THE DETERMINATION OF BLOOD AMMONIUM BY A MODIFICATION OF THE CONWAY TECHNIC

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SINCE the latter part of the nineteenth century the existence of ammonium in normal circulating blood has been questioned from time to time. Now it is generally agreed that minute amounts of certain substances do exist in the blood, which appear as gaseous ammonia after alkalization of the blood sample. This base is present in two forms: the ammonium ion, NH_4^+, and free ammonia, NH_3. The relationship between the two forms may be represented by the equation

$$NH_3 + H^+ \rightleftharpoons NH_4^+$$

Because a proton is involved, the equilibrium is sensitive to pH and might be expected to shift greatly in pathologic conditions characterized by acidosis or alkalosis. Evidently this does not occur. According to Bessman,[1] even at the extremes of physiologic pH variation more than 99.9 per cent of this base is in the form of the ion. Thus the term *ammonium* is preferable to *ammonia,* and will be used throughout this report to signify the summation of both forms.

Numerous methods for the quantitative estimation of ammonium in blood have been devised and have been presented in the chemical and medical literature.[2-4] The accurate determination of the minute amounts of ammonium that exist in blood is difficult by any technic because of certain potential and subtle sources of error. The four principal sources of unreliability in all methods are: (1) minuteness of quantities of ammonium present in the blood; (2) ease of contamination of the specimen; (3) continuous increase of ammonium in blood after its withdrawal from the body; (4) alkaline hydrolysis of labile ammonia-producing compounds.

In any blood specimen, even one in which the ammonium concentration is high, only microgram quantities are present. For this reason, any practicable method of analysis must be extremely sensitive, and therein lies its vulnerability to contamination. Infinitesimal amounts of ammonia in the laboratory air, from reagents, from urine specimens, or even tobacco smoke are potential contaminants. Furthermore, in any of the methods that depend upon the basicity of ammonia for measurement, errors may arise through trace quantities of soaps, detergents, or other nonvolatile alkalies in the glassware used for the tests.

Conway[2] on the basis of his experimental work stated that blood at the instant of withdrawal from the body contains no ammonium, but that ammonium is produced rapidly during the first five minutes and then more slowly for many hours afterward. In 1954, McDermott, Adams, and Riddell[5] called attention

to the early, rapid release of ammonium from blood of cirrhotic patients, and attributed this largely to the rapid breakdown of unstable compounds, particularly adenosine. In 1957, Seligson and Hirahara[3] presented convincing clinical evidence that ammonium is actually present in freshly drawn blood. Whatever the initial concentration, there is little doubt that blood ammonium concentration becomes greater on standing, though the rate of increase varies from sample to sample.

The fourth cause of error in measurement arises from the susceptibility of unidentified ammonia-producing compounds of blood to alkaline hydrolysis, as demonstrated by Seligson and Hirahara.[3] This can lead to erroneously high results in the determination of blood ammonium.

Attempts to measure blood ammonium by any method are somewhat arbitrary because some of the factors involved are not completely understood nor are all of them capable of being adequately controlled. However, analyses can be performed with reasonable accuracy if each of the potential sources of error is recognized and appropriately dealt with. The purpose of this report is to describe a simple and reliable modification of the Conway technic in which provision has been made to eliminate or to minimize each of the four potential sources of error. This laboratory has performed more than 1300 analyses during the two and one-half years we have used this method.

Materials

1. A microburet (capable of delivering fluid in increments of 0.0001 ml.).
2. Conway microdiffusion units *(Fig. 1).*
3. Ostwald-Folin pipets (0.5 ml.).

