ANALYSIS OF GASES IN BLOOD WITH THE MASS SPECTROMETER. IV. TOTAL EXTRACTION OF GASES FOR DETERMINATION OF CARBON DIOXIDE AND OXYGEN IN BLOOD *†

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IN previous papers (1, 2) dealing with the technic for the determination of the concentration of ether and nitrous oxide in blood the process was divided into two basic steps. The first step was the preparation of a sample of gases from the blood in which the gases to be analyzed were in known proportion to their original concentrations in blood. The second step was the actual analysis of the sample of gases so obtained on the mass spectrometer. In the case of nitrous oxide or ether as considered in the previous papers the portion of gas released by equilibration with a sample of blood containing that gas was determined solely by the solubility of the gas in the blood at the temperature of equilibration. This is the application of Henry's law. The same procedure may be applied for the determination of any gas whose concentration in blood is strictly in accordance with Henry's law.

This reasoning fails, however, when it is applied to the consideration of gases entering into some chemical combination with elements of the blood as in the case of carbon dioxide and oxygen. The proportion of these gases in a supernatant sample of gases following equilibration with blood depends on a variable function commonly portrayed graphically as a dissociation curve. Thus the division of oxygen (or carbon dioxide) between the blood and the gas phase when in equilibrium at a constant temperature is in accordance with a ratio which varies with the pressure of the gas in question. It is apparent, therefore, that the technics applied to analysis of blood for ether and nitrous oxide are not feasible for analysis of carbon dioxide, oxygen, or any gas entering into chemical combination with some element of the blood.

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A search was made, therefore, for a method to obtain a sample of all the gases in blood. The basis for such a technic was found in the work of W. C. White who has made application of mass spectrometric technics in another field of blood chemistry (3). Essentially this method requires the elimination of any chemical bond existing between blood and the gas to be analyzed followed by the total evaporation of the blood into a previously evacuated system and the absorption of the water vapor released in the process. Assuming that the constituents of the gas sample obtained in this manner can be determined with some accuracy, it is apparent that analysis of the concentration of the gases originally in the blood may be made without resorting to equilibration and without prior knowledge of solubility or distribution ratio.

EXTRACTION OF GASES FROM BLOOD

The process of total extraction of gases from the blood by desiccation was carried out in the apparatus illustrated in figure 1. Essentially it consists of a vacuum system including a chamber for the admission of a sample of blood and a Toepler pump for mixing the gases and for moving them through a drying chamber filled with calcium chloride. Provision is made for the addition of a measured quantity of argon to the total gas sample after it has been dried and before a portion of it is admitted to a bulb suitable for attachment to the manifold of the mass spectrometer.

As in the Van Slyke method for determination of oxygen, potassium ferricyanide was used to release the oxygen from hemoglobin. No reagent was required to release carbon dioxide because of the increasing acidity of hemoglobin as gases are released during the process of extraction. Before potassium ferricyanide was introduced into inlet *A*, the three-way stopcock, *a*, was placed in an off position. After the reagent was introduced, the stopcock was adjusted to connect the lateral arm of chamber *A* with the vacuum in the system. Thus the reagent was rendered air-free without admitting it to the expansion chamber and the admission chamber was sealed by appropriate rotation of the stopcock. The blood inlet chamber *A* is sealed by a rubber stopper protected against gas leakage by a mercury cup. When a sample of blood was introduced through a needle inserted into chamber *A* while it was in a high state of vacuum, an inconstant amount of blood and gas in the blood was drawn from the bore of the needle and the unemptied portion of the syringe by the vacuum. To eliminate this error, the small (2 to 3 ml.) inlet chamber *A* was filled with an inert gas (helium) to approximately atmospheric pressure and the remainder of the system evacuated of all gas. After the introduction of the helium the measured amount of blood was injected through the rubber diaphragm into the inlet chamber *A* where it mixed with potassium ferricyanide. Stopcock *a* was turned to connect chamber *A* with globular expansion

chamber *B* which provided a means for more rapid extraction of gases from the blood by exposing a larger surface. Stopcock *b* was rotated to a position connecting tonometer *D* and those parts of the system designated *A*, *B* and *C*. The tonometer *D* is a large glass sphere (capacity 200 ml.) connected by way of a glass tube 75 cm. in length to a sealed mercury reservoir of 300 ml. capacity. The supernatant air in the reservoir can be connected alternately to the outside atmosphere and to a source of vacuum. Thus, the mercury is allowed to rise into

