ON THE NITROGEN METABOLISM IN EXPERIMENTAL SUBACUTE ARSENIC AND ANTIMONY POISONING.

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The variations of nitrogen metabolism, especially urea, in diseases of the liver due to bacterial and chemical causes as well as conditions produced by removing the liver or cutting it off from circulation partially or completely have been studied of late with varying results. The effect of fatty degeneration of the liver lobules produced chemically by P, As, Sb, alcohols, CHCl₃, ether, cyanohydrogen, hydrazine, trinitrotoluene, dinitrobenzene, on the non-protein nitrogen of blood and urine has been investigated by various workers. It is without doubt, that many other chemicals may produce degeneration in liver cells. Wells (1) pointed out that any poison which does not directly cause death, but which causes a severe injury to the liver cells without at the same time destroying the autolytic enzymes, so that the cells die and undergo rapid autolysis, may produce a condition identical with or similar to acute yellow atrophy.

In 1876 Kossel (2) found an increase of total non-protein nitrogen in urine in liver degenerations following experimental arsenic poisoning and suggested it to be due to urea increase. Löffler (3) working with isolated liver found no abnormal variation in urea formation in phosphorus poisoning and consequent fatty degeneration of liver lobules; in chloroform and alcohol poisoning, he found that the urea formation is actually affected. Also Luciano (4) reported a decrease of urea formation in chloroform poisoning. Marshall and Rowntree (5) noted definite and sometimes marked increase in non-protein nitrogen, urea, and amino acid nitrogen in

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I wish to express my gratitude to Professor Folin for his permission to carry on this work in his laboratory, and for his advice and interest, and to the International Education Board for making this opportunity possible.
it the blood in phosphorus poisoning. Jackson and Pearce (6) working with dogs with necrosis of liver produced by hemotoxic serum did not notice any variations in urea formation and concluded that this "factor of safety" is very important in the chemistry of liver diseases. The ratio of urea, ammonia, and amino acid is not always disturbed.

The results are far from uniform and with a view to elucidate the relation of urea to non-protein nitrogen in blood and urine in degeneration of liver, the following experiments were undertaken.

Throughout rabbits were used as experimental animals and in one group a subacute poisoning by sodium arsenite and in the other by antimony potassium tartrate was produced. During the period of experiments the rabbits were fed on 75 cc. of milk and 50 gm. of glucose per kilo of weight of rabbit per day in two meals, one at 10 a.m. and one at 10 p.m. When the animals were unable to take food they were fed by stomach tube. The urines were collected from metabolism cages every 24 hours and preserved with toluene. At regular intervals blood was taken from the marginal ear vein. The dose of sodium arsenite and antimony potassium tartrate given per os in solution was 10 and 15 mg., respectively, per kilo of body weight. Rabbit 1 was given the toxic dose of 10 mg. per kilo of body weight all at once and it died 3 days later; to prolong the period of poisoning both of the poisons were, therefore, administered in several increasing doses in the other rabbits.

Chemical Methods Used.—The preparation of protein-free blood filtrate has been made by the Folin-Wu method (7). Non-protein nitrogen and urea nitrogen were determined by Folin's colorimetric methods (7); also, for the determination of total nitrogen, urea, and ammonia nitrogen, Folin's colorimetric methods were used (8-10).

Tables I and II show the average figures for blood and urine before and after the poisoning. The figures under "before" represent the average data calculated for a period of 6 days prior to the administration of poison.

The non-protein nitrogen in the blood of normal rabbits varied between 30.74 and 33.82 mg., the urea nitrogen between 12.28 and 13.21 mg. per 100 cc. of blood. The urea nitrogen quotient \[
\frac{\text{urea nitrogen}}{\text{non-protein nitrogen}}
\] in the blood of normal rabbits is between
37.4 and 43.3 per cent. The daily output of total nitrogen in urine in the period prior to administration of poison was between 269 and 675 mg., the urea nitrogen quotient between 72.1 and 85.3 per cent. The ammonia nitrogen excreted in a 24 hour period was between 2.75 and 7.66 mg., the ammonia nitrogen quotient was 0.4 to 1.9 per cent.

