THE EFFICIENCY OF VARIOUS DIURETICS IN THE ACUTELY NEPHROPATHIC KIDNEY, PROTECTED AND UNPROTECTED BY SODIUM CARBONATE. II.*

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Plates 3 to 6.

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In a recent paper it was shown that intravenous injections of a solution of sodium carbonate inhibited the toxic action of uranium nitrate for the kidney. It was furthermore shown in this paper that the use of such an alkaline solution would protect the kidney of young animals acutely nephropathic from uranium against the toxic effect of an anesthetic. Animals protected in such a manner formed a much larger quantity of urine in a given time limit than did animals of the same age which had received the same amount of uranium nitrate per kilo but which received in place of a solution of carbonate a 0.9 per cent solution of sodium chloride. A histological study of the kidneys of these two groups of animals showed that the animals receiving the carbonate protection failed to develop severe degenerative changes in the renal epithelium, while those animals which were not protected by the carbonate had a renal epithelium which was acutely swollen and undergoing necrosis. In these experiments no information was obtained which would explain the cause of the swelling and necrosis of the epithelium or the relative efficiency of an alkaline solution to furnish a protection against an anesthetic as compared with the efficiency of an equal volume of a solution of sodium chloride.

In Part I of the present study I have been able to show by inducing uranium intoxications in animals of different ages that the severity of the uranium intoxication was dependent upon the degree of acid

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intoxication which developed in the different animals, and furthermore that the older animals developed a severer acid intoxication than did the younger animals.

The object of the present study is twofold: first, to determine the changes in the acid-base equilibrium of the blood induced by an anesthetic in normal animals, in animals acutely nephropathic from a constant quantity of uranium per kilo and protected by the intravenous use of an alkali, and in animals nephropathic from the same quantity of uranium but unprotected by an alkali; second, in such a series of animals to study the efficiency of various diuretics and the relative severity of the pathological changes which have taken place in the kidney.

EXPERIMENTAL.

Forty-six animals were used in this series of experiments. The animals varied in age from pups 8 months old to animals 11 years and 4 months old. Thirty of the animals that were employed in these experiments were used in Part I of this paper. The animals of the present series that are not included in these experiments were subjected to an experimental technique similar to that previously described. Ten of the animals were normal dogs which varied in age from 1 to 5 years. These animals were kept in metabolism cages for 3 days before they were used for experimental purposes, were fed on bread with a small amount of meat, and were given 500 cc. of water daily by stomach tube. The remaining thirty-six nephropathic animals were given the same diet and the same amount of water.

Tables I and II furnish an outline of the observations which have been made on two of the normal animals and thirteen of the acutely nephropathic animals. The animals have been selected according to their age in order to show the influence of this factor in determining the toxicity of an anesthetic and in determining the ability of an alkali to protect the nephropathic kidney against an anesthetic. In the experiments on nephropathic animals, two dogs were used in each experiment. One of the animals received the alkaline solution, while the other animal of the same age received an equal volume of sodium chloride solution and served as a control. On the day of the experiment, prior to the use of an
WILLIAM DEB. MACNIDER

TABLE I.