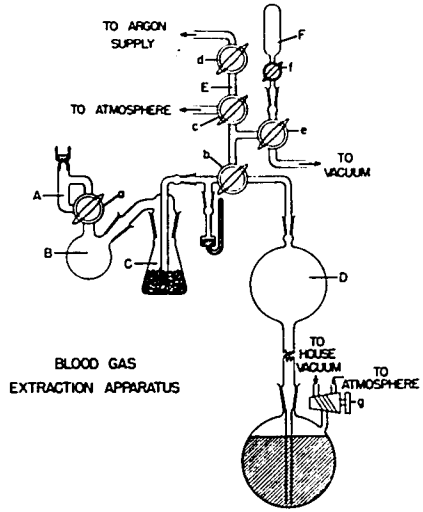

FIG. 1. Gas extraction apparatus. A complete description of this apparatus is given in the text.

Chamber *A* admits the blood and ferricyanide in helium at atmospheric pressure; it is surmounted by a rubber cap filled with mercury through which the admitting needle is inserted. *B*. The desiccation chamber which may be warmed gently. *C*. Calcium chloride for drying the gas sample. *D*. 200 ml. tonometer for mixing gas sample. *E*. Calibrated argon chamber. *F*. Sample bulb.

the evacuated portion of tonometer *D* when stopcock *g* is turned to atmosphere and the mercury will fall from tonometer *D* when stopcock *g* is turned to vacuum. By turning stopcock *g* alternately to atmosphere and to vacuum, the gases released from the blood may be mixed to and fro in the entire extraction system through calcium chloride in chamber *C*. The gases were quickly released and the blood was dried in a few minutes while the entire gas contents were moved to and fro. Chamber *B* may be warmed gently in a water bath to hasten desiccation.

After the blood was completely dried and the systems containing chambers *E* and *F* were evacuated, argon was admitted to chamber *E* and retained there by stopcocks *c* and *d*. Care was taken to insure that the argon was at room temperature and atmospheric pressure. Chamber *E* had previously been calibrated and had an accurately known volume. With stopcock *c* in an off position, chamber *E* was connected with the remainder of the gas system *A*, *B*, *C*, and *D*, through stopcock *b*. The argon was mixed with the total blood gas sample by using the mercury pump and tonometer *D*. When mixing was complete, a sample of the mixture was admitted to sample bulb *F* through stopcock *e*. The sample bulb *F* was then closed at stopcock *f* and the sample was ready for transfer to the manifold of the mass spectrometer.

THE QUANTITATIVE DETERMINATION OF CARBON DIOXIDE AND OXYGEN IN A GAS MIXTURE

Inasmuch as the concentrations of carbon dioxide and oxygen in blood are commonly reported in terms of volume per 100 ml. rather than milligrams per 100 ml. as is the case with nitrous oxide and ether, the equations for calculation of these concentrations are presented in terms of volume rather than mass. The complete extraction of these gases from a known quantity of blood eliminates the necessity for introduction of a distribution ratio and permits the rather simple calculation of their respective volumes per 100 ml. directly from the voltage ratios determined by the mass spectrometer.

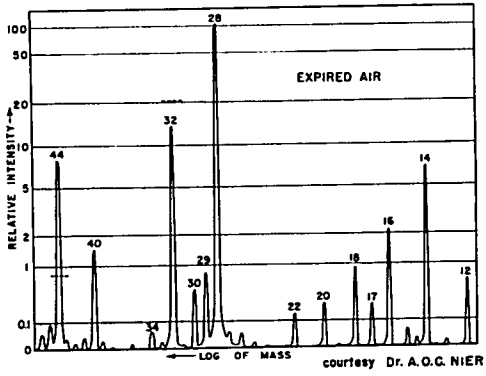

FIG. 2. Mass spectrum of expired air. Peaks 16 and 32 represent chiefly oxygen. The chief contributor to peaks 14 and 28 is nitrogen. Peak 44 is made up largely of the ionized carbon dioxide molecule while peak 40 represents the argon which is present in the air to the extent of 0.93 per cent.

