Closed-System Apparatus for Aeration-Diffusion: Application to Determination of Blood Ammonia

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An apparatus is proposed primarily for comparison with other methods for determination of "blood ammonia," and studies of involved factors. It employs the aeration-diffusion method of Van Slyke and Cullen, but in a closed system which avoids loss of ammonia and contamination with ammonia from the outside. Incomplete liberation of ammonia is avoided by the use of a moving thin film ever renewed in the donor section, and incomplete absorption of ammonia in the receiver is avoided by recirculating the air. Because the aeration may be done at any reduction in pressure, the apparatus lends itself to use in studies of the dynamics of aeration-diffusion of ammonia and other gases. A curve is presented which reconfirms the observation that ammonia liberated from blood after addition of reagents increases after 15 min. To avoid such increase, the time between blood collection and analysis is reduced to less than 2 min. A tentative upper limit of normal is set at 0.14 mg./100 ml.

Determination of "blood ammonia" often employ either the Conway or the Seligson diffusion cells for liberation of "free" ammonia from blood. Using a modification of Seligson’s apparatus, Carl Smith, at this hospital, has obtained some recoveries of ammonia from ammonium sulfate of over 90% in 3 min. and of about 98% in 5 min. Such a rapid rate of diffusion tends to confirm the suspicion that a trace of ammonia (which may constitute a large percentage of the trace present in even pathological blood) may be lost, even though the cell is open only momentarily for addition of the alkaline reagent. Smith used a modification of Seligson’s apparatus in which the test solution and the saturated potassium carbonate were separated by a well until after the stopper bearing the receiver had been tightly inserted. He also employed, as the receiver, 2 or 3 parallel rods in close contact, which held the drop of sulfuric acid solution by capillary action, from which the ammonia was more readily recovered than from a ground glass-tipped rod.

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Unsuccessful attempts to eliminate the drawback of the Conway cell (the need to open it momentarily during addition of the alkaline reagent) included modifications in which, with the cell positioned at a slant, the blood and the alkaline reagent were separated by a crescent-shaped wall, or horizontally by a transverse barrier, and then mixed after the cover was put in place. The ammonia diffused into a disk of minute glass beads one layer thick, fused into the cover. Apparently the ammonia could not be completely washed out of the sintered disk. There was one objection to Smith's modification of Seligson's cell in routine clinical use: the technologist might inadvertently touch the side of the cell in withdrawing the receiver. In both methods, the sample is so small and the amount of ammonia so minute that a trace of ammonia lost, or gained by contamination, constitutes a larger percentage error.

The apparatus presented was designed to preserve the specificity attained by a combination of diffusion with the use of Nessler's reagent, and by examining larger samples, to minimize the error caused by visually undetectable turbidity after Nesslerization. A further intent was to avoid the errors due to lapse of time during which more than the original amount of ammonia is said to appear in the blood before the alkaline reagent is added, and an additional amount produced by "decomposition" after its addition.\(^*\)

The form of apparatus most used at this hospital is shown in Fig. 1. It is carried to the bedside. Only 1.5-2.0 min. elapse from the time the syringe is filled until 5 of the 6 ml. of blood drawn enters the apparatus with the 15 ml. of saturated potassium carbonate solution, and the aeration-diffusion process is started. Prior to using this apparatus, we had never been able to get the specimen to the laboratory in less than 3 min., and in most cases it took over 10 min.

**Apparatus**

The donor chamber \((D, \text{Fig. 1})\) is a flat spiral of one 4-ft. length of "\(\frac{5}{8}\)"-in.\) heavy-walled borosilicate glass tubing whose inside diameter is about 15 mm. The heavy wall makes it easier to bend a smoothly curved spiral and gives it added strength. Upright tubes about 50 mm. long are situated at right angles to this spiral and about 60 mm. apart at the ends. The openings in the upright tubes are straight (fire-polished), because it is difficult to tool a perfect gas-tight taper. The rubber stoppers, of 13-mm. diameter at the bottom, fit tightly in the slightly smaller inside diameter of the glass tubes. Because rubber absorbs ammonia, the area of

\(^*\text{Work published since this report was submitted may have a bearing on the increase of ammonia in the sample both before and during analysis (1).}\)
rubber exposed inside the apparatus is kept to a minimum, and polyethylene tubing (inside diameter about 3 mm.) is used to conduct the air carrying the ammonia to the receiver (R in Fig. 1).