<table>
<thead>
<tr>
<th>No.</th>
<th>Experiment</th>
<th>Age</th>
<th>yrs.</th>
<th>mg.</th>
<th>cc.</th>
<th>Pa.</th>
<th>R. Pa.</th>
<th>Carbon dioxide tension</th>
<th>Na₂CO₃ conc. per kilo.</th>
<th>Pa in half hr.</th>
<th>R. Pa in half hr.</th>
<th>Carbon dioxide tension in half hr.</th>
<th>Urine flow per min.</th>
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<td>635</td>
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<td>43</td>
<td>0</td>
<td>60</td>
<td>7.45</td>
<td>8.1</td>
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</tr>
<tr>
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<td>5</td>
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<td>7.25</td>
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<td>34 Na₂CO₃</td>
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<td>8.1</td>
<td>39</td>
<td>8</td>
<td></td>
</tr>
<tr>
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<td>8</td>
<td>5</td>
<td>509</td>
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<td>8.0</td>
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<td>60</td>
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<td>7.95</td>
<td>34</td>
<td>0</td>
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<td>35 Na₂CO₃</td>
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<td>Control</td>
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<td>765</td>
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<td>8.0</td>
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<td>5</td>
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<td>34 Na₂CO₃</td>
<td>60</td>
<td>7.45</td>
<td>8.1</td>
<td>37</td>
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<td>900</td>
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<td>8.0</td>
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<td>7.9</td>
<td>30</td>
<td>0</td>
<td></td>
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<td>5</td>
<td>575</td>
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<td>415</td>
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<td>7.95</td>
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<td>7.9</td>
<td>30</td>
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<td>3+</td>
<td>5</td>
<td>362</td>
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<td>470</td>
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<td>7.9</td>
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<td>7.9</td>
<td>35</td>
<td>0</td>
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<td>5+</td>
<td>5</td>
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<td>7.85</td>
<td>26 Na₂CO₃</td>
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<td>7.35</td>
<td>8.0</td>
<td>45</td>
<td>0</td>
<td></td>
</tr>
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<td></td>
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<td>5</td>
<td>340</td>
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<td>7.85</td>
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<td>60</td>
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<td>8.0</td>
<td>45</td>
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<td>310</td>
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<td>60</td>
<td>7.45</td>
<td>8.0</td>
<td>45</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Anesthetic, the hydrogen ion content of the blood, the alkali reserve of the blood, and the tension of alveolar air carbon dioxide were determined by the methods previously described. The nephropathic animals were then given intravenously either 25 cc. per kilo of a 3 per cent solution of sodium carbonate or the same amount of a solution of sodium chloride equimolecular with 3 per cent sodium carbonate. The normal animals were not given either of these solutions. In such animals it was possible to obtain information cover-
TABLE II.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Diuretic</th>
<th>Urea</th>
<th>NaCl</th>
<th>Carbonate</th>
<th>Control</th>
<th>Carbonate</th>
<th>Control</th>
<th>Carbonate</th>
<th>Control</th>
</tr>
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<td>1 Normal.</td>
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<td>7</td>
<td>7.4</td>
<td>8.1</td>
<td>Urea</td>
<td>0.9%</td>
<td>64</td>
<td>7.45</td>
<td>8.1</td>
</tr>
<tr>
<td>2 &quot;</td>
<td>Pituitrin, 0.5 cc.</td>
<td>10</td>
<td>7.3</td>
<td>8.1</td>
<td>NaCl</td>
<td>5%</td>
<td>10 cc.</td>
<td>7.2</td>
<td>8.0</td>
</tr>
<tr>
<td>3 Carbonate</td>
<td>0.5 &quot;</td>
<td>28</td>
<td>7.35</td>
<td>8.1</td>
<td>10 Urea</td>
<td>0.9%</td>
<td>38</td>
<td>7.3</td>
<td>8.0</td>
</tr>
<tr>
<td>4 Control.</td>
<td>&quot; 0.5 &quot;</td>
<td>0</td>
<td>7.35</td>
<td>7.9</td>
<td>0 &quot;</td>
<td>0.9%</td>
<td>0</td>
<td>7.35</td>
<td>7.9</td>
</tr>
<tr>
<td>5 Carbonate</td>
<td>Theobromine, 1%</td>
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<td>7.35</td>
<td>8.1</td>
<td>1 NaCl</td>
<td>5%</td>
<td>22</td>
<td>7.35</td>
<td>8.0</td>
</tr>
<tr>
<td>6 Control.</td>
<td>&quot; 1%</td>
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<td>7.25</td>
<td>7.85</td>
<td>0 &quot;</td>
<td>5%</td>
<td>0</td>
<td>7.25</td>
<td>7.85</td>
</tr>
<tr>
<td>7 Carbonate</td>
<td>1% &quot;</td>
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<td>7.5</td>
<td>8.0</td>
<td>5 &quot;</td>
<td>5%</td>
<td>41</td>
<td>7.3</td>
<td>7.95</td>
</tr>
<tr>
<td>8 Control.</td>
<td>&quot; 1%</td>
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<td>7.2</td>
<td>7.8</td>
<td>0 &quot;</td>
<td>5%</td>
<td>0</td>
<td>7.2</td>
<td>7.8</td>
</tr>
<tr>
<td>9 Carbonate</td>
<td>1% &quot;</td>
<td>13</td>
<td>7.35</td>
<td>8.0</td>
<td>5 Urea</td>
<td>0.9%</td>
<td>44</td>
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</tr>
<tr>
<td>10 Control.</td>
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<td>7.2</td>
<td>7.8</td>
<td>0 &quot;</td>
<td>0.9%</td>
<td>0</td>
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<td>7.75</td>
</tr>
<tr>
<td>11 Carbonate</td>
<td>&quot; 1%</td>
<td>0</td>
<td>7.3</td>
<td>7.9</td>
<td>0 NaCl</td>
<td>5%</td>
<td>0</td>
<td>7.25</td>
<td>7.8</td>
</tr>
<tr>
<td>12 Control.</td>
<td>&quot; 1%</td>
<td>0</td>
<td>7.1</td>
<td>7.8</td>
<td>0 &quot;</td>
<td>5%</td>
<td>0</td>
<td>7.05</td>
<td>7.75</td>
</tr>
<tr>
<td>13 Carbonate</td>
<td>&quot; 1%</td>
<td>0</td>
<td>7.3</td>
<td>7.85</td>
<td>0 &quot;</td>
<td>5%</td>
<td>0</td>
<td>7.2</td>
<td>7.8</td>
</tr>
<tr>
<td>14 &quot;</td>
<td>&quot; 1%</td>
<td>0</td>
<td>7.3</td>
<td>7.9</td>
<td>0 &quot;</td>
<td>5%</td>
<td>0</td>
<td>7.2</td>
<td>7.8</td>
</tr>
<tr>
<td>15 &quot;</td>
<td>&quot; 1%</td>
<td>0</td>
<td>7.3</td>
<td>7.9</td>
<td>0 &quot;</td>
<td>5%</td>
<td>0</td>
<td>7.2</td>
<td>7.8</td>
</tr>
</tbody>
</table>

