UREA CONCENTRATIONS IN THE BLOOD OF THE RAT IN RELATION TO PREGNANCY AND LACTATION ON DIETS CONTAINING VARYING CONCENTRATIONS OF PROTEIN.*

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The determination of the blood urea is of considerable importance as an indicator of the metabolic activity of the organism and was of especial interest in the present investigation because of the possible significance attached to fluctuations in its concentration under the conditions of these experiments (Parsons, Smith, Moise, and Mendel, 1930) where both the excretory power of the kidney and a heightened protein metabolism were under scrutiny. The possible extent of its fluctuations in the blood under conditions of normally functioning kidneys in response to variations in the protein intake has been recognized (Addis and Watanabe, 1917; Wang, Hawks, and Wood, 1927).

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Experimental Procedure.¹

Animals.—The technique in regard to the care and handling of the rats has been fully described (Parsons, Smith, Moise, and Mendel, 1930). In addition to the rations already cited, two others were used: a high liver ration composed of beef liver 74.5 per cent, sodium chloride 1.0 per cent, calcium carbonate 1.5 per cent, butter fat 3.0 per cent, commercial dried brewery yeast 20.0 per cent, cod liver oil 1 cc. daily; and a modification of the Steenbock stock ration consisting of two-thirds by weight of the dry ingredients in that ration and one-third dried whole milk powder. This modification has also been employed by Waddell and Steenbock (1928) as an experimental ration.

Methods of Analysis.—Determinations of the urea concentration of the blood were made according to the micro urease method of Van Slyke (1927) by means of a portable manometric gas apparatus.

The blood samples for the determination of total solids were dried to constant weight in weighing flasks at 106°. Drying usually consumed 3 or 4 days time.

Methods of Taking Blood Samples.—Two methods were used in obtaining blood samples. For determinations both of urea and of total solids in the blood of the living animal samples were obtained from the tail, inasmuch as heart stabs were inadvisable in the case of the pregnant females. The perfect diffusion of urea makes the former method valid although it is less satisfactory for samples used for the determination of formed elements. One 0.2 cc. sample at a time was delivered into the cup of the Van Slyke apparatus except in the case of blood exhibiting a particularly rapid coagulation time. In such cases two 0.1 cc. samples were used. In a few instances a 0.2 cc. sample of blood was deposited in a small test-tube containing 1 cc. of 0.02 N lactic acid, was kept on ice, and later was transferred to the gasometric machine.

At the time the animal was sacrificed, it was anesthetized lightly and the skin dissected back from the ventral portion of the neck. An incision was made in the jugular vein and carotid artery

¹ Thanks are due to Dora Hesse Goldschmidt and Eunice Kelly for technical assistance in the care of the experimental animals and in operative procedures.
on one or both sides with sharp pointed scissors. The blood was collected in a depression in a block of paraffin, care being used to hold the rat in such a position that the blood flowed over cut surfaces as little as possible, and drained directly from the elevated body of the rat. Samples were transferred to weighing bottles before clotting occurred.

Time of Taking Blood Samples.—The selection of the time intervals at which urea determinations were made depended on the possible significance of these intervals. In the first place a "baseline" was sought against which to measure possible fluctuations occurring in other periods. For this purpose determinations of the urea concentration were made on the blood of the rats while they were still on the modified Sherman stock ration immediately before they were nephrectomized and changed to the new experimental conditions and diet. It would have been desirable to include another control determination at a given interval after operation and the change in diet, but before gestation. However, the possibility of breeding some of the animals in so short a time as a week after the operation made this period impractical.

The first experimental period selected for determining the concentration of urea in the blood was as early in the first 2 weeks of gestation as convenient. A second period was one shortly before parturition. Inasmuch as the beginning of gestation had been determined by the vaginal smear, and the length of gestation usually was 22 or 23 days although occasionally extending to even 25 days, the time of its probable termination could be foretold with relative accuracy. This period is known to be a time of rapidly forming tissue in the fetus. Two periods during lactation, namely near the 14th and the 21st days, were also chosen for urea determinations in the case of most of the females with litters, with a view to including the time of greatest need for food on the part of the mother rat and therefore presumably the height of protein metabolism. Samples were obtained in a few instances following weaning.

The opportunity to determine the urea concentration in the blood of both intact and partially nephrectomized rats afforded a possible criterion of the adequacy of the physiological adjustments to the conditions of the experiment. A greater concentration of urea in the blood of the latter animals might reasonably be
interpreted as the degree of failure to compensate on the part of
the remaining kidney.