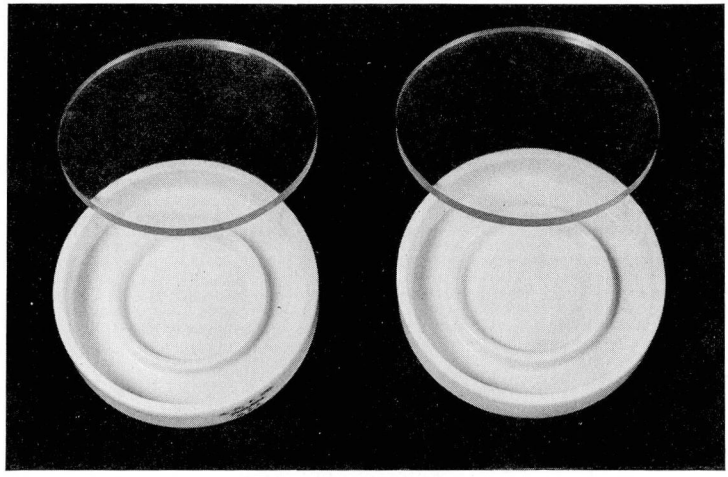

Fig. 1. Conway microdiffusion units.

Reagents

1. Hydrochloric or sulfuric acid (approximately 0.01 N, accurate standardization is unnecessary).

2. Boric acid stock solution (2 per cent).

Dissolve 10 g. of boric acid crystals in about 200 ml. of water with the aid of heat and agitation. Cool the solution to room temperature and dilute to 500 ml.

3. Bromcresol green indicator (0.1 per cent).

Place 0.1 gm. of bromcresol green crystals in 100 ml. of 95 per cent ethanol and stir until they are dissolved.

4. Boric acid-plus-indicator.

Add 12.5 ml. of the bromcresol green indicator to 100 ml. of the boric acid stock solution. Dilute to 400 ml. with water. Then adjust the solution to a yellow-green with the hydrochloric or sulfuric acid (reagent No. 1). Prepare new reagent every few weeks.

5. Buffered alkali.

Place 17 gm. of anhydrous potassium carbonate and 10 gm. of potassium bicarbonate in a beaker. Add 48 ml. of water. Stir and warm gently until all crystals have dissolved. Upon cooling, a small amount of crystalline material may precipitate which does not impair the value of the reagent.

6. Ammonium sulfate stock solution [0.300 mg., ammonium-nitrogen per milliliter (NH_4^+-N/ml.)].

Dissolve 0.1415 gm. of ammonium sulfate in a few milliliters of water. Transfer the solution quantitatively to a 100-ml. volumetric flask and dilute to the mark. This solution is stable indefinitely if stored in a refrigerator.

7. Ammonium working standard (300 μg. of NH_4^+-N/100 ml.)

Pipet 1 ml. of the ammonium sulfate stock solution into a 100-ml. volumetric flask and dilute to the mark. This standard may be kept at room temperature but should be made up every few weeks.

8. Ucon lubricant, 75-H-90,000.*

Procedure

1. Draw about 2 ml. of blood into a clean dry 5-ml. syringe containing 1 drop of liquid sodium heparinate.† Cap the syringe and mix the blood with the heparin. Leave the specimen in the syringe (at room temperature) until time for analysis.

2. Apply Ucon lubricant lightly to the rims of four Conway units.

3. Place 1 ml. of boric acid-plus-indicator in the central compartments of the units. If the acid changes color, remove the acid by aspiration and add a fresh portion. Repeat this procedure until no color change occurs.

* *Supplied through the courtesy of the Carbide and Carbon Chemicals Company, 30 East 42d Street, New York 17, New York.*

† *Organon, Inc.*

4. Pipet 0.5 ml. of buffered alkali into the outer compartments of the units. Keep this reagent confined as well as possible to a small portion of the circumference. Avoid contamination of the central compartments with the alkali.

5. Twenty minutes after collection of the blood specimen, mix it gently in the syringe and dispense it into a 5-ml. beaker. With an Ostwald-Folin pipet, measure 0.5 ml. of blood into the outer compartment but on the side opposite to that of the alkali. Perform the analysis in duplicate.

6. Remove the boric acid completely by aspiration and replace it with 1 ml. of a fresh sample of boric acid-plus-indicator.

7. Place the lid on the unit and thoroughly mix the blood with the alkali by gentle manual rotation of the unit. Start an interval timer and allow diffusion to proceed for exactly 20 minutes.

8. Run ammonium standards in duplicate as described for blood. Use 0.5 ml. of the ammonium working standard. Diffuse exactly 20 minutes.

9. At the end of the diffusion period, remove the lid and, using standard acid, titrate the boric acid back to the original color. For color comparison use 1 ml. of a fresh portion of boric acid-plus-indicator in a well-rinsed unit.