ing the natural susceptibility of the kidney to the anesthetic. All the animals were anesthetized by Gréhant's anesthetic in 60 per cent strength. When the animals had become completely anesthetized the abdomen was opened and cannulas were tied into both ureters. The flow of urine was recorded in drops per minute. Half an hour after giving the anesthetic observations were made on the acid-base equilibrium of the blood and these observations were repeated every half hour during the experiment. The experiments were terminated at the end of 1½ hours. At each half hour period during the experiments the animals were given, intravenously, one of the following diuretic solutions, a record being made of the relative efficiency of these substances in the different groups of animals: theobromine, 1 per cent, 1 cc. per kilo; pituitrin (Parke, Davis and

2 The animal is given 0.25 cc. per kilo of a 4 per cent solution of morphine. This is followed in half an hour by 10 cc. per kilo of the following mixture: chloroform, 50 cc.; alcohol and water, each 500 cc.
Company), 0.5 cc. regardless of weight; urea solution, 0.9 per cent, in 0.9 per cent sodium chloride, 10 cc. per kilo; sodium chloride; 5 per cent, 10 cc. per kilo.

The Efficiency of Diuretics in the Normal Animal Anesthetized by Gréhant’s Anesthetic.

In Experiments 1 and 2 of Tables I and II an outline is given of the blood changes, the changes in carbon dioxide tension, and the efficiency of several types of diuretic substances in two of the ten normal animals which were anesthetized with Gréhant’s anesthetic. The animals were respectively 1 and 4 years old. Both these animals were freely diuretic prior to the anesthetic. The hydrogen ion content of the blood, the alkali reserve, and the tension of carbon dioxide in alveolar air were normal. Half an hour after the animals were anesthetized no change had taken place in these readings except a reduction of the tension of carbon dioxide of 1 mm. The urine flow from each animal was two drops per minute. The animal of Experiment 1 was given 1 cc. per kilo of a 1 per cent solution of theobromine, while the animal of Experiment 2 received 0.5 cc. of pituitrin. The flow of urine was increased by theobromine to seven drops per minute and by pituitrin to ten drops per minute. At the end of the second half hour period no change had taken place in the alkali reserve of the blood. The combined volatile and non-volatile acid content of the blood as indicated by the hydrogen ion determinations had increased in the animal 1 year old from 7.45 to 7.4, while in the animal 4 years old this reading was 7.3, as compared with the normal of 7.45. At this stage of the anesthesia the animal of Experiment 1 was given 10 cc. per kilo of a 0.9 per cent solution of urea, and the animal of Experiment 2, 10 cc. per kilo of a 5 per cent solution of sodium chloride. The diuresis from both these substances was marked. The urea solution increased the flow of urine to sixty-four drops per minute and the sodium chloride solution to 10 cc. per minute. At the end of the experiments, 1½ hours after the animals were anesthetized, the flow of urine from both animals was in excess of the normal. In the young animal of Experiment 1 no change had taken place during the period of anesthesia in the hydrogen ion con-
tent or alkali reserve of the blood and practically no change in the tension of alveolar air carbon dioxide. The tension had been reduced 1 mm. In the animal of Experiment 2, 4 years old, the hydrogen ion content had increased from the normal of 7.45 to 7.2 and the alkali reserve showed a reduction from 8.1 to 8.0. The tension of carbon dioxide had decreased from the normal of 43 to 39 mm.

