วารสารเทคนิคการแพทย์ เชียงใหม่
BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY

May 1972
Vol. 5 No. 2

# Effect of Time and Temperature on Plasma Ammonia Determination

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## Abstract

**By means of this method it has been demonstrated that the distribution of ammonia in plasma of normal individuals increases after drawing. The influence of temperature on in-vitro generation of ammonia in plasma was evaluated. It was found that when blood was placed in a freezer with the temperature between -20° C and -25° C there was no significant rise of blood ammonia levels. There wes gradual rise when blood was placed in a refrigerator with the temperature about 4° C and more rapid rise at room temperature. The inhibition of ammonia production at temperature below -20° C facilitates a laboratory procedure when it is necessarily delayed.**

## Introduction

Blood ammonia is formed in the gastrointestinal tract and eliminated as urea by the liver. In cases of liver damage or disease, cirrhosis, and occasionally in severe heart failure, azotemia, corpulmonale, and erythroblastosis fetalis, the ammonia is not properly converted and the ammonia level of the plasma rises. Repeated ammonia determinations have been found especially useful in the monitering of patients suffering from hepatic coma. The method used to determine ammonia is fairly simple for any laboratory, but it must be performed as soon as possible following collection of the sample. Only freezing of the sample provides an adequate mean of storate as was demonstrated in this experiment.

* This experiment and report were presented by Chatchawadee Kunarak in fullfillment of the requirements for a B.Sc. (M.T.) from Chiang Mai University 71-72.

**Method**

The method used in this experiment represents a modification of the Seligson and Seligson microdiffusion method, the principle being that when saturated potassium carbonate is added to an aliquot of plasma, the alkali causes the release of any ammonia present in the plasma. The ammonia is trapped in hydrochloric acid in specially designed diffusion bottles. After an appropriate time interval, the acid drop and the absorbed ammonia are rinsed into cuvettes with Nessler's reagent and the optical density read. A standard of known concentration is prepared and treated in the same manner and the final results computed by the standard formula:

$$\frac{\text{O.D.}_{unk} \times \text{Value of Std}}{\text{O.D.}_{Std.}} = \text{Value unknown}$$

To show the effect of time and temperature on the level of ammonia in plasma, samples were taken from nine normal individuals with no history of liver damage or disease. EDTA has been found to be the ideal anticoagulant for ammonia studies and was used in this case. Plasma was separated immediately and an immediate determination done in order to determine a baseline value for each sample. Plasma must be separated before freezing because freezing and thawing of whole blood results in the hemolysis of red cells which causes a false elevation due to the presence of ammonia in the red cells. The samples were then divided into three sets. Set 1 was kept at room temperature (25° C) and retested 3 hours, 6 hours and 9.5 hours after collection. Set 2 was refrigerated and retested at 6, 12 and 23.5 hours. The third set was placed in the freezer and tested again at 24, 48 and 72 hours.

**Results**

It was found that the ammonia content of plasma prepared and maintained at room temperature is not constant. Plasma in contact with air in vitro continues to form ammonia. This reaction is markedly inhibited by keeping plasma in the freezer. It was observed that the ammonia levels of plasma frozen in the freezer immediately after shedding, if measured promptly after thawing, remain less changed than that of refrigerator stored or plasma kept at room temperature.

In Set 1, in which plasma was left at room temperature, there was a gradual rise of ammonia content starting at 10 minutes. At the end of 6 hours, the concentration of blood ammonia had risen to 2 or 3 times the normal level. When blood was kept in the freezer, there was no significant increase in ammonia level.

In Set 2, the temperature of ordinary refrigeration apparently could not prevent the enzymatic hydrolysis of blood, when

was a gradual increase of ammonia although not to the same degeee as in these specimens left at room temperature.

In Set 3, the period of freezing was extended to 4 days. There was no significant increase of ammonia content in the blood, when compared with the normal. A comparison of curves indicated that the ammonia levels were less altered during the period of freezing than storage at either room temperature or refrigeration.

The mean values of varied conditions were :

| % of plasma ammonia increase per hour | |
|---|---|
| Room temperature | 28.1 % |
| Refrigerator | 9.3 % |
| Freezer | 0.834 % |

**Conclusion :**

This investigation showed that the ammonia concentration of freshly drawn plasma was insignificantly altered by rapid freezing but that at room temperature, the plasma ammonia concentration remains constant for 15-40 minutes after blood is withdrawn from the body and then rapidly increases. Because ammonia levels increase rapidly on standing, the test should be run as soon as possible following collection of blood. If processing must be delayed more than 20 minutes, plasma should be quick frozen and kept in the freezer until ready for determination. Testing should not be delayed more than 3 days as even in the frozen state, the ammonia increases after that time. The sample should be thawed at 37°C for no more than 5 minutes and analysed immediately. Nowadays, freezing helps laboratories to be able to determine plasma ammonia when specimens have been drawn at night or on weekends because they may be stored for subsequent analysis. It also affords a means of preserving blood specimens during transportation from hospitals at which blood ammonia determinations are not available to central laboratories. In research laboratories it allows investigators to collect serial specimens for subsequent analysis without requiring repeated interruptions for immediate processing of individual samples. The determination of plasma ammonia has wide applicability in clinical medicine and research. Nevertheless, many hospital laboratories do not perform ammonia determinations because of difficulties encountered in the collection and analysis of specimens. A major reason for excluding this procedure has been the necessity of performing the analysis within 10-30 minutes of obtaining the sample.

Comparison of percentage increase in plasma ammonia at various times

Kept plasma at room temperature.

| Sample | Time | | |
|---|---|---|---|
| | 3 hours | 6 hours | 9.30 hours |
| 1 | 138.9% | 186.9% | 140.0% |
| 2 | 144.1% | 172.1% | 28.6% |
| 3 | 15.7% | 374.1% | 120.2% |
| Average/hour | 33.2% | 40.7% | 10.3% |

Comparison of percentages increase in plasma ammonia at various times.