10. Calculate the quantity of ammonium-nitrogen in the unknown sample by means of the equation:

$$\frac{\text{Milliliter of acid for unknown}}{\text{Milliliter of acid for standard}} \times 300 = \text{Micrograms of } NH_4^+\text{-N per 100 ml.}$$

Comment. Great sensitivity to free ammonia is an essential characteristic of any technic for the accurate measurement of blood ammonium. In this respect the modification described here is similar to most others. However, the concentration of boric acid solution, the strength of the indicator, the absolute and relative volumes of blood and alkali have all been carefully adjusted in our procedure so that optimum sensitivity and accuracy of measurement may be attained. A microburet capable of delivering titrating fluid in increments of 0.0001 ml. per scale division is an essential feature of this modification and constitutes an important factor for enhancing the accuracy of analysis. Less precise instruments should not be used.

To avoid errors from contamination of the specimen and the glassware, several precautions are taken. The blood specimen is left in the syringe until time for diffusion and is then dispensed into a thoroughly clean beaker. Boric acid is dispensed from a buret and therefore is not exposed to several, possibly contaminated pipets during the course of analysis. Finally, the Conway unit is rinsed several times with boric acid-plus-indicator in order to neutralize any trace of nonvolatile alkali in the unit before the test is started.

Evaluation of the Method

The observation of Conway[2] that the ammonium content of blood increases with the passage of time, after venipuncture, has been confirmed by others.[4,6]

However, there is no general agreement regarding the extent or the rate at which this increase takes place. Such information is of importance in establishing the greatest amount of time that could be allowed to elapse before starting the test without incurring the risk of introducing intolerable errors. To evaluate this time factor, experiments were carried out on a number of random blood samples from normal persons. Blood was drawn into heparinized syringes and remained in the syringes for the lengths of time shown in *Figure 2.*