The experiments on the normal animals show that the normal kidney is relatively non-susceptible to the toxic effect of Gréhant's anesthetic. Animals anesthetized by this mixture, the active anesthetic ingredients of which are chloroform and alcohol, remain diuretic during a period of 1½ hours and respond to such diuretic substances as theobromine, pituitrin, and solutions of urea and sodium chloride. The experiments also indicate that in the older normal animals there is a tendency for the anesthetic to increase the hydrogen ion content of the blood and reduce the alkali reserve of the blood. The extent to which these changes have developed in the normal animals has not been indicated by any reduction in the response of the kidneys to various diuretics.

At the end of the experiments on normal animals and prior to the death of the animals the kidneys were removed and studied histologically. The findings were negative.

The Efficiency of Diuretics in Nephropathic Animals Anesthetized by Gréhant's Anesthetic and Protected against the Anesthetic by a Sodium Carbonate Solution as Compared with Animals of the Same Age Which Were Unprotected.

Thirteen of the thirty-six experiments on nephropathic animals are included in Tables I and II. Ten of the experiments were conducted in pairs, one animal receiving intravenously prior to the anesthetic 25 cc. per kilo of a 3 per cent solution of sodium carbonate, while the other animal which was used as a control was given an equivalent volume per kilo of a solution of sodium chloride equimolecular with the carbonate solution. All the animals had been intoxicated by uranium in the dose of 5 mg. per kilo which was given on the 2 days prior to the day of the experiment. During the last day of the uranium intoxication all the animals were diuretic. The output of urine varied between a minimum of 310 cc. to a maximum
of 960 cc. The urine was collected at 9 a.m. and the animals were anesthetized for the experiments in the afternoon of the same day. At this time the hydrogen ion and alkali reserve determinations were made. These determinations have therefore no association with the output of urine as recorded in Table I. It will, however, be noted that the older animals with a well marked acid intoxication show a decrease in the output of urine.

A study of the animals included in Table I, before they were anesthetized, confirms the observation made in Part I of this investigation. The older animals at the end of a 2 day period of intoxication by uranium show a severer grade of acid intoxication than do the younger animals. The animals over 1½ years of age show a more constant increase in the hydrogen ion content of the blood, and the alkali reserve of the blood has been drawn upon to a greater extent than has been the case with the animals under 1½ years of age. The carbon dioxide tension shows a greater reduction in the older animals than in the young animals.

Following these determinations the animals were given either the carbonate solution or the solution of sodium chloride, and at once anesthetized with Gréhant's anesthetic in 60 per cent strength. The observations which follow were made at half hour intervals during the experiments.

Reference to the tables of experiments shows that depending upon the relative susceptibility of the kidney to this anesthetic the animals may be divided into two groups. (1) The control animals. All these animals, even though they had first received 25 cc. per kilo of a solution of sodium chloride, became anuric by the end of the first half hour period of the experiment. This applies to animals of all ages. (2) The animals which received a solution of sodium carbonate. These animals may be subdivided into two groups. (a) The nephropathic animals not over 1½ years old which received the alkaline solution remained diuretic during the experiments, while (b) those animals over this age became anuric and remained anuric during the entire experiment. A study of the changes in the blood and alveolar air of these different groups of animals shows the following variations.