The number of determinations performed rendered uniformity
in the time of day at which these were done practically impossible.
The suggestion arose of subjecting the rats to a preliminary fast
before the blood sample was drawn. Since the time of day of
sampling varied, a fast, unless it lasted from 14 to 22 hours, would
give only an appearance of accomplishing uniformity without
actually doing so. This is because the distribution of the food
intake by the rat is so uneven during the 24 hours, two-thirds or
more of the total food ingested being consumed at night. More-
over, a period of fasting would have defeated one purpose of the
experiment; namely, to determine the actual degree of protein
mobilization in these animals under the conditions imposed in so far as this was reflected in the concentration of urea in the blood.

**Fig. 2.** The significance of the symbols and heavy line is the same as for Fig. 1.

**Results.**

Variations in Urea of Blood at Different Periods.—The "normal" values in this experiment for the concentration of urea in the blood of rats on a stock ration immediately before nephrectomy...
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ranged from 23 to 48 mg. per 100 cc. of blood, values roughly comparable to those obtained in the Yale laboratory for male rats under similar conditions. In striking contrast to these low figures, however, were the wide fluctuations in the urea content of the blood of these same animals when subjected to the experimental conditions. Figs. 1 to 3 present the results of an extensive series of urea determinations in the blood of partially nephrectomized and intact rats with both high and low protein intake.

By comparing the records of the rats on high protein rations with those on low protein it can be seen that dietary protein exerts a profound influence on the blood picture in regard to urea content. Similarly, a comparison of the figures for animals on any of the diets during lactation as compared with intervals of rest shows that periods of milk production are associated with an increased concentration of urea in the blood. The results are inconclusive in regard to the influence of gestation alone, since this was not differentiated clearly, and there is no reason to interpret the data as showing any influence of this factor. Certainly the influence of
lactation is of so much greater magnitude in comparison that the
former is relatively insignificant. A comparison of the intact with
the partially nephrectomized animals on high protein rations
showed that both groups respond to this dietary influence with an
increase in concentration of urea in the blood but that the latter
group did so to a much greater degree. The results would seem to
indicate that a single remaining kidney after nephrectomy, even
though hypertrophied, is not the equivalent in function to the two
normally present.

Testing Various Hypotheses to Account for Wide Variations
Observed.—While considerable information had thus been compiled
concerning concentrations of urea in the blood of rats under vary-
ing conditions of diet, reduction of kidney tissue, and stages of
reproduction, it was highly desirable to arrive at a better under-
standing of the mechanism regulating such striking fluctuations as
had been observed.

A careful examination of the data gave assurance that the time of
day of taking samples was not in itself responsible for the highest
and lowest values recorded, although this possibility might be
suggested in view of the predominately heavy night feeding of the
rat. Even when samples had been taken at more than one time of
day from the same lactating females no clear tendency was observed
for the peak to fall at any one time although great differences (as
high as 30 mg.) were noted between concentrations of urea in the
blood of the same rat at different times of day.

A detailed record was next kept of the daily food intake of a
group of twenty nephrectomized rats, some pregnant and some
lactating. On the days the urea determinations were made, the
rats fasted from 9 a.m. until the blood sample was drawn. For
intakes of 17 to 14 gm. of food the blood urea concentrations were
uniformly high, 132 to 104 mg. When 1 gm. or less of food was
eaten, the urea values were uniformly low, 46 to 25 mg., but be-
tween these extremes, urea values from 127 to 48 mg. seemed to be
scattered impartially without reference to previous food intake or
time of day of taking the sample. In the case of three samples
taken at 5.30 p.m. for example, the urea concentration of one
was 81 mg. with a food intake of 13 gm., 105 mg. for another with
the same food intake, and 104 mg. for the third with an intake of
only 3 gm. of food. Clearly, some other factor besides the total
consumption of food was causing the variations.
Two other hypotheses were entertained. It might be that the consumption of food by some rats was concentrated in a short space of time either early or late in the night interval. On the other hand it was possible that the excretory capacity of the kidney differed markedly from rat to rat, resulting in varying degrees of retention of urea. Accordingly an experiment was planned to test the validity of these general hypotheses by controlling more carefully the exact time of consumption of a given amount of food. 7 gm. of food were offered to each of seven rats and the uneaten portions were ascertained at the end of a 3 hour interval and again at the end of a second 3 hour interval, at which time any uneaten portion was withdrawn and fasting continued throughout the remainder of the night and the following day. The 6 hours allowed for feeding began for different rats at 6 p.m., 9 p.m., 12 p.m., and 3 a.m., respectively. The curves given in Fig. 4 were plotted from the two or more urea determinations made at intervals during the following day for each animal but they are arranged on the sheet not relative to the actual hour of the taking of the sample but with reference to the interval elapsing after food was offered to the animals at some given time in the night.

