the fetus and with amniotic fluid. Since the concentration of sodium thiocyanate in the blood serum never decreased by more than 0.2 mg/100 ml during a period of 3 hr after the end of equilibration, it was concluded that urinary and other losses of injected SCN during the equilibration period must have been very slight. They have therefore been neglected in calculations of SCN space.

After equilibration, rats were anesthetized with ether, exsanguinated from the abdominal aorta for determination of serum SCN and sodium concentrations and osmolality, then killed. Fetuses were delivered alive just after bleeding the dams, examined, weighed, and killed with ether. In ten separate experiments the entire gravid uterus and its contents was removed on the expected day of delivery and the dams were kept alive for 2 weeks further of sodium balance studies. No determinations of SCN space at term were made on these rats. The removed uteri plus their contents were weighed and wet-ashed for determination of their sodium content.

Thiocyanate blanks were similar in nonpregnant and pregnant rats and averaged 0.0 mg sodium thiocyanate/100 ml blood serum, with a range of 0.4-1.4 mg/100 ml. Thiocyanate space was calculated by dividing the equilibrium concentration of sodium thiocyanate in the blood serum (corrected by subtracting from it the thiocyanate blank) into the amount injected. Analysis of serum for SCN was done by a modification of the method of Eder (4). The modification consisted of adding 0.2 ml of serum or standard SCN solution to 2.0 ml of 10% trichloracetic acid as the initial step in the procedure. Osmolality was determined on fresh samples of blood serum with a cryoscopic osmometer.

TABLE 1. Weight gain and accumulation of sodium in each third of gestation in the rat

<table>
<thead>
<tr>
<th>Days</th>
<th>Weight gained, g</th>
<th>% of total</th>
<th>Na(^+) accumulated, mEq</th>
<th>% of total</th>
<th>mEq Na(^+)/kg wt. gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-7</td>
<td>15.6±2.59</td>
<td>18.0</td>
<td>0.81±0.034</td>
<td>11.9</td>
<td>52.1</td>
</tr>
<tr>
<td>8-14</td>
<td>19.0±1.47</td>
<td>22.0</td>
<td>1.61±0.049</td>
<td>23.3</td>
<td>84.6</td>
</tr>
<tr>
<td>15-21</td>
<td>52.0±3.42</td>
<td>60.0</td>
<td>4.48±0.041</td>
<td>64.8</td>
<td>86.3</td>
</tr>
</tbody>
</table>

* Mean ± s.e.m. 23 rats.

Quantitative analysis for sodium was done with a flame photometer using a lithium internal standard.

In addition to those on normal pregnant rats, observations were also made on seven rats subjected to interference with circulation to the gravid uterus and on seven mock-operated control rats. Uterine arteries and veins were ligated bilaterally, caudal to the site of implantation of the lowest-lying placenta in each cornu, on the 14th day of gestation. After this procedure, exchange of blood in the uteri of these rats takes place only via the still-intact uterine branches of the ovarian arteries and veins of each side. Mock operations, involving the same manipulations of uterine vessels as in the rats described above but not including actual ligations, were carried out concurrently also on the 14th day of gestation.

RESULTS

Timing of sodium accumulation and weight gain. Accumulation of sodium by dam and fetuses closely paralleled weight gain throughout pregnancy (Table 1). Net accumulations of sodium and weight in each third of gestation were respectively about 15, 23, and 62% of the totals for the whole of gestation. It is clear that the greatest net accumulations of sodium and weight occurred in the last third of pregnancy.

Ratio of sodium accumulation to weight gain. The amount of sodium saved by 32 rats (dams plus fetuses) in the last third of pregnancy was significantly correlated with the weight gained in the same period (Fig. 1). Operated and unoperated rats behaved essentially in the same fashion. The ratio of sodium saved to weight gained by 22 dams throughout the whole of gestation was 82.9±12.8 mEq/kg. In comparison, direct analysis of term uteri plus contents in ten of these pregnancies showed...
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TABLE 2. Isotonic saline administration, per cent of dose excreted in urine in 6 hr

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Water</th>
<th>No. of Rats</th>
<th>Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant*</td>
<td>21</td>
<td>74.36±3.28</td>
<td>18</td>
</tr>
<tr>
<td>Term pregnant</td>
<td>44.80±2.88†</td>
<td>76.38±3.72</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>26.58±5.43</td>
<td>-9.14±3.72</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SEM. † Significantly different from nonpregnant at 1% level of confidence.

the presence of 81.7 ± 0.6 mEq of sodium/kg wet weight of tissue.

Amount of sodium accumulated by dam alone. Net negative sodium balance by dams during the immediate postpartum period may be taken as an indirect indication that there has been previous retention of sodium in the tissues of the dam over and above any retention of sodium by the fetuses. This test was applied to ten dams which had shown average net retention of 6.65 ± 1.26 mEq of sodium in the 3 weeks of gestation. In the first 2 weeks postpartum rather than losing sodium, they showed further retention of an average of 0.99 ± 0.77 mEq of sodium. Direct analysis of the sodium content of the gravid uteri at term showed that they contained an average of 5.22 ± 0.44 mEq of sodium. This figure is not significantly less than the 6.56 ± 1.26 mEq of sodium accumulated by dams plus fetuses.

