Changes in Ammonia Values of Protein-free Filtrates of Blood During Storage in the Frozen State

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Protein-free filtrates of blood treated with trichloracetic acid were divided into two or three parts. The ammonia concentration of one of these was determined immediately by the ninhydrin method described by Nathan and Rodkey (1). The others were kept frozen for periods of 1-8 days and then thawed and analyzed. The blood ammonia concentrations calculated from these filtrate values were compared. A significant change in ammonia level during frozen storage was demonstrated.

The rate of change of ammonia concentration in shed blood is controversial, but there is general agreement that significant ammonia formation does occur and that analysis should, therefore, be carried out immediately. However, some ammonia methods are quite tedious, and determination could be made much more efficiently if samples could be stored and run in groups. This paper reports an attempt to evaluate the effect of freezing and storing on the ammonia in protein-free filtrates of blood.

Methods

The ammonia method used was that described by Nathan and Rodkey (1). The blood was drawn without stasis into a dry, 5-ml syringe

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to the drawer's best quick estimate of a 3-ml. volume, the error usually amounting to 0–0.2 ml. and rarely exceeding 0.3 ml. Although no attempt was made to exclude the 0.1–0.2 ml. of dead space air in the syringe, the sample was discarded if the air was in the form of many small bubbles or foam. Within 10-15 sec. of the start of the drawing, the sample was ejected into an ice-cold centrifuge tube containing trichloracetic acid and held by an assistant who immediately replaced the rubber stopper, mixed the contents of the tube thoroughly and returned it to an insulated container of chipped ice. Within one hour, the samples were centrifuged and the filtrates removed. Each was divided into two or three equal portions in small, glass test tubes tightly closed with rubber stoppers. The analysis was then carried out on one of these aliquots (hereafter referred to as "control") while the others were frozen, either in a large insulated container used for storing Dry Ice or in the freezing compartment of an electric refrigerator (using dichlordifluoromethane as a refrigerant) at about −15°. On subsequent days, these were thawed and analyzed. The laboratory in which analyses were performed was on a different floor from the clinical laboratories and its other work was mainly limited to blood gas analyses. No urine samples or other materials known to liberate ammonia were allowed in the laboratory.

The absorbencies were read at 560 mµ on the Beckman Model B spectrophotometer and the ammonia content of the blood calculated from that of the filtrate, using the formula of Nathan and Rodkey. Thus, all values mentioned in this report will be expressed as the derived, whole-blood ammonia concentration rather than the ammonia concentration of the filtrate. It should be noted that, although the frozen filtrates were run at various times up to 8 days after shedding, not more than two stored samples were analyzed for any given control.

The values used in the present study are the result of single determinations, each filtrate being considered only in comparison with its own frozen and stored aliquots on subsequent days. However, the usual procedure for the determination of blood ammonia in our laboratory is to take the average of analyses of three separate samples, obtained as described earlier, using one venipuncture and three separate syringes. Thus, it is possible to determine the variability of the laboratory technic by an estimate of sigma based on the difference between the high and low readings for each set of triplicates. The mean of the differences in 200 samples is 24.8 ± 14.7 µg./100 ml. (± 1 S.D.). For values under 100 µg./100 ml., between 100 and 200, and over 300,
the mean differences are 14.0 ± 8.3, 22.2 ± 13.1, and 38.4 ± 22.7, respectively.

The blood samples were taken from 30 in-patients of whom 10 had normal blood ammonias and no liver disease. Usually, both arterial and peripheral venous samples were obtained and a few samples were from other venous sites. A total of 136 initial analyses and 179 analyses of frozen filtrates is included in this study, which was carried out over a 10-month period.

Results

The results for days when more than 10 frozen filtrates were analyzed are summarized in Table 1. In order to combine the data, it was necessary to reduce all figures to similar terms. This was accomplished by considering each value obtained from the analysis of a frozen filtrate as a percentage of the control value. Since only one or two frozen filtrates were subsequently examined for each control, one may not consider the figures as necessarily signifying a day-to-day progression of change. However, in 26 samples, frozen aliquots were analyzed on both the first and second days after drawing the blood. Values obtained, expressed as percentages of the mean control value (129 µg./100 ml.), were as follows: Day 0, 100%; Day 1, 116%; and Day 2, 134%. A few filtrates were examined on 2 other days after the control analyses (Fig. 1). Here it is permissible to compare the value for each day with that of each of the other days.