Within half an hour following the anesthetic the control animals
show an increase in the hydrogen ion content of the blood and a begin-
ing reduction in the alkali reserve of the blood. The tension of carbon dioxide either remains constant or is decreased. In the control animals in this short period the anesthetic has increased the degree of acid intoxication which existed prior to the use of the anesthetic. All the animals that received the carbonate solution show at this period of the experiment a decrease in the hydrogen ion content of the blood, an increase in available alkali, and an increase tension of carbon dioxide. Those animals over 1½ years old which resisted the toxic effect of the anesthetic and remained diuretic show a more marked decrease in the hydrogen ion content of the blood and a larger amount of available alkali than do the animals over this age which became anuric from the anesthetic. For instance, in the diuretic animal of Experiment 9 the carbonate solution changed the hydrogen ion reading prior to the anesthetic from 7.25 to 7.55 and the alkali reserve from 7.9 to 8.2. In the anuric animal of Experiment 13 the hydrogen ion determination was changed by the carbonate from 7.25 to 7.35 and the alkali reserve from 7.85 to 8.0. The inability of the alkaline solution to protect the nephropathic kidney against the anesthetic is associated with its failure to decrease the acid intoxication of the anuric animals to the same extent to which the intoxication is modified in the diuretic group.

At this stage of the experiments, the end of the first half hour period, two of the animals were given pituitrin and the remaining eleven animals were given theobromine. The control animals remained uniformly anuric from these diuretic substances. The carbonate-protected animals under 1½ years of age which had not shown a severe acid intoxication prior to the anesthetic and which following the anesthetic and the intravenous injection of the alkaline solution gave no evidence of an acid intoxication were freely diuretic to both pituitrin and theobromine. The older animals, those over 1½ years of age, which prior to the anesthetic had shown a severe grade of acid intoxication and which following the anesthetic and the carbonate solution gave less evidence of protection against the acid intoxication have remained anuric and unresponsive to these diuretic substances. A solution of sodium carbonate is able to protect the kidney of a nephropathic animal against Gréhant's anesthetic up to
a certain age limit. After this age limit is reached the older animals
are not afforded a protection by the carbonate but become anuric
as do the control animals which have received a solution of sodium
chloride.

A study of the tables of experiments from this first half hour period
following the anesthetic, through the remaining periods to the ter-
mination of the experiments, shows the variations which occur in
the acid-base equilibrium of the blood in the control animals, in the
carbonate-protected animals which remain diuretic, and in those
animals receiving the carbonate which become anuric; it also shows
the relative efficiency of diuretics in these three groups of animals.

It will be observed that all the control animals remain anuric through
the remaining hour of the respective experiments and that none of
these animals show any diuretic effect from a 0.9 per cent solution
of urea or a 5 per cent solution of sodium chloride. It will also be
observed that in the control animals the degree of acid intoxication
tends to increase progressively in severity as the age of the animal
increases. The control animal of Experiment 4, 8 months old, at
the end of the experiment had a hydrogen ion content of 7.35 and an
alkali reserve of 7.9. The carbon dioxide tension was 29 mm. The
control animal of Experiment 12, 3 years and 7 months old, had a
hydrogen ion content of 7.05, an alkali reserve of 7.75, and a carbon
dioxide tension of 24 mm. Nephropathic animals anesthetized by
Gréhant's anesthetic and unprotected against the toxic action of the
anesthetic by an alkali show the same type of response to the anes-
thetic as the animals of different ages have shown to uranium. The
toxicity of the anesthetic increases with the age of the animal.

During the remainder of the experiments the nephropathic animals
which received the sodium carbonate protection permit the same
division into two groups. The animals not over 1½ years of age re-
main diuretic to the conclusion of the experiment, while those over
this age remain anuric.

At the end of the second half hour period of the experiments all the
animals which had received the carbonate protection were given
either 10 cc. per kilo of a 0.9 per cent solution of urea or 10 cc. per
kilo of a 5 per cent solution of sodium chloride. The functional re-
sponse of the kidneys to these diuretics was clear-cut. The younger
group of animals which were previously diuretic to either theobromine or pituitrin and which at the present stage of the experiment showed a hydrogen ion concentration not over 7.35 and an alkali reserve not below 8.0 were freely diuretic to both 5 per cent sodium chloride and a 0.9 per cent solution of urea. The older animals, which earlier in the experiments had shown no diuretic effect from theobromine, at this later stage of the experiment also remained anuric to the solutions of urea and sodium chloride. In this latter group of anuric animals the injected carbonate solution had been rapidly used up so that the blood showed an alkali reserve of not over 7.8.