**Non-Protein Nitrogen, Urea, and Ammonia Nitrogen in Blood and Urine after Administration of Sodium Arsenite.**—There was a marked increase of non-protein nitrogen in the blood in all four rabbits (Table I). The urea nitrogen also showed a rise in all cases. The urea nitrogen quotient increased in all cases except in Rabbit 4 where it was of almost the same value as before the poisoning. The rise of non-protein nitrogen in the blood of poisoned rabbits (except No. 4) was due to the rise of urea nitrogen. The changed blood picture was not associated with the same changes in urine. The total nitrogen in all cases increased, also the urea nitrogen with the exception of Rabbit 2. The urea

### TABLE I.
**Showing the Average Data for Each Rabbit before and after Administration of Sodium Arsenite.**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Duration of poisoning (g)</th>
<th>Loss of body weight (mL)</th>
<th>Whole blood per 100 cc.</th>
<th>Urine of 24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>gm.</td>
<td>Non-protein N.</td>
<td>mg.</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>15</td>
<td>Before.</td>
<td>33.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After.</td>
<td>36.50</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>10</td>
<td>Before.</td>
<td>30.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After.</td>
<td>45.40</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>22</td>
<td>Before.</td>
<td>32.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After.</td>
<td>42.78</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>35</td>
<td>Before.</td>
<td>31.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After.</td>
<td>36.24</td>
</tr>
</tbody>
</table>
Nitrogen Metabolism

Nitrogen quotient increased in Rabbits 1 and 4, and decreased in the other two cases. The ammonia nitrogen increased but not proportionally to total nitrogen; the ammonia nitrogen quotient was, therefore, lower.

Non-Protein Nitrogen, Urea, and Ammonia Nitrogen in Blood and Urine in Rabbits after Administration of Antimony Potassium Tartrate.—In general the rise with antimony potassium tartrate was not so great as with sodium arsenite, but still there was a rise in non-protein nitrogen and urea nitrogen in blood and urine (Table II). The rise of non-protein nitrogen was not entirely on account of the rise of urea nitrogen as was the case in arsenic poisoning. The difference in urea nitrogen quotients was only 1 to 2 per cent, while the difference in quotients in arsenic experiments was 4 to 21 per cent.

The total nitrogen in urine increased in all cases except in Rabbit III. The urea nitrogen quotient was almost the same in urines of Rabbits II and III; in Rabbit I there was a fall and in

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Duration of poisoning (days)</th>
<th>Loss of body weight</th>
<th>Whole blood per 100 cc</th>
<th>Urine of 24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nitrogen &amp; Urea N.</td>
<td>Nitrogen &amp; Urea N.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg. mg. mg. per cent</td>
<td>mg. mg. mg. per cent</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>105</td>
<td>Before: 31.87 12.46 39.0</td>
<td>561 466 83.0 2.75 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After: 34.69 13.99 40.3</td>
<td>664 485 71.5 5.01 0.7</td>
</tr>
<tr>
<td>II</td>
<td>13</td>
<td>20</td>
<td>Before: 32.80 12.28 37.4</td>
<td>495 387 78.1 7.21 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After: 33.78 13.50 39.9</td>
<td>542 424 78.2 6.34 1.1</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>15</td>
<td>Before: 32.00 12.46 38.9</td>
<td>675 557 82.5 7.66 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After: 38.70 15.66 40.4</td>
<td>479 394 82.2 6.52 1.3</td>
</tr>
<tr>
<td>IV</td>
<td>22</td>
<td>140</td>
<td>Before: 30.72 12.98 42.2</td>
<td>280 202 72.1 4.47 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After: 36.44 14.30 39.24</td>
<td>527 428 81.2 5.62 1.0</td>
</tr>
</tbody>
</table>
Rabbit IV a marked decrease. The ammonia nitrogen quotient fell in two cases (Rabbits II and IV) and there was a rise in Rabbits I and II. The increased ammonia nitrogen was noticed mostly in cases where the urea nitrogen quotient showed decrease. From the experiments an acceleration of protein metabolism is evident, followed by an increase of non-protein nitrogen in blood and an increased total nitrogen output in urine. This acceleration is attributed mainly to increased autolysis of tissues. Evidently, as shown by the examination of pathological changes of liver parenchyma, the progressive degeneration of liver cells is not always accompanied with marked changes in urea content in blood and urine. Our results are in harmony with those of Jackson (6), Marshall (5), and Chasatzky (11), as far as these investigators claim the relatively little change in urea formation in liver insufficiency.

I am including some brief clinical observations and protocols of autopsies and histological examinations of livers and kidneys. The duration of poisoning, in spite of the same dose for each rabbit, is varying within broad limits; also the loss of body weight. In antimony poisoning the loss of weight is comparatively much greater. Icterus was noticed during last days of poisoning. In two rabbits anuria of short duration occurred, followed by albuminuria. In all cases of poisoning, rabbits showed diarrhea, the excreta being changed in color and consistency.