The curves in Fig. 4 showing the decline in the urea content
of the blood at given intervals after the ingestion of food are remarkably uniform. There would seem to be no indication of any retention of urea in these seven rats. The rapidity of the decrease in the concentration of urea and the relatively low values reached in the blood of these rats after the withdrawal of food indicate that the functional capacity of their surviving renal tissue after the

![Diagram](https://example.com/diagram.png)

**Fig. 5.** Changes in the urea concentrations of the blood of rats soon after feeding, showing the initial increases to be roughly proportional to the intake of food.

removal of one kidney is essentially unimpaired, after certain intervals on a high protein diet.

On the other hand there seemed to be some possibility that distribution of food intake in point of time might account for the apparent discrepancies which had been encountered previously in attempting to correlate total food intake with urea content of the
blood. If the curves in Fig. 4 are arranged, not with reference to time after feeding but with reference to the time of taking the blood samples, it is evident that, at a certain hour of the day, the content of urea in the blood of Rat 154 was 105 mg. per 100 cc. of blood, and for Rat 161, 53 mg. (interpolations on the curves), although the amount of food eaten was 5 gm. in the one case and 6 gm. in the other.

Since such uniform results were obtained for the rapid decrease in concentration of urea during an interval after feeding, it was of great interest to determine and plot the rise as well as the fall of urea in the blood after a definite amount of food intake. Two rats were deprived of food during the night time and in order to reverse as far as possible diurnal conditions, the rats were kept under a strong electric light during the night and in the morning were placed in a dark room with a weighed amount of food. Within half an hour approximately 2 gm. of food had been consumed by each rat after which time the rats appeared sleepy and not inclined to eat more. Food was, therefore, withdrawn and urea determinations were begun immediately. The graphs (Rats 156 and 165, Fig. 5) show the results of four successive urea determinations for each animal.

Since even these prompt determinations had apparently not caught the initial part of the response to the ingestion of food, the ration was withheld from four other rats during the night, and the following morning before any food was offered determinations were made of the fasting level of urea in the blood (Fig. 5). These fasting values were found to be comparatively low. After taking the blood sample, food was offered to the rats and the time when they actually began eating was recorded as the beginning of the food intake period. 0.7 gm. of ration was consumed by each of two rats, Nos. 151 and 155, 0.8 gm. by Rat 157, and 6.4 gm. by Rat 163, the latter eating this amount in 17 minutes.

It is apparent from Fig. 5 that even in the short interval of half an hour after the beginning of food consumption a distinct increase in the concentration of urea in the blood had occurred. This would seem to indicate an amazing speed of digestion, absorption, and deamination. The curves also show a surprising uniformity. The intake of even relatively small portions of a high protein ration after a preliminary fast and the drawing of a control blood
sample is thus shown to be followed by a rapid increase in the concentration of urea in the blood of partially nephrectomized rats, from a relatively low fasting value to a concentration roughly proportional to the amount of food consumed. The concentration subsequently decreases but at a somewhat slower rate than the increase.

Although the experiments just described were suggestive, they seemed inadequate to explain fully the very high urea concentrations of 160 to 180 mg. which had been observed in the blood of