During the course of the anesthesia all the animals which have received the carbonate protection show an increase in the hydrogen ion content of the blood and a reduction in the alkali reserve. The degree to which these changes take place varies in the diuretic and anuric groups of animals. In the younger group of animals which remain diuretic the reduction in the alkali of the blood takes place gradually so that during an anesthesia lasting 1½ hours the alkali reserve of the blood has not shown a reading below 7.95 and the hydrogen ion content has not been over 7.35. The older group of animals in which no protection was afforded by the sodium carbonate solution and which remained anuric throughout the period of anesthesia have shown a rapid depletion of the blood of the injected alkali, so that by the termination of the experiment this group of animals has had a hydrogen ion content as high as 7.2, and an alkali reserve as low as 7.8.

The changes in carbon dioxide tension in alveolar air of the two groups of animals have shown in general a correlation with the change in the alkali reserve of the blood. In the group of young animals which show less reduction in the alkali reserve the tension of carbon dioxide has varied between 30 to 37 mm. In the older group of animals with a more marked reduction in the alkali reserve the tension of carbon dioxide has varied between 26 to 29 mm.

The foregoing brief review of the differences in the acid-base equilibrium of the blood in the diuretic and anuric groups of animals shows that the establishment of a state of anuria in an animal nephropathic from uranium and anesthetized by Gréhant's anesthetic is associated with the development of a tissue acidosis and with a
failure of the carbonate solution to maintain the reaction of the blood near the point of neutrality.

The Pathology of the Kidney in Nephropathic Animals, Protected and Unprotected against the Toxic Effect of Gréhaut's Anesthetic by Sodium Carbonate.

At the termination of the experiments and before the death of the animals the kidneys were removed for histological study. Tissue was fixed in 10 per cent formalin and in mercuric chloride-acetic acid. Sections for routine histological study were stained with eosin and hematoxylin. Frozen sections were stained for fat by Herxheimer's modification of the Scharlach R stain.

Depending upon the severity of the pathological changes which have developed in the kidneys, these organs may be classified into three groups: the kidneys of animals which have received the carbonate protection and which have remained diuretic and responsive to diuretic substances; the kidneys of animals which also received the carbonate protection but which during the development of the anesthesia became anuric and remained unresponsive to diuretic substances; and finally, the group of control animals all of which following the anesthetic became anuric.

The kidneys of the animals which were successfully protected by the carbonate show no degenerative change in the glomerular vessels. These vessels are usually distended with blood and when compared with the glomerular vessels in the anuric group of animals the difference in the degree of distention of the loops is very evident. The epithelium of the convoluted tubules is shrunken. The nuclei of these cells are large and hyperchromatic. The lumen of the tubules is prominent. Occasionally tubules are observed in which the cells show an accumulation of granules, or more rarely vacuoles, and an early swelling. Stainable fat has not been found in the epithelium of the convoluted tubules, the glomerular vessels, or in the connective tissue of the kidney. The loops of Henle usually show a trace of fat (Figs. 1 and 2).

The kidneys of those animals which received the carbonate solution but which were not afforded any protection against the toxic effect
of the anesthetic show a pathological response which is striking when compared with the changes in the kidneys that have been protected by the alkaline solution. The vascular element of the kidney shows no evidence of degeneration. The capillary loops of the glomeruli are certainly not engorged with blood, and when compared with the glomerular vessels of the previous group of animals protected by the carbonate, the glomerular vessels of this anuric group contain less blood. The epithelium of the convoluted tubules is severely swollen. The cell cytoplasm has not become necrotic and the nuclei have not undergone fragmentation. In many of the tubules the lumen has become completely obliterated by the swollen cells (Fig. 3). The epithelium of the convoluted tubules has not contained any clearly defined stainable fat. The loops of Henle show a large amount of fat in the form of droplets, which frequently fuse together.