Autopsy and Histological Changes in Livers and Kidneys of Rabbits Poisoned by Sodium Arsenite.

Rabbit I.—Gastrointestinal tract showed hemorrhages in stomach and small intestine; liver enlarged, of a dark brown color; kidneys without any macroscopic changes.

Microscopical Examination.—Liver: Slight congestion in the vessels and capillaries. No fatty changes in cells, nuclei fairly well stained. Kidneys: The vessels and capillaries were moderately congested. Convoluting tubules were swollen.

Rabbit 2.—Hemorrhages in mucosa of stomach and small intestine. Liver showed bright yellow color at the margin of the lobes. Kidneys showed hemorrhages in the cortex.

Microscopical Examination.—Liver: Complete degeneration of parenchymal cells, mostly about the central veins. Fatty changes very evident. Kidneys: The degenerative changes in the glomeruli and tubules were very marked. The lumina of the tubules were filled with degenerated cells.
Rabbit 3.—Hemorrhagic changes in gastrointestinal tract. Liver of yellow color in peripheral parts of the lobes. Kidneys showed hemorrhages in the cortex.

Microscopical Examination.—Liver: The cells of the lobules were full of fat. Some cells showed only very slight degenerative changes. Kidneys: Degenerative changes in the tubules and glomeruli. The lumina obliterated with necrosed cells.

Rabbit 4.—Hemorrhages in stomach and small intestine. Liver atrophied; of yellow color. Kidneys showed hemorrhages in the cortex.

Microscopical Examination.—Liver: Fatty changes in parenchymal cells very marked. Only few cells in periphery of lobules without fat. Kidneys: Hemorrhages in cortex; congestion of wall of the glomeruli.

The microscopical examination showed no fatty degeneration of liver in Rabbit 1; in the other three rabbits the fatty changes were apparent; most severe destruction of liver cells was in Rabbit 2. The kidneys of Rabbit 1 showed a state of hyperemia, the other three rabbits showed marked glomerulonephritis. The chemical changes in blood and urine indicated some relation to the pathological changes in liver and kidneys. The increase of non-protein nitrogen in blood was most apparent in Rabbit 2, also the urea nitrogen in blood and urine in this rabbit was very low on the last day of the period of poisoning.

Autopsy and Histological Reports of Changes in Liver and Kidneys of Rabbits Poisoned by Potassium Antimony Tartrate.

Rabbit 1.—Hemorrhages in stomach and small intestine. Liver atrophied, of slight yellow color in the margin of the lobes. Kidneys were without pathological changes macroscopically.

Microscopical Examination.—Liver: Parenchymal cells in the intermediary zone of the lobules contained fat; those at the periphery were in a state of beginning fatty degeneration. Kidneys: Slight congestion of tubules and glomeruli.

Rabbit II.—The same pathological changes in gastrointestinal tract as in Rabbit I. Liver slightly congested and atrophied. Kidneys showed hemorrhages in the cortex.

Microscopical Examination.—Liver: Fatty degeneration of cells in the intermediary zone of lobules marked. The peripheral cells without fat. Kidneys: The lumina of tubules obliterated with necrosed cells.

Rabbit III.—Hemorrhages in stomach and small intestine. Liver showed marked atrophy and yellow color. Kidneys showed hemorrhages in the cortex.


Rabbit IV.—Congestion with hemorrhages in stomach and small intestine. Liver showed slight atrophy, yellow color. Kidneys showed the periphery of the cortex lighter in color.
Microscopical Examination.—Liver: Parenchymal cells in the intermediary zone of the lobules contained fat; those of the periphery contained none. Kidneys: Glomeruli showed slight congestion.

SUMMARY.

Experimental subacute poisoning in four rabbits by sodium arsenite and in four rabbits by antimony potassium tartrate has been induced and the changes in ratio between urea nitrogen and non-protein nitrogen in blood, and between urea and ammonia nitrogen and total nitrogen in urine have been followed.

It has been found that there is an increase of non-protein nitrogen in blood after administration of both poisons. In arsenic poisoning the increase is more apparent. In arsenic poisoning there is a rise of non-protein nitrogen due to rise of urea nitrogen. The urea nitrogen quotient rises with the rise of urea nitrogen.

The rise of non-protein nitrogen and urea nitrogen in blood is associated with an increase of these constituents in the urine. The ammonia nitrogen quotient in the urine of poisoned rabbits seems to be inversely proportional to the urea nitrogen quotient.

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