The receiver (upper part of Fig. 2) is constructed entirely of 14-mm. tubing. Before it is assembled, the body of the tube is ground on the inside by rotating and sliding an 11-mm. tube against the inside surface with fine carborundum between them. One end is closed, the short upright tubes are attached about 45 mm. apart on centers, and then the other end is closed. (Unavoidably, small areas of ground surface are lost by being re-fused in assembling the receiver.)

The cup (C in Fig. 1), with a stopcock below, is used in evacuating the apparatus and then in admitting the blood sample and the reagent. A bore of 2 mm. in the stopcock is satisfactory.

Method

Although the method used is subject to choice, it is here given as used at this hospital.

Cleaning the Apparatus

It was assumed that the detergency of the potassium carbonate would be sufficient to remove blood constituents. Tap water is flushed through the donor until all visible color is removed. If water fails to drain from the apparatus in an unbroken film, the donor is filled with sodium dichromate-sulfuric acid and left overnight, but ordinarily, acid cleaning solutions are avoided because they might leave a trace of ammonium salts which would contaminate the next analysis. Because tap water may contain ammonia or amines from its original source or added during chlori-
nation, the apparatus, including the receiver, is rinsed 6 times with ordi-
nary distilled water; because ordinary distilled water often contains
substantial traces of ammonia, the entire apparatus is rinsed 4 times
with water from a special still. This still* produced water of 1.5–2.0
megohms resistance, with no ammonia detectable on testing with Nessler’s reagent. After cleaning, the receiver is attached to prevent con-
tamination from the air, and the apparatus set aside until used. Im-
mediately before use, the apparatus is again flushed at least twice with
the special distilled water, and drained for about 2 min. in an ammonia-
free atmosphere.

A 10-ml syringe is rinsed at least 4 times with sterile special distilled
water, and partially dried by pumping in ammonia-free air.

Procedure

After the last flushing, 0.20 ml. of 2 N sulfuric acid is introduced into
the moist receiver, which is then connected with the donor. The system
is evacuated through the cup (C in Fig. 1) by a mechanical pump capable
of reducing the pressure to 3 or 4 mm., or by a water-ejection pump. The
apparatus is then carried to the bedside.

About 6 ml. of blood is collected in a syringe (prepared as stated under
Cleaning of Apparatus) with as little tension as possible applied to the
plunger. These precautions are often overlooked unless the blood is
taken by someone familiar with the technic. A 5-ml. sample is delivered
into the cup. It is immediately drawn into the apparatus until the blood
enters the top of the capillary tube above the stopcock. Then 15 ml. of
saturated potassium carbonate solution are delivered into the cup from
a serologic pipet with a large opening in the tip. It is admitted as far as
the top of the capillary if the aeration is to be done under reduced pres-
sure, or air is admitted before the stopcock is closed if atmospheric
pressure is to be used. In view of the increase of ammonia commonly as-
sumed to occur in blood on standing, speed in handling is much more im-
portant than overconcern about small volumetric errors. The entire
operation, from removal of the needle from the vein to delivery of the
potassium carbonate into the apparatus, should require no more than 90
sec. An assistant should be present to take care of the patient so that the
analyst can give immediate full attention to the analysis.

The analysis is started immediately, at the bedside. The apparatus is
tipped at an angle of about 45° so that the blood and reagent collect in

*Description of the still may be found in References 2–4. Probably adequately ammonia-free
water can be produced from dilute aqueous solution of potassium permanganate and sodium
hydroxide in an ordinary borosilicate glass still, by discarding the first third distilled over
through a hot condenser, and saving the second third recovered through the same condenser when
cooled.
the bottom of a loop of the donor chamber and act as a piston to drive air through the receiver as the flat spiral, which remains inclined at a 45° angle throughout, is rotated clockwise and counterclockwise. The direction of rotation is reversed when the blood reaches the angle of the upright tube; it should not enter the tube. The aeration is continued for 11 min. at the bedside, or preferably, during transport to the laboratory.