It is evident that ammonia levels did not remain unchanged during the period of study. There was an early substantial rise and later an apparent fall to near control levels by the eighth day. The data regard-

Table 1. Derived Blood Ammonia Levels of Filtrates Analyzed Immediately after Preparation and After Frozen Storage

<table>
<thead>
<tr>
<th>Day</th>
<th>Arterial</th>
<th>Venous</th>
<th>Combined arterial and venous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean</td>
<td>No.</td>
</tr>
<tr>
<td>0</td>
<td>71</td>
<td>100 (148)</td>
<td>65</td>
</tr>
<tr>
<td>1</td>
<td>43</td>
<td>130</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>135</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>139</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>121</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>105</td>
<td>9</td>
</tr>
</tbody>
</table>

Numbers in parentheses are mean control values (µg. 100 ml.). All other values are means of percentages of these control values.
ing the late fall, however, are dependent on relatively small numbers of filtrates and, because of considerable variability between samples, have not been found to be statistically significant. There was considerable variability between filtrates in the degree and timing of the change during frozen storage and, indeed, in some there was no increase or there was a decrease from the control level. There was no clear relation between the change and the height of the initial blood ammonia level or the venous or arterial site of origin of the blood. The continuing nature of the change, illustrated by the data in Fig. 1, suggests that it is not the result of the freezing or thawing processes. The early rise and apparent late fall suggest that formation and loss of ammonia may be occurring simultaneously.

Since the data presented thus far do not lend themselves readily to statistical analysis, the data on the 76 filtrates which were tested after 24 hours' freezing were analyzed using a t-test for paired data to compare the values for Day 0 and Day 1. It was found that the difference between the ammonia concentrations on Days 0 and 1 is statistically significant ($p < .001$).

**Discussion**

Reported observations on storage of blood for ammonia analysis can be summarized briefly. Ammonia is not formed in significant
amounts during the first 20 min. if the blood is drawn and kept anaerobically in a syringe (2), and it has been found (3) that collection and storage of blood under carbon dioxide inhibits formation of ammonia.

Merchant et al. (4), using a modification of the Conway method with whole blood, concluded that there was no significant elevation in ammonia concentrations in 24 hr. in samples which had been quick-frozen in a bath of Dry Ice and alcohol. The samples were obtained from 10 normal subjects and 1 patient with an elevated ammonia level. The mean ammonia concentration of the frozen and stored blood samples was about 135% of the mean of the original unfrozen samples (or 120% of the immediately frozen, thawed, and analyzed "controls"). These increases approximate those reported here.

If ammonia is liberated by the action of enzymes on blood constituents and by the alkaline hydrolysis of proteins, early removal of the blood proteins, including enzymes, suggests itself as a means of interrupting ammonia formation. Nathan and Rodkey (1) found no change in the ammonia content of iced, protein-free filtrates in trichloracetic acid over a period of 80 min. Nathan’s data (5) suggest that blood filtrates are not stable at 0° for 24 hr. and that frozen brain tissue is not stable at -70°. However, Bromberg et al. (6), also using the Nathan and Rodkey method, found no significant changes in ammonia levels over a period of several weeks in bloods from two dogs, one with normal ammonia levels and one with high levels as a result of an infusion of ammonium acetate. Their filtrates were apparently not quick-frozen and were kept at -10°.

Summary

The ammonia concentrations of protein-free filtrates of blood were compared with those of aliquots of the same filtrates which had been frozen and stored for periods up to 8 days. The rise in ammonia levels (amounting to an average of 33%) which occurred during the first day is statistically highly significant and the data suggest that the levels continue to change.

References
5. Nathan, D. G., Personal communication.