Stored plasma in the refrigerator.

| Sample | Time | | |
|---|---|---|---|
| | 24 hours | 48 hours | 72 hours |
| 1 | 7.02% | 70.2% | 71.4% |
| 2 | 5.4% | 0% | 108.6% |
| 3 | 148.9% | 257.1% | 274.4% |
| Average/hour | 12.5% | 9.1% | 6.4% |

Comparison of percentage increase in plasma ammonia at various times.

Stored plasma in the frozen state.

| Sample | Time | | |
|---|---|---|---|
| | 24 hours | 48 hours | 72 hours |
| 1 | 0% | 0.3% | 90.9% |
| 2 | 58.4% | 0% | 178 9% |
| 3 | 14.83% | 0% | 74.6% |
| Average/hour | 1.01% | 0.002% | 1.5% |

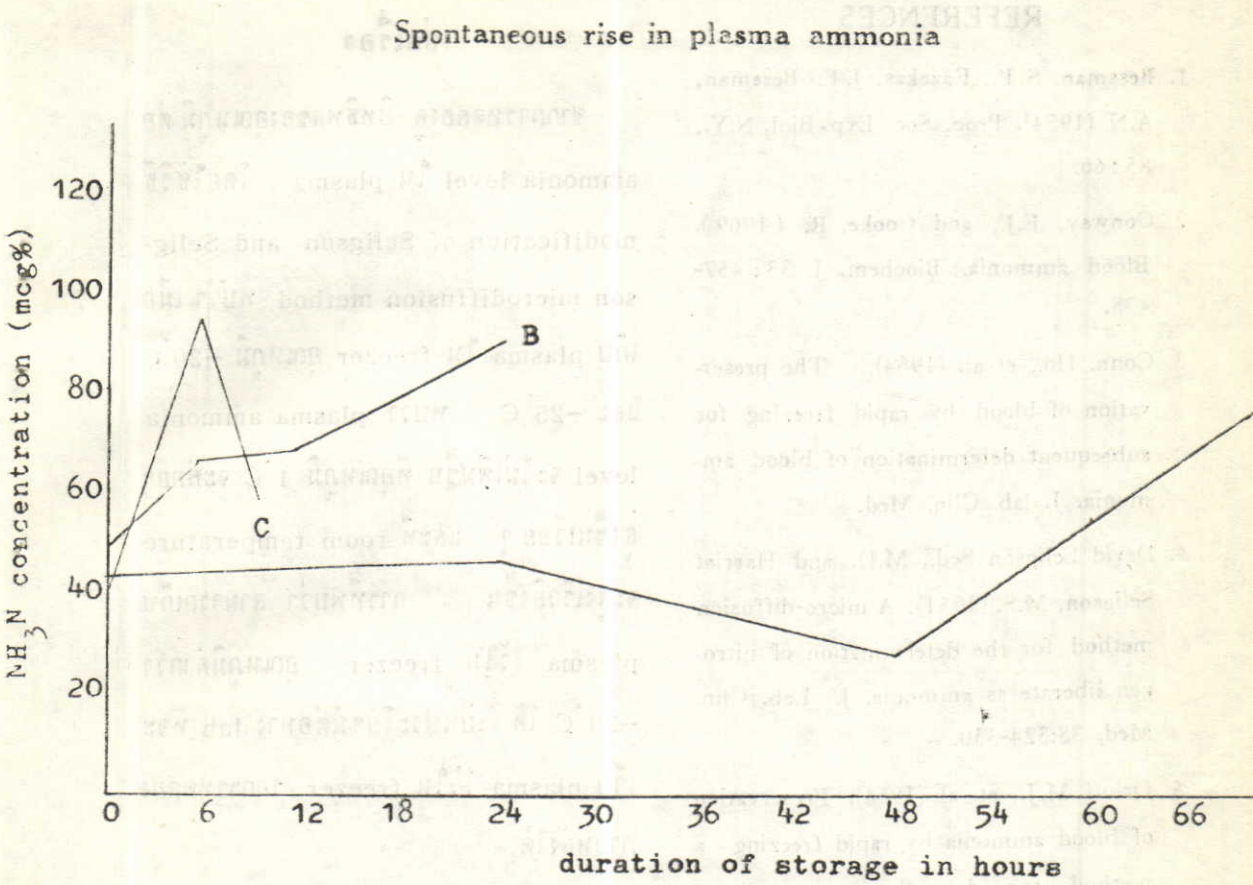


Fig II. Ammonia concentration in nine samples of blood as a function of time after shedding.

curve A. – plasma kept in the freezer

curve B. – plasma kept in the refrigerator.

curve C. – plasma kept at room temperature.

Each curve base on the mean plasma ammonia concentration of nine specimens determined serially after various period of storage.

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## ย่อเรื่อง


จากการทดลองดู อิทธิพลของอุณหภูมิ ต่อ ammonia level ใน plasma โดยใช้วิธี modification of Seligson and Seligson microdiffusion method พบว่า เมื่อเก็บ plasma ใน freezer อุณหภูมิ −20°C และ −25°C พบว่า plasma ammonia level จะไม่เพิ่มขึ้น ที่อุณหภูมิ 4°C จะค่อยๆ สูงขึ้นเรื่อยๆ และที่ room temperature จะสูงเร็วยิ่งขึ้น การที่พบว่า สามารถเก็บ plasma ไว้ใน freezer อุณหภูมิต่ำกว่า −20°C ได้ เป็นประโยชน์ต่อทาง lab ที่จะเก็บ plasma ไว้ใน freezer รอการทดลองภายหลังได้