The blood and reagent leave a thin film on the walls of the donor chamber from which ammonia diffuses rapidly into the air and is carried to the receiver. The rotation is carried on as rapidly as is possible without causing bubbles to pass through the blood; this might cause films of alkaline blood to pass up the upright tubes into the receiver, and might not drive enough air through the receiver. As soon as possible after the aeration period ends, the receiver is disconnected, leaving the polyethylene tubes attached to it to prevent inward diffusion of any ammonia from the outside air.

In the laboratory, 5.00 ml. of an ammonium sulfate standard sufficient to contain 0.025 mg. of ammonia (equivalent to 0.50 mg./100 ml. of blood) is pipetted into a cuvet, followed by 0.20 ml. of 2 N sulfuric acid, to bring the final alkalinity as near as possible to that of the unknown. Then 0.40 ml. of Nessler's reagent are added, and the contents mixed by swirling. Allowing 0.20 ml. for moisture left in the receiver by rinsing before the analysis, 4.80 ml. of water and 0.40 ml. of Nessler's reagent are added directly into the receiver. The receiver is rocked to-and-fro several times, and the contents poured into a cuvet. At 3 min. after adding the Nessler's reagent to each, the standard and the unknown are read in a photoelectric colorimeter.

**Earlier Models**

In an earlier attempt, a model was designed which could be given an oscillatory-rotatory motion in a fixed inclined plane: rotated clockwise to almost a complete circle, then similarly counterclockwise. A simple but bulky mechanical device could be set up to impart this motion, mounted on a table with wheels so that it could be moved to the bedside. However, the blood-reagent mixture barely filled the cross-section of the longer arc which it occupied and often permitted air to pass through the liquid; the objections were evident.

A device was designed to permit continuous rotation in one direction on an inclined turntable. A trap below the upright tubes held back a small part of the blood-reagent mixture, thus diverting the gases through the receiver while the rest of the liquid acted as a piston to drive the gases along. Thus all of the gas passed through the receiver without
returning and mixing with gas from the subsequent cycle. Because of
the continuous one-directional flow of gases, the circle of the donor cham-
ber could be made rather small, about 6 in. in diameter. However, the
apparatus had a marked tendency to form foam in the trap.
At least a score of other devices were tested and found to be entirely
inadequate.
In one, by raising and lowering a mercury leveling bulb while turning
a 3-way stopcock, the air was forced in one direction through a donor
chamber and receiver, accompanied by reduction of pressure through
half the cycle. To pump through an estimated ½ as much air as used in
Van Slyke's aeration method required 45 min., which raised the question
of decomposition of blood.
An attempt to adapt a method to the Van Slyke manometric blood gas
apparatus was unsuccessful.
Many modifications of U-tubes connected with receivers, (as in Fig. 1
or otherwise) were tested, with or without beads in the donor chambers.
Only half of the air passed through the receiver when the apparatus was
rocked in either direction. In addition, no matter how slowly the liquid
was made to displace air in the beads, foaming always resulted.
Five or six models were tested in which air was pumped between donor
and receiver by mechanically induced vortex stirring. They included
vortices which acted like the rotor in a centrifugal pump, placed in either
the receiver or donor or both, or vortices which entrained air from the
donor and forced it through a tube to the receiver whence it returned to
the donor. The rate of movement of air in this type of apparatus could
not be estimated. When strong hydrochloric acid was placed in the donor
and silver nitrate in the receiver, some models showed a precipitate in
the silver nitrate in a few seconds, but these same models failed to show
yellow color in over 30 min. when ammonia equivalent to that in patho-
logic blood was placed in the donor and dilute Nessler's reagent in the
receiver.
These failures are cited so that their repetition may be avoided.

Discussion

There is legitimate suspicion that ammonia may be lost if the blood is
collected in an evacuated tube, especially if a small specimen is collected
in a large tube. Also, more time elapses than when the blood is trans-
ferred directly from a syringe into the apparatus.
Syringes sterilized and dried in the autoclave should be rinsed several
times with special distilled water and partially dried by pumping in am-
monia-free air, because boiler steam often contains volatile amines in-
troduced into the boiler water to prevent corrosion. Housekeeping departments in hospitals are not informed of the danger of contamination in analyses by ammonia in the air. Ammonia or ammonia-bearing detergents must not be used for cleaning windows, floors, etc. The laboratory should test all cleaning solutions.