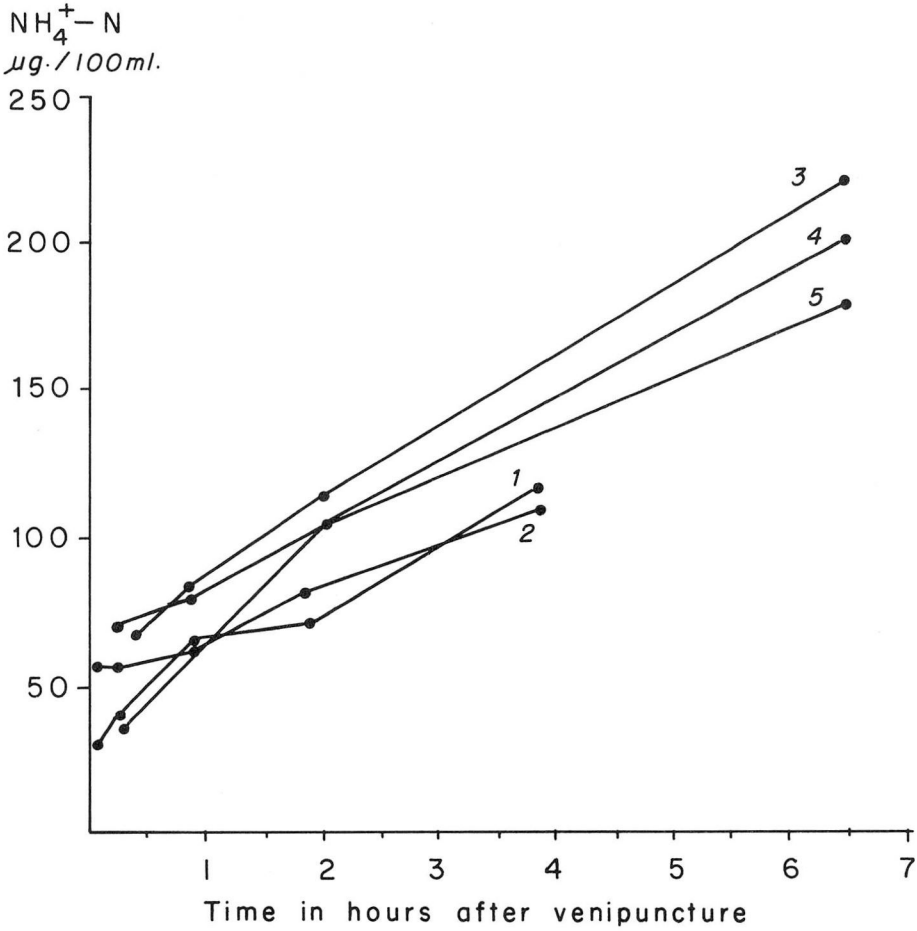

Fig. 2. Graph showing spontaneous rise in ammonium concentration in random samples of blood from five normal persons.

It is evident that the ammonium increased in all samples tested, and that the rate was different in each case. From these observations it might be concluded

that this source of error could be eliminated by starting the test immediately after venipuncture. Since this is not feasible, 20 minutes was arbitrarily selected as a suitable period. In this interval, the rise of ammonium is probably small. Twenty minutes does permit time to bring the specimen back to the laboratory and to prepare for the analysis.

Effect of alkalies on ammonia evolution. Seligson and Hirahara[3] reported that exposing blood to dry crystals of potassium carbonate not only caused the preformed ammonia to be liberated from ammonium compounds but also the hydrolysis of labile substances to yield additional ammonia. Presumably the hydrolysis was caused by an excessively high pH. They further reported that hydrolysis could be virtually eliminated by substituting a mixture of potassium carbonate and potassium bicarbonate crystals for potassium carbonate alone. This principle was applied to the Conway technic by employing as the alkalizing agent a solution of potassium carbonate and potassium bicarbonate saturated with respect to both chemicals.

Table 1 shows the pH values of various solutions and mixtures. These measurements were made with a Cambridge Micro condenser type glass electrode in conjunction with a Cambridge Research model pH meter.*

Table 1.—*The pH values of alkalies and blood-alkali mixtures*

Material	pH
Saturated potassium carbonate	13.1
Buffered alkali	10.5
Saturated potassium carbonate plus whole blood	11.5
Buffered alkali plus whole blood	9 9

Figure 3 compares the effects of the two alkalizing agents on blood from normal persons. The diffusion times were 10, 20, 40, and 60 minutes. Standards were run concurrently with each set of analyses. They were diffused for the same lengths of time. All analyses were performed at room temperature. Values of ammonium were calculated in each instance by reference to a standard exposed to the same alkalizing agent for the same length of time as the sample. The use of buffered alkali resulted in fairly consistent values of ammonium regardless of the diffusion period up to one hour. But when saturated carbonate was employed, continuously rising concentrations were obtained. These observations may be interpreted as indicating the hydrolysis of compounds in the sample to produce additional ammonium.