**TABLE I.**

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Identity*</th>
<th>Sample for total solids</th>
<th>Date of sample for total solids</th>
<th>Amount of total solids per cent</th>
<th>Urea mg. per 100 cc.</th>
<th>Date of sample for urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>106</td>
<td>NH</td>
<td>Tail.</td>
<td>Apr. 6</td>
<td>21.6</td>
<td>188</td>
<td>Simultaneously.</td>
</tr>
<tr>
<td>62</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot; 4</td>
<td>21.7</td>
<td>182</td>
<td>&quot;</td>
</tr>
<tr>
<td>77</td>
<td>&quot;</td>
<td>Carotid.</td>
<td>1</td>
<td>20.9</td>
<td>170</td>
<td>Mar. 30.</td>
</tr>
<tr>
<td>101</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Mar. 28</td>
<td>21.1</td>
<td>161</td>
<td>&quot; 27.</td>
</tr>
<tr>
<td>103</td>
<td>&quot;</td>
<td>Tail.</td>
<td>Apr. 6</td>
<td>21.9</td>
<td>136</td>
<td>Simultaneously.</td>
</tr>
<tr>
<td>119</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Mar. 29</td>
<td>22.1</td>
<td>133</td>
<td>&quot;</td>
</tr>
<tr>
<td>74</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot; 29</td>
<td>22.1</td>
<td>129</td>
<td>Mar. 29.</td>
</tr>
<tr>
<td>74</td>
<td>&quot;</td>
<td>Carotid.</td>
<td>&quot; 30</td>
<td>20.9</td>
<td>129</td>
<td>Mar. 29.</td>
</tr>
<tr>
<td>102</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot; 29</td>
<td>21.3</td>
<td>115</td>
<td>&quot; 27.</td>
</tr>
<tr>
<td>65</td>
<td>&quot;</td>
<td>Tail.</td>
<td>Apr. 10</td>
<td>23.4</td>
<td>112</td>
<td>Simultaneously.</td>
</tr>
<tr>
<td>126</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Mar. 29</td>
<td>21.0</td>
<td>87</td>
<td>&quot;</td>
</tr>
<tr>
<td>134</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot; 28</td>
<td>20.7</td>
<td>68</td>
<td>&quot; 27.</td>
</tr>
<tr>
<td>124</td>
<td>IH</td>
<td>Tail.</td>
<td>Apr. 10</td>
<td>21.5</td>
<td>67</td>
<td>Simultaneously.</td>
</tr>
<tr>
<td>137</td>
<td>&quot;</td>
<td>Carotid.</td>
<td>&quot; 3</td>
<td>20.3</td>
<td>63</td>
<td>Mar. 31.</td>
</tr>
<tr>
<td>136</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot; 3</td>
<td>20.7</td>
<td>54</td>
<td>&quot; 31.</td>
</tr>
<tr>
<td>129</td>
<td>NL</td>
<td>Tail.</td>
<td>Apr. 10</td>
<td>20.4</td>
<td>74</td>
<td>Simultaneously.</td>
</tr>
<tr>
<td>117</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot; 10</td>
<td>21.2</td>
<td>71</td>
<td>Apr. 5.</td>
</tr>
<tr>
<td>125</td>
<td>&quot;</td>
<td>Carotid.</td>
<td>&quot; 6</td>
<td>20.7</td>
<td>47</td>
<td>Simultaneously.</td>
</tr>
</tbody>
</table>

* The identity is indicated by the symbols as follows: NH, rat nephrectomized and fed high protein diet; IH, rat intact and fed high protein diet; NL, rat nephrectomized and fed low protein diet.
some of the lactating rats. It is conceivable that in the lactating rats fed the high protein rations there would be a relative anhydremia, not only due to the large mobilization of fluid necessary for excreting metabolites in the urine but also to the elimination of water in the milk secreted. Accordingly determinations were made of the total solids in the blood of certain of the rats as recorded in Table I.

From the third and fifth columns in Table I it is plain that there is no direct correlation between the concentration of urea and of total solids in the blood of these animals. Variations in the water content of the blood are therefore not an explanation for the very wide fluctuations in the concentrations of urea observed in the blood of nephrectomized rats fed diets rich in protein.

It had been noted that the lactating mother rats whose daily food intake reached as high as 19 or more gm. consumed an appreciable amount of food during the daytime. It seemed possible that the extremely high urea values of 160 to 180 mg. per 100 cc. of blood which had been observed previously might have resulted from a summation of metabolites from small portions of food consumed throughout the day superimposed on the heightened urea concentration due to the large intake of food during the night. Accordingly observations were made on two lactating females. The first determinations of urea showed approximately the same concentrations in the blood of each. The food cup was allowed to remain in the cage of Rat 153, and a sharp increase in the concentration of urea in the blood of this animal was observed at the end of an hour's time. Food was withdrawn from Rat 151 and the urea concentration was found to have fallen considerably at the end of a 5 hour interval. At the outset, therefore, these results seemed to be in harmony with the hypothesis that the excessively high concentration of urea which had been observed in the blood of lactating rats could be adequately explained in the basis of the effect of small portions of food eaten frequently during the day, after heavy night feeding; however, later results made a different interpretation of this experiment necessary. Simultaneous observations were next made on Rats 150 and 153, both of which were suckling litters. Food was withdrawn and a comparable decline in the urea concentration was observed to take place in both. Following this preliminary determination of the trend of the curve,
food was offered to Rat 150 but was still withheld from Rat 153. Quite contrary to expectation, the next determinations showed a sharp increase in the concentration of urea in the blood of Rat 153 from which food had been withheld and a continued decline in the blood of Rat 150 which had eaten food.