Swirling is recommended for the mixing of the standard with Nessler’s reagent. Inverting against the thumb, washed and rinsed with special distilled water, did not increase the values obtained, but there seems to be a remote possibility of contamination. For reasons unknown, use of a vortex mixer apparently results in values that are too high.

A standard in the high pathologic range of 0.50 mg./100 ml. of blood brings the reading into a more accurate range of the electric colorimeter. 

As 3 min. may be too short a time for maximum development of color in Nesslerization, the temperature and timing must be exactly the same for standard and unknown. Invisible turbidity, too faint to be seen even by the Tindell effect, was feared if longer time were to be allowed.

Nessler’s reagent was added directly into the receiver because apparently the products of the reaction are more completely removed from the ground glass than ammonium sulfate is by water alone.

Aeration as a method for transferring ammonia from donor to receiver was borrowed from Marshall’s and Van Slyke and Cullen’s methods for urea by liberation of ammonia. The closed system prevents loss of or contamination by ammonia during the aeration. Less than ½ as much air is required to pass through the apparatus (estimated) as was specified by Van Slyke for the open-ended bubbling aeration. This may be partly explained by the formation of thin films in the donor.

There appears to be some “magic” in diffusion of ammonia from a thin film. In his modification of Seligson’s cell, Smith recovered 97-99% of the ammonia from ammonium sulfate in 5 min. On the other hand, in the Conway cell about an hour is required for diffusion from a relatively thick film. In the apparatus shown in Fig. 1, as high a yield was obtained in 5 min. as in 15 min. (Fig. 3), while in the apparatus in which a vortex with extensive surface and vigorous agitation was generated, the transfer to the receiver was negligible or nil. A possible explanation is that in a thick film more gas diffuses to-and-fro within the liquid, mostly parallel to the film and in a very narrow space. Consequently a larger proportion must diffuse across the interface into the space above the thin film.

In models rejected because of foaming, the foam might have been suppressed by an antifoaming compound such as caprylic alcohol. While such antifoaming compounds do not dissolve enough ammonia to make an appreciable error in urea determinations, it was feared that they
might make a relatively large error in the trace detected as "free" ammonia. This factor is still open to investigation.

Figure 3 confirms the well known phenomenon of increase in the yield of ammonia sometime after 15 min., thought to be due to decomposition of some constituent of blood by the alkaline reagent. No significance is attached to the initial drop from 0.13 to 0.11 mg/100 ml.; it is well within the estimated error of the method.

We consider the present trend toward reporting "blood ammonia" in micrograms per 100 ml. to be unrealistic to the point of absurdity. It implies an accuracy far beyond the limits of present methods under all but ideal conditions. At this hospital, we estimated consistency at plus or minus 0.02 mg./100 ml. but questioned accuracy in the second decimal place.

We have tentatively set 0.14 mg./100 ml. of blood as the upper limit of the normal range, on the basis of numerous determinations on our own blood; the values decreased with improvement of technic until 0.11–0.14 mg./100 ml. was consistently obtained. This is higher than the value set by a laboratory in a neighboring hospital, which considered 0.075 as a normal value, using the Conway cell for diffusion.

In the few cases compared, our laboratory and the neighboring one were in substantial agreement except that in 1 case, we obtained 0.24 mg./100 ml. and they obtained 75 μg./100 ml.; these were blood samples from the same patient taken 2 or 3 days apart. The blood sample which we analyzed was taken by the described method. The blood sent to them was collected by an unknown member of our medical staff; in all likelihood, it was drawn into a Vacutainer* tube. We can only speculate as to which of a number of undetermined factors was responsible for this discrepancy. It is even possible that the patient returned to normal in the interim. The results from the other laboratory were accepted and our method was discredited.

That such an incident could occur is one of many reasons for presenting the present method and apparatus as a different approach for the

*Beckton, Dickinson and Co., Rutherford, N. J.
study of factors involved in the determination of blood ammonia, with the hope of discovering errors and sources of errors in the various methods and establishing accurate standards of normal and abnormal values.

References