* *Cambridge Instrument Co.*

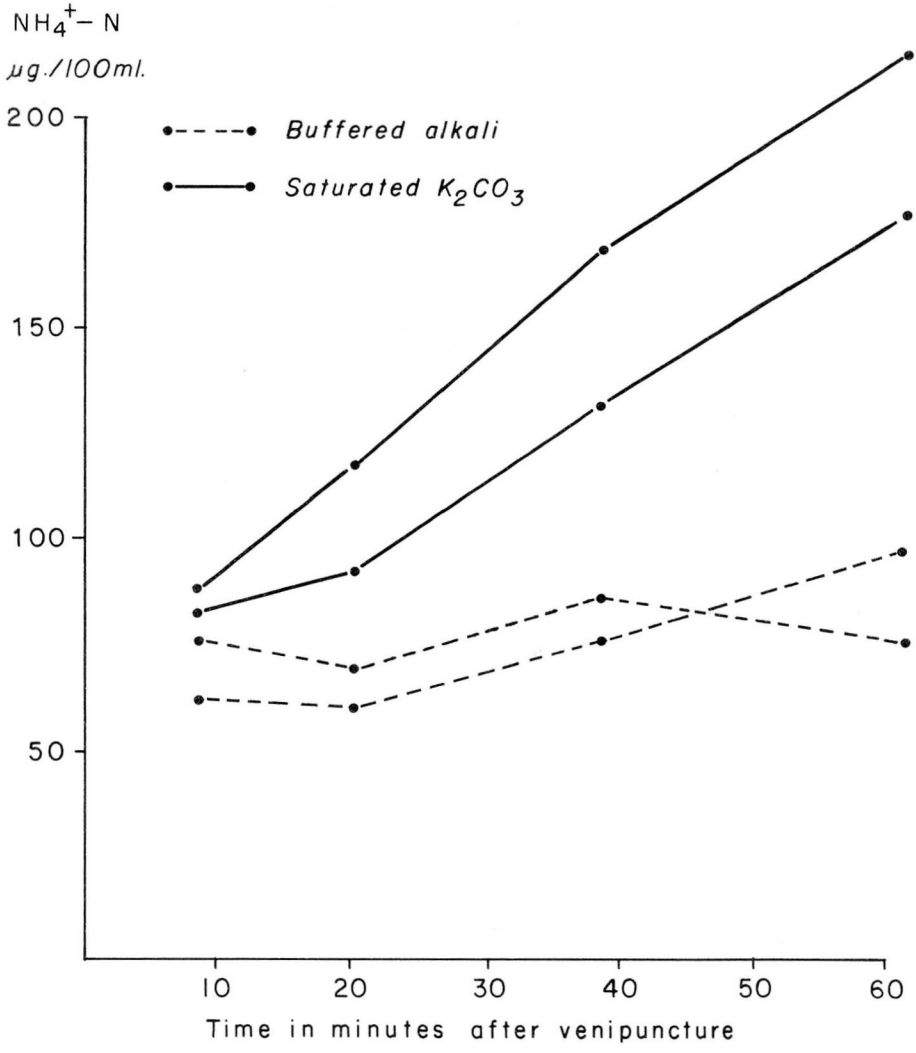

Fig. 3. Graph showing effect of alkalizing agents on ammonia evolution in two samples of normal blood.

Table 2 presents supplementary data that reinforce the conclusion implicit in *Figure 2,* namely that potassium carbonate causes the production of "extra" ammonium. The additional ammonium observed in some samples after 60 minutes' exposure to buffered alkali is attributed to the spontaneous production of ammonia that was occurring regardless of the presence or absence of any type of alkali.

Table 2.—*Effect of buffered alkali and saturated carbonate on blood ammonium in eight normal persons*

Sample number	NH_4^+-N in blood samples, μg./100 ml.					
	Tested with buffered alkali			Tested with saturated carbonate		
	20 min.	60 min.	Increase	20 min.	60 min.	Increase
1	69	67	—	115	203	88
2	177	174	—	174	322	148
3	58	41	—	97	152	55
4	63	64	1	98	168	70
5	77	91	14	95	179	84
6	116	143	27	171	214	43
7	53	84	31	80	160	80
8	93	144	51	170	218	48

Precision of the method. To test the reproducibility of the method, an ammonium sulfate solution containing 200 μg. of ammonium per 100 ml. was analyzed on different days over a period of one and one-half months. *Table 3* presents the data obtained.

The mean value for the 20 analyses was 206 μg. per 100 ml. with a standard deviation of 13.5 and a mean deviation of 6.0.

Recovery studies. An experiment to appraise the attainable accuracy of the method was performed. Ammonium sulfate was added in various quantities to three different blood samples as indicated in *Table 4.*

Normal blood ammonium values. The blood ammonium concentrations from 18 apparently healthy adult human beings were determined with this method. The range was from 36 to 75 μg. with a mean of 55 μg. per 100 ml. The standard deviation was 10. *Table 5* gives the values for each person in the series.

In contrast to values in normal individuals are those observed in patients in hepatic coma or in stupor as shown in *Table 6.*

Clinical Applications of the Method

In the past decade there has been great interest in the importance of ammonium intoxication in patients with hepatic coma. The ingestion of ammonium chloride is known to produce stupor and coma in cirrhotic patients,[7] and elevated blood ammonium concentrations in patients with gastrointestinal bleeding is almost pathognomonic of cirrhosis.[8]

Chalmers,[9,10] in an excellent review of the problem, points out that hepatic coma is a complicated derangement of metabolism in which prolonged elevations