The kidneys of the control animals, which instead of receiving the alkaline solution were given a solution of sodium chloride equimolecular with the carbonate solution, show a pathological response resembling that of the anuric group of animals which failed to receive any protection from the carbonate solution. The pathological changes in the kidneys of this control group show more advanced evidence of degeneration. The glomerular vessels are not distended with blood. The cells of the convoluted tubules are severely swollen, granular, and frequently hydropic. The nuclei are hypochromatic and undergoing fragmentation. The cytoplasm of many of the cells has become necrotic (Fig. 4). In these cells stainable fat has frequently been demonstrated. The relative amount of fat in the loops of Henle is far greater in this control group of animals which were anuric than in the anuric group of animals that received the carbonate solution.

**SUMMARY.**

The outline which has been given of the relative toxicity of Gréhant's anesthetic in normal animals, in animals that were nephropathic from uranium and protected against the anesthetic by an alkaline solution, and in those animals which were unprotected by such a solution, furnishes the basis for the following summary.

The kidney of the normal dog is relatively non-susceptible to the
toxic action of Gréhant's anesthetic. The kidneys have failed to show any change in their histological structure during the period of anesthesia. These animals have remained diuretic during the period of anesthesia and have responded to diuretics such as theobromine, pituitrin, and solutions of urea and sodium chloride.

Normal animals anesthetized with Gréhant's mixture for 1½ hours usually show at the end of the experiment either no change, or only a slight variation from the normal, in the hydrogen ion content of the blood, the alkali reserve of the blood, and in the tension of carbon dioxide. In several normal dogs which were over 4 years of age, by the end of an anesthesia of such a duration the animals have shown a reduction in the alkali reserve of the blood and also a decrease from the normal in the carbon dioxide tension of alveolar air. From this observation it would appear that even in a normal animal Gréhant's anesthetic tends to induce an acid intoxication, and as was the case with normal animals which were being intoxicated by uranium, such an intoxication is more readily induced in an old animal than in a young one.

The nephropathic animals which have been anesthetized by Gréhant's anesthetic and in which an attempt has been made to protect these animals against the toxic effect of the anesthetic by the use of a solution of sodium carbonate fall into two clear-cut groups. Those animals of the series not over 1½ years old have shown at the end of the uranium intoxication and prior to the use of the anesthetic a less severe acid intoxication than have the animals of the series which were over 1½ years old. In this younger group of animals the intravenous injection of a 3 per cent solution of sodium carbonate immediately before the animals were anesthetized has succeeded in protecting these animals against the toxic action of the anesthetic. During the following 1½ hours of anesthetization these animals have not developed a severe grade of acid intoxication, and in several of the animals at the end of the experiment the alkali reserve of the blood was in excess of what it was at the end of the uranium intoxication and before an anesthetic was administered. Animals of this protected group have remained diuretic throughout the experiment and have shown an active diuresis from pituitrin, theobromine, and solutions of urea and sodium chloride. The kidneys of such animals
have shown histologically a normal vascular tissue, a convoluted tubule epithelium which gave the appearance of being hyperactive, and only occasionally were tubules encountered which showed signs of an early epithelial degeneration.

The nephropathic animals of the series in which a solution of sodium carbonate failed to afford any protection against Gréhan's anesthetic were animals over 1½ years old in which the uranium intoxication had resulted in a severer grade of acid intoxication than in the younger animals. When these older animals were given intravenously the carbonate solution and were anesthetized, it was found impossible to increase the alkali reserve of the blood to the same extent as was possible in the younger animals. Furthermore, the alkaline solution during the period of anesthesia is rapidly used up so that by the termination of these experiments the animals may have an alkali reserve of the blood which may be even lower than was the alkali reserve before the use of the carbonate. These animals have remained completely anuric throughout the experiments and have shown no diuretic effect from those diuretics which in the animals that were successfully protected by the carbonate induced free diuresis. The kidneys of these anuric animals show no degenerative changes in the glomerular vessels. The capillaries are not distended with blood as has been the case with the diuretic group. The epithelium of the convoluted tubules is acutely swollen. The swelling has frequently taken place to such an extent that the lumen of the tubules has become obliterated.

The nephropathic animals of the series which served as control animals and which were given a solution of sodium chloride equimolecular with the carbonate solution, following Gréhan's anesthetic became completely anuric. The sodium chloride solution furnished no protection against the anesthetic. The animals of all ages became anuric and unresponsive to the diuretic substances which have been used during this study. With the establishment of a state of anuria in these control animals the hydrogen ion content of the blood has increased, the alkali reserve of the blood has been rapidly depleted, and associated with this change the carbon dioxide tension has been reduced. The rapidity with which these changes develop
and the degree of acid intoxication which is induced is more marked in these animals than in any of the other series.