Rat 153 had been suckling a litter at the time that the blood sample was taken for the third determination so that the young

![Graph showing changes in the concentration of urea in the blood of rats with reference to suckling their young.](http://www.jbc.org/)

**Fig. 6.** Changes in the concentration of urea in the blood of rats with reference to suckling their young. The solid lines denote values for mother rats without food or young. The dotted lines denote that the young were allowed to suckle for part of the time. Rats A, B, C, and D had one kidney; Rat E, two kidneys. Rats A and B were fed a high casein ration; Rat C was fed a high liver ration; Rats D and E were on stock rations.
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had to be literally shaken free from the mother's nipples. It seemed barely possible that the act of nursing might itself have some influence although the results of previous determinations of blood solids gave no support to the hypothesis that unusual concentrations of the blood of the mother was the factor responsible. Therefore, a series of determinations of blood urea was made on all of the individual rats of the group with litters. Food was withdrawn in the morning at as early an hour as possible and the mother rat segregated from her young. Urea determinations were made immediately and at succeeding intervals during the day. When a considerable decline in the concentration of urea in the blood had been observed in each rat, the litter of young was restored to the mother's cage without, however, the food cups being replaced. The graph for Rat A on Fig. 6 is representative of the response obtained from each of the six mother rats in this group.

It is seen that following the decline in the urea content of the mother's blood, a sharp increase occurs after the young have suckled. The rise was observed at even as short an interval as 20 minutes after the beginning of suckling. The conclusion seems warranted that the puzzling observation made earlier, of the rise in concentration of urea in the blood of Rat 153 without food was due to the active nursing of the young. It is also probable that the same explanation accounts for the increase first observed for this same rat, thought at that time to be due solely to an intake of food.

Although the results demonstrated that prompt and striking increases in the concentration of urea in the blood of partially nephrectomized rats on a high casein ration may be produced in response to the act of withdrawal of milk by the young even though food has not been consumed by the mother rat for several hours previously, it was not clear whether the phenomenon was general in nature or occurred only under the special conditions of high casein feeding and partial nephrectomy. Accordingly, further experiments were carried out on lactating rats fed rations rich in casein, liver, or egg albumin, as well as rations relatively low in protein. Both intact rats and those in which one kidney had been removed were fed stock rations.

The physiological response was found to be decidedly general inasmuch as distinct increases in the urea content of the blood were
observed in each type listed above. Of the 55 periods of suckling studied, forty-six showed an augmented concentration of urea in the blood of the mother following a half hour to an hour of nursing. Typical graphs of the individual experiments are shown in Fig. 6.

The type of protein fed seemed to exert some influence. The average concentration of urea in the blood of the mother rat at an early hour of the day when the experiments were begun was greater in those cases where high casein or high egg albumin was fed than with a ration containing an approximately equal concentration of nitrogen from liver. Furthermore, the magnitude of the average increase following nursing was greatest after previous feeding of casein-rich rations. These relationships are made clear in Table II.

An important point to be determined because of its bearing on the nature of the phenomenon observed was whether or not the degree of increase in urea concentration in the blood is proportional