Table 3.—*Values obtained on ammonium sulfate samples containing 200 μg. of NH_4^+-N per 100 ml.*

Sample	NH_4^+-N, μg./100 ml.
1	226
2	191
3	228
4	197
5	185
6	208
7	208
8	194
9	194
10	218
11	229
12	206
13	205
14	224
15	207
16	192
17	200
18	212
19	189
20	212

Table 4.—*Recoveries of ammonium added in the form of ammonium sulfate to three samples of blood*

Blood sample	NH_4^+-N, μg./100 ml.			Percentage of recovery of calculated total NH_4^+-N, per cent
	Initially	Added	Finally	
1	113	52	170	103
	113	80	202	105
	113	87	203	108
2	56	90	154	105
3	86	90	185	105

Table 5.—*Blood ammonium values from 18 normal persons*

Sample	NH_4^+-N, μg./100 ml.
1	45
2	45
3	64
4	36
5	54
6	54
7	45
8	68
9	56
10	66
11	52
12	75
13	50
14	49
15	69
16	53
17	48
18	74
Mean	55
S.D.	10

Table 6.—*Blood ammonium concentrations in three patients in coma and two in stupor*

Patient	Clinical condition	NH_4^+-N, μg./100 ml.
1	Coma	600
2	Coma	425
3	Coma	225
4	Stupor	294
5	Stupor	234

of blood ammonium may cause central nervous system symptoms, but frequently may be only one of many biochemical factors responsible for symptoms.

A reliably accurate determination of blood ammonium concentration is of great clinical value in the differential diagnosis of stupor and coma,[11] particularly in cases of unsuspected cirrhosis. In the management of patients in coma the blood ammonium concentration is a useful guide in treatment with intravenous arginine when hyperammonemia is present. As shown in *Figure 4,* adequate

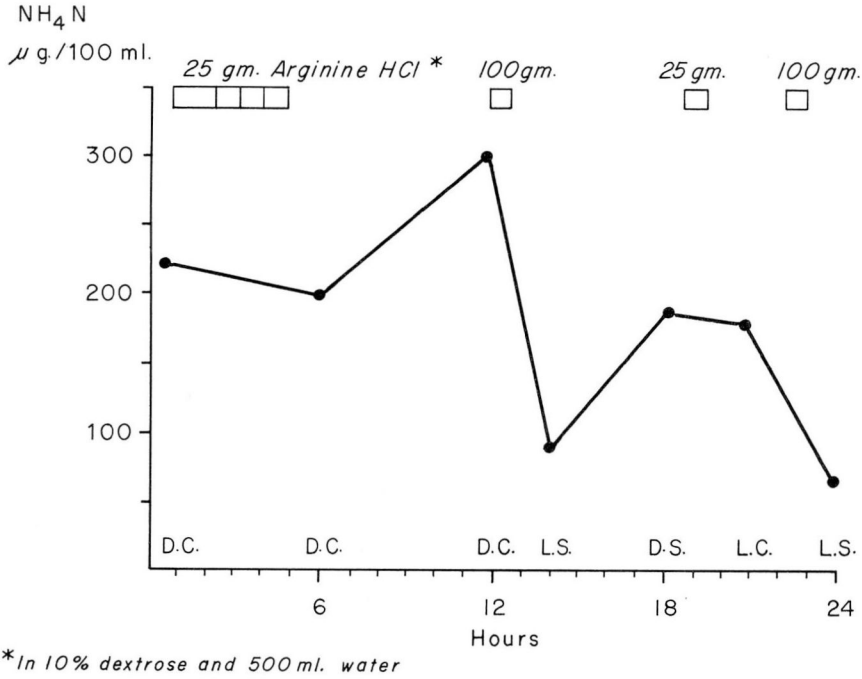

Fig. 4. Graph showing the effect of dosages of arginine intravenously injected, and infusion times on blood ammonium concentration and central nervous system symptoms in a patient with hepatic cirrhosis and gastrointestinal bleeding. Abbreviations are: L.S., light stupor (easily aroused); D.S., deep stupor (confused, disoriented); L.C., light coma (responsive to painful stimuli); D.C., deep coma (unresponsive to painful stimuli).

amounts of arginine resulted in significant lowering of ammonium concentrations in the blood. Although this response is not always followed by a rapid change in depth of coma, all measures are of value which will help to restore consciousness and an effective cough.