The kidneys of the control animals show the severest grade of degeneration of any of the nephropathic animals. The epithelium of the convoluted tubules is not only severely swollen but the cells frequently show necrosis. The loops of Henle contain more stainable fat than has been demonstrated in the kidneys of the carbonate animals.

CONCLUSIONS.

1. The toxicity of Gréhant's anesthetic for the nephropathic kidney is associated with the ability of the anesthetic to induce an acid intoxication. The severity of the acid intoxication increases with the age of the animal.

2. The efficiency of a solution of sodium carbonate to protect the kidney against the toxic effect of Gréhant's anesthetic depends upon its ability to prevent the development of an acid intoxication. The efficiency of a solution of sodium carbonate to furnish such a protection decreases as the age of the animal increases.

3. The inability of a solution of sodium carbonate to protect the kidney against Gréhant's anesthetic has been characterized by the development of an acute swelling of the renal epithelium which is later followed by necrosis. These animals develop an acute anuria and are unresponsive to diuretics.

4. The ability of a solution of sodium carbonate to protect the kidney against Gréhant's anesthetic has been associated with the histological preservation of the renal epithelium. These animals remain diuretic and responsive to various diuretic substances.

5. The inference should not be drawn from the observations which have been made that the epithelial damage is the sole cause for the anuria and the lack of response of some of the animals to diuretic substances. It is difficult to conceive that such gross changes in the volume of the renal epithelium could occur without seriously affecting the functional response of the vascular mechanism of the kidney.
EXPLANATION OF PLATES.

PLATE 3.

Fig. 1. Camera lucida drawing, Zeiss oc. 3, obj. 6. The figure is from the kidney of the carbonate animal of Experiment 3, Table I. The glomerular vessels show no degeneration and the capillary loops are well filled with blood. The epithelium of the convoluted tubules, a, is shrunken; the lumen of the tubules is prominent. The nuclei are large and hyperchromatic. At b the epithelium shows an early swelling. The animal was successfully protected against Gréhant's anesthetic by the carbonate solution, and was freely diuretic to both pituitrin and a 0.9 per cent solution of urea.

PLATE 4.

Fig. 2. Camera lucida drawing, Zeiss oc. 3, obj. 6. The figure is from the kidney of the carbonate animal of Experiment 5, Table I. The pathology of the kidney is in general similar to that shown in Fig. 1. The glomerular vessels fill the subcapsular space and the capillary loops are well filled with blood. The epithelium of the convoluted tubules, a, is shrunken; the nuclei of the cells are large and hyperchromatic. At b these cells show a beginning swelling. The animal was successfully protected against the anesthetic by the alkaline solution; it had a marked diuresis from both theobromine and a 5 per cent solution of sodium chloride.

PLATE 5.

Fig. 3. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the carbonate animal of Experiment 11, Table I. The glomerulus is partially compressed by the swelling of the tubular epithelium. The capillaries are not distended with blood. The epithelium of the convoluted tubules, a, is severely swollen. The epithelium of the junctional tubules, b, fails to show the swelling. The animal was not protected against Gréhant's anesthetic by the carbonate solution. Early in the experiment it became anuric and showed no diuretic effect from theobromine or from a 5 per cent solution of sodium chloride.

PLATE 6.

Fig. 4. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the control animal of Experiment 12, Table I. The animal received 25 cc. per kilo of a solution of sodium chloride equimolecular with a 3 per cent solution of sodium carbonate. The glomerular capillaries are not filled with blood. The epithelium of the convoluted tubules at a shows a severe grade of swelling. At b the cells have become necrotic. The junctional tubules at c
have an epithelium which is not swollen and in which the nuclei stain normally. During the development of a state of anesthesia the animal became anuric and remained anuric throughout the experiment. No diuretic effect was obtained from either theobromine or a 5 per cent solution of sodium chloride.
FIG. 1.

(MacNider: Toxicity of uranium. II.)
FIG. 2.

(MacNider: Toxicity of uranium. II.)
FIG. 3.

(MacNider: Toxicity of uranium. II.)
(MacNider: Toxicity of uranium. II.)