<table>
<thead>
<tr>
<th>Type ration fed</th>
<th>Rats with one kidney</th>
<th>Rats with two kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of periods of nursing studied</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Average increase in concentration of urea in blood of mother rat following nursing period, mg. per 100 cc. blood</td>
<td>18.6</td>
<td>6.5</td>
</tr>
<tr>
<td>Greatest single increase in urea in blood of mother following nursing period, mg. per 100 cc. blood</td>
<td>50.0</td>
<td>31.0</td>
</tr>
<tr>
<td>No. of nursing periods not followed by increase in urea content of blood</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Average concentration of urea in blood at beginning of experimental day, mg. per 100 cc. blood</td>
<td>138.8</td>
<td>107.3</td>
</tr>
<tr>
<td>Highest single determination of urea during day, mg. per 100 cc. blood</td>
<td>191.0</td>
<td>150.0</td>
</tr>
</tbody>
</table>
to the amount of milk obtained by the young in nursing. While this question could not be settled with certainty by any of the methods that suggested themselves, significant data bearing on this point were obtained by weighing the young just before and just after nursing. The amount of milk estimated by this method showed so imperfect a correlation with the apparent vigor of the efforts of the young to nurse that later, in thirty-two instances, the weights of the mother rats were recorded also. In twenty-five of these, the increase in weight of the litter failed to equal the loss in weight of the mother by from 3 to 7 gm., for some undetermined reason. A loss of weight of the young due to excreta does not seem to be the factor involved. However, in seven of the thirty-two cases, these discrepancies were not found.

Three cases were selected where a lack of decrease in weight on the part of the mother gave assurance that no significant amount of milk was obtained by the young, for a group on the one hand; four others were selected where the loss of weight on the part of the mother was practically equalled by the gain in weight on the part of the young, thus lending validity to the figures as a true estimate of the amounts of milk obtained, on the other hand. A comparison of the increases in the concentration of urea in the blood of the mother rats in the two groups would then be a crucial test of the question raised above as to whether these increases in urea are proportional to the amount of milk nursed.

### TABLE III.

*Fluctuations in Urea Content of Blood of Mother Rats in Relation to Amount of Milk Nursed by Young a Short Time Previously.*

<table>
<thead>
<tr>
<th>Increase in concentration of blood urea per 100 cc. between samples before and after nursing period.</th>
<th>Loss in weight of mother rat.</th>
<th>Gain or loss in weight of whole litter.</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg.</td>
<td>gm.</td>
<td>gm.</td>
</tr>
<tr>
<td>19.5</td>
<td>0.0</td>
<td>-4.0</td>
</tr>
<tr>
<td>15.0</td>
<td>0.0</td>
<td>4.0</td>
</tr>
<tr>
<td>20.0</td>
<td>0.0</td>
<td>-2.0</td>
</tr>
<tr>
<td>8.4</td>
<td>-3.0</td>
<td>+2.0</td>
</tr>
<tr>
<td>3.2</td>
<td>-4.0</td>
<td>+3.0</td>
</tr>
<tr>
<td>10.4</td>
<td>-8.0</td>
<td>+6.0</td>
</tr>
<tr>
<td>6.3</td>
<td>-13.0</td>
<td>+12.0</td>
</tr>
</tbody>
</table>
From the data in Table III it seems obvious that the increases observed in the urea content of the blood of lactating rats are not proportional to the amount of milk nursed. While the figures for urea cannot be taken to represent exactly the total increases for each period, inasmuch as the maximum height of the increase may not have been revealed by the sample obtained, nevertheless the fact that increases in urea content occurred at all when evidently no milk was obtained shows that the increases in urea and the amount of milk nursed are not correlated. This observation is of importance inasmuch as the first hypothesis assumed was that the source of the urea causing the increased concentration might be incident to a rapid formation of milk during or following nursing to replace that withdrawn by the young. This hypothesis therefore cannot be correct. It is, however, not inconceivable that the sensation of pulling at the teats might normally initiate the mobilization of protein for milk formation through some reflex mechanism inasmuch as this sensation would usually be accompanied by the withdrawal of milk from the mammary.