Serial blood assays after ingestion of 1.0 gm. of ammonium chloride have been of value in determining the patency of portacaval shunts *(Fig. 5).*

Summary

A modification of the Conway technic for the determination of blood ammonium with a high degree of accuracy and reproducibility is presented. In this

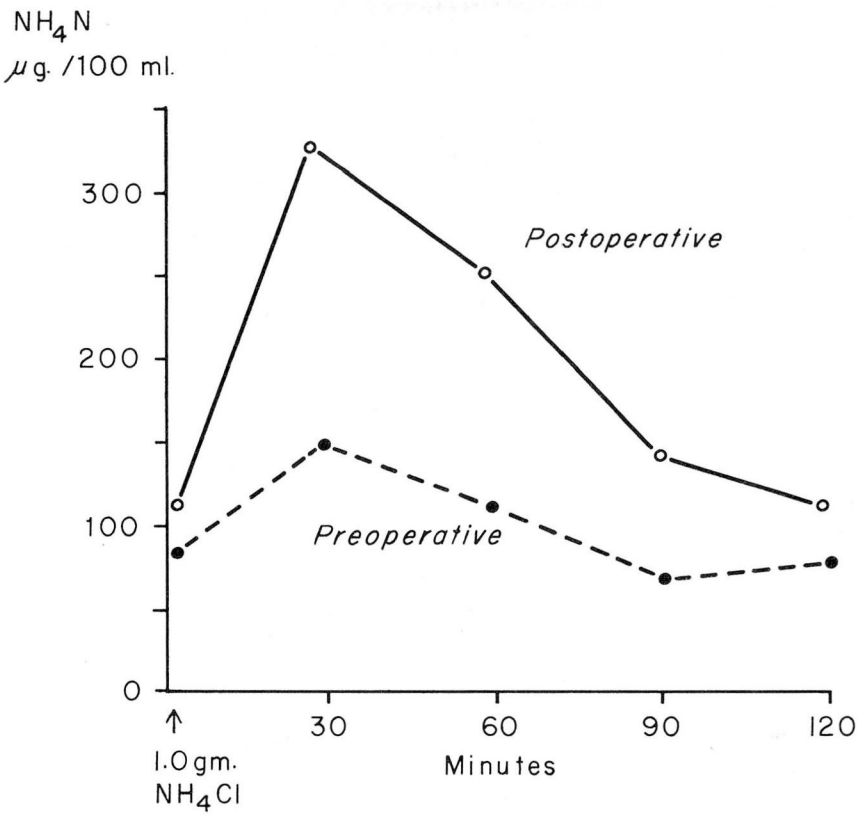

Fig. 5. Graph showing blood ammonium levels in one patient after ingestion of 1.0 gm. of NH_4Cl before and after portacaval shunt.

method the four chief sources of error have been eliminated or their effects have been minimized. These errors arise from such factors as the infinitesimally small quantities of ammonium present in blood, the spontaneous increase in concentration that occurs after venipuncture, the possibility of contamination during performance of the test and, finally, the danger of hydrolysis of labile ammonium-producing compounds in the blood by the use of excessively strong alkalizing agents.

The inherent difficulty in measuring the extremely small quantities of ammonium present in blood is lessened by carefully adjusting the concentrations and the relative quantities of reagents and by using a precise microburet for the titration. Errors arising through the spontaneous production of ammonia, although not susceptible to control, can be minimized by starting the test at a short but fixed time after venipuncture. Contamination of the specimen or of the glassware is virtually eliminated by keeping the sample in the syringe until time for analysis,

and by rinsing the unit thoroughly with absorbing fluid before starting the test. Alkaline hydrolysis of labile ammonium-producing compounds in the blood is largely prevented by using a buffered alkalizing agent.

The measurement of blood ammonium is of clinical significance in the differential diagnosis of unsuspected cirrhosis with central nervous system symptoms, in the evaluation of treatment of patients with arginine glutamate, in the determination of the patency of portacaval shunts, and in specialized studies involving the measurement of blood distribution.

Acknowledgment

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