Increase in extracellular sodium. Increases in extracellular sodium content of dams plus fetuses during the last third of pregnancy have been calculated and compared with the total gain in sodium as obtained from balance studies. Total extracellular sodium content at term has been computed as the product of serum sodium concentration and SCN space on day 21 of gestation. The amount of extracellular sodium on day 14 of gestation has been computed similarly under the assumptions: a) serum sodium concentration of day 14 was the same as in a separate group of six normal pregnant rats on day 14, and b) SCN space on day 14 was equal to the product of relative SCN space in the same six rats (31.5% of body wt.) and the actual body weight on day 14 of gestation. The difference between the values calculated for days 21 and 14 has been taken as an approximate measurement of the change (increase) in extracellular sodium. In 16 rats the amount of sodium added to the extracellular fluid in the last third of gestation was 3.32 ± 0.65 mEq compared to 3.27 ± 0.67 mEq of sodium saved overall by dam plus fetuses during the same period.

Excretion of isotonic saline. Table 2 shows the results of oral administration of isotonic saline in terms of the percentage of the dose of water and sodium excreted in the urine within 6 hr. At term there was significantly less ability to dispose of water than in the same rats before mating. In contrast, the ability of rats at term to dispose of sodium was not essentially changed.

Serum sodium concentration, osmolality, and SCN space at term. In 12 rats at term the serum sodium concentration was slightly less than that before mating, and serum osmolality was significantly decreased (Table 3). In addition, SCN space was significantly expanded both relatively (to body weight) and absolutely, as the rats were heavier at term than before mating. This expansion of SCN space was greater in all seven rats with ligations of the uterine vessels than in the other pregnant rats at term. Insofar as concentration of the blood serum and distribution of SCN give information about the extracellular space, both dilution and some degree of expansion occurred in pregnancy.

DISCUSSION

Net saving of sodium occurred during pregnancy in the rat. Accumulation of sodium occurred at the same time as, and in direct proportion to, weight gain. The amount of sodium saved per kilogram weight gain of the pregnant dams was identical to the amount of sodium found in a kilogram of gravid uterus plus contents at term. The absolute amount of sodium saved by the pregnant dams also was not significantly different from the total amount of sodium present in the fetal tissues. These considerations, plus the fact that there was no significant postpartum loss of sodium, strongly suggest that there was no net accumulation of sodium in the maternal tissues during pregnancy. This conclusion applies equally well to pregnant rats with ligations of uterine vessels and to normal pregnant rats.

A fairly recent balance study shows that two normal pregnant women consuming about 9 mEq sodium/day accumulated 50 mEq sodium/kg increase in weight in the last trimester of pregnancy (5). This figure might have been higher if the intake of sodium had not been so restricted. Two other normal pregnant women studied in the same hospital saved 61 and 76 mEq of total exchangeable sodium (Na+ space times serum sodium concentration) per kilogram weight gain between weeks 15 and 38 of gestation (6). Making due allowance and for errors of measurement, these figures agree with the present data in rats.

Calculations show that all of the sodium accumulated by the pregnant rats in the last third of gestation could have been accommodated in the augmented extracellular fluid space of dams plus fetuses, so that there is no reason to postulate accumulation of significant amounts of intracellular or osmotically inactive sodium. This is in agreement with conclusions based on evidence from human studies (7).

In the first 6 hr after administration of isotonic saline

TABLE 3. Serum sodium concentration, osmolality and SCN space at term

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Serum [Na+] mEq/l</th>
<th>Serum Osmolality mOsm/l</th>
<th>SCN space % body wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant*</td>
<td>6</td>
<td>140.5</td>
<td>297.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±6.8</td>
<td>±8.4</td>
</tr>
<tr>
<td>Term pregnant</td>
<td>12</td>
<td>134.5</td>
<td>285.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1.10</td>
<td>±1.07</td>
</tr>
</tbody>
</table>

* Mean ± SEM. † Significantly different from nonpregnant at 1% level of confidence.
the pregnant rats near term had a relative antidiuresis without accompanying antinatriuresis, as compared to the results in the same rats when nonpregnant. In contrast, it has been reported that late pregnancy in intact or hypophysectomized Sprague-Dawley rats does not alter the rate of excretion of a water load (8). The water load employed was an oral dose of distilled water equal to 5% of the body weight, whereas in the present work, an equal dose of isotonic saline solution was used. It is possible that administration of isotonic saline rather than water could permit a tendency to relative antidiuresis, caused by pregnancy, to be expressed.

The present results are consistent with the known fact of oliguria in late pregnancy in humans, and like it, have at present no truly adequate explanation. Whether or not as a result of antidiuresis without any defect in sodium excretion, there were slight depressions in serum sodium concentration and meaningful depressions in serum osmolality in all pregnant rats at term.

REFERENCES

3. MAYER, A. Arch. Gynakol. 137: 1, 1929.

The thiocyanate space of rats at term was expanded in comparison with that of nonpregnant rats, particularly in the seven rats with ligations of their uterine vessels. These rats did not appear to differ from the other pregnant rats in other respects such as accumulation of sodium during pregnancy or ability to excrete water or sodium at term. In humans, significant expansion of the radiosodium space and increase in total exchangeable sodium of mother plus fetus has been shown in toxemic pregnancies but not in normal pregnancies (9). This is the best established example to date of a clear-cut difference in fluid handling between toxemic and normal pregnant women, and it is interesting to speculate that there might be a connection between this finding and the present data in rats.

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