The discovery that the fluctuations in urea values in the blood are not proportional to the amount of milk withdrawn suggested, however, the possibility that the whole phenomenon might be a response of the animal to the emotional excitement incident merely to obtaining the blood samples. This possibility had seemed at first to have been obviated by the taking of two or more blood samples before nursing, following the withdrawal of food, and finding so pronounced rates of decline in the urea concentration in the blood in this preliminary period. The question had not been entirely answered, however, inasmuch as the intervals between the preliminary samples were usually not so short as the intervals between the samples taken before and after nursing respectively and hence the possibility still remained that a rise in urea content had followed the taking of the former samples, but had been of so brief duration as to have escaped detection. The reason that the two or more preliminary blood samples were not taken more closely together in the experiments from the first was that it was thought important to disturb the rat as little as possible just before nursing in order to avoid excitement and induce an adequate flow of milk. However, in certain of the experiments this consideration was set aside and blood sam-
amples were taken at brief intervals without nursing. In only thirteen of the 95 intervals without nursing where the trend of the urea content was determined was there any increase found. Of these, eight instances occurred at an early hour in the day following the removal of the ration cup and may have been due entirely to the metabolism of protein recently eaten. In five cases, however, illustrated by rat B on Fig. 6, increases seem really attributable to the disturbance incident to the blood sampling, and may be a confirmation of Tashiro's results. He has shown (1925, 1926) that the urea nitrogen concentration of the blood of rabbits is increased by parasympathetic stimulants such as pilocarpine and choline, or by directly stimulating the peripheral stump of the vagus but is decreased by injections of atropine and adrenaline or by the direct stimulation of the splanchnics or the central stump of the vagus. He noted also a prompt increase in the urea concentration of the blood of rabbits during the process of binding them. After 3 to 5 hours of binding, the urea concentration was sometimes found to be doubled. He attributed this phenomenon to the central stimulation of the vagus. Astanin and Rubel (1928) confirmed the results obtained by Tashiro by stimulation of the peripheral end of the vagus in contrast to the failure to increase the urea concentration of the blood on stimulating the sympathetic nerves. They suggested the possible nervous influence on some gland of internal secretion.

The results of the experiments with rats in the present instances, however, clearly demonstrate that the increases in urea in the blood following nursing are of unmistakably greater magnitude than those resulting from the blood sampling itself. This is illustrated in Fig. 6 by the graph of Rat C, where repeated samples of blood taken at close intervals during the day showed a steady decrease in the urea content of the blood; but on the other hand, the sample taken following a nursing period late in the day showed the typical response of augmented urea.

DISCUSSION.

In view of the multiplicity of the influences which have been shown in this investigation to have a bearing on the urea concentration in the blood, and the wide fluctuations occurring during even an hour's time, there seems no justification for an attempt to
formulate an exact mathematical expression of the values for urea in the blood of these experimental animals, such as has been devised by MacKay (1928) to express the relationship between the renal weight of her experimental rats and the concentration of urea in their blood derived from food protein, based on single determinations.

On the other hand these experiments are suggestive of such possible relationships in a qualitative way. The larger relative size of the kidneys of the young of mother rats on high protein rations (Parsons, Smith, Moise, and Mendel, 1930) as well as the very striking enlargements of the kidneys of these females themselves in comparison with non-lactating rats, seems to be correlated with blood changes. One might postulate an influence due to the concentration of nitrogenous metabolites circulating in the mother's blood and perhaps passing into her milk in more than usual amounts. But although the fluctuations in the blood urea were the observed phenomena associated with nursing, it would be an unwarranted assumption to restrict the possible agent in the renal changes in mother and offspring to urea itself, inasmuch as the concentrations of other metabolites were not determined simultaneously. It is conceivable that some substances more physiologically active than the relatively inert urea are also involved in the mobilization of nitrogen of which the urea fluctuations in these experiments seem to be an index.

SUMMARY.

Striking fluctuations were observed in the urea concentration in the blood of rats subjected to varying conditions in respect to reduction of kidney tissue, concentration of dietary protein, and the burdens of reproduction.

The highest concentration of urea, 191 mg. per 100 cc. of blood, occurred in a lactating rat with one kidney, on a high casein ration.

It has been shown that the increased food intake of the lactating females accounts only in part for the extreme values observed. A definite influence of the act of suckling itself on the concentration of urea during fasting has been demonstrated in the blood of intact rats as well as those with only one kidney, when the ration consumed previous to the fast was rich in casein, liver, or egg albumin, or contained only moderate amounts of protein.
Inasmuch as the increases in the concentration of urea in the blood of the lactating rat following the suckling of the young seem not to be closely correlated with the amount of milk nursed, it is possible that they result from an emotional response to the act of suckling itself rather than from the formation of milk in the mammary gland.

An occasional slight increase in the urea concentration occurred following the taking of the blood sample without nursing, and may be related to the effect on blood urea shown by others to result from manipulation of the experimental animal. However, the increases in urea in the blood of rats following nursing were demonstrated to be of unmistakably greater magnitude than those resulting from the blood sampling itself.

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UREA CONCENTRATIONS IN THE BLOOD OF THE RAT IN RELATION TO PREGNANCY AND LACTATION ON DIETS CONTAINING VARYING CONCENTRATIONS OF PROTEIN
Helen T. Parsons


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