The dotted lines indicate the abundance of peaks 32 and 44 representing oxygen and carbon dioxide respectively in fresh air.

The fragment pattern characterizing expired air (fig. 2), which contains substantial quantities of carbon dioxide and oxygen, reveals that peak 40 is no greater than one might expect from the amount of argon present in air (0.93 per cent). Neither carbon dioxide nor oxygen contributes materially to this peak. The proximity of peaks 32 and 44 to that of argon, 40, and the abundance of their masses make them suitable for use in the spectrometric determination of oxygen and carbon dioxide respectively. The procedure for the analysis of a mixture of gases containing carbon dioxide will be described.

Previous to the analysis a mixture of known amounts of argon and carbon dioxide must be prepared. The volume ratio of carbon dioxide to argon in this mixture is known. This known mixture of gases is then analyzed on the mass spectrometer, and the ratio of the voltage at peak 44 to the voltage at peak 40 can be determined. From these two ratios, a constant, k, is obtained, which may vary in different spectrometers and to a small extent at different times in the same spectrometer. Thus:

$$\frac{\text{Vol } CO_2}{\text{Vol A}} = k \frac{V_{CO_2}}{Va} \tag{1}$$

when: Vol CO_2 = Volume of carbon dioxide
Vol A = Volume of argon
V_{CO_2} = output voltage on the peak 44
Va = output voltage on the peak 40
k = a calibration constant.

When the value of k is known and a known volume of argon is added to the mixture of gases from blood to be analyzed, the volume of carbon dioxide in that mixture can be determined. Thus:

$$\text{Vol } CO_2 = kR \text{ (Vol A)} \tag{2}$$

when: R = V_{44}/V_{40} or the mass spectrometric voltage ratio of carbon dioxide to argon in the mixture to be analyzed.

The following equation may then be applied to convert to volumes o carbon dioxide per 100 ml. of blood and corrected to standard temperature and pressure:

$$\text{Vol } CO_2 \text{ per 100 ml.} = \frac{kR \times \text{Vol A} \times 273.2 \times BP \times 100}{T \times 760 \times Vol_b} \tag{3}$$

when: T = temperature of argon when added to gas sample
BP = barometric pressure
Vol_b = volume of blood sample.

For the quantitative determination of oxygen the voltage ratio obtained as V_{32}/V_{40} is considered as R. The calibration constant, k, as

determined for a mixture of known amounts of argon and oxygen is substituted for the carbon dioxide constant, k.

In the analysis of blood for carbon dioxide and oxygen in the presence of nitrous oxide or ether or both, correction must be made for the additive effects of the latter gases to peaks 44 and 32. The voltage ratio of any two peaks in the mass spectrum for any gas is constant. Inasmuch as mass 31 was chosen for the determination of ether, the spectrometric measurement of the voltage at peak 31 in the case of

TABLE 1

COMPARISON OF REPEATED MASS SPECTROMETRIC DETERMINATIONS OF THE CARBON DIOXIDE CONTENT OF BLOOD TO THOSE OBTAINED BY THE MANOMETRIC METHOD OF VAN SLYKE-NEILL*

Van Slyke-Neill, Vol. per 100 Ml.	Mass Spectrometer, Vol. per 100 Ml.	Van Slyke-Neill, Vol. per 100 Ml.	Mass Spectrometer, Vol. per 100 Ml.
36.0	37.0	45.9	44.9
	36.6		43.9
	37.3	57.8	58.4
	36.8		
47.4	49.1	59.3	58.4
	46.5		58.5
	46.0		58.5
	46.0		58.5
	46.4	55.4	55.5
	48.1		53.9
	48.1		54.8
	46.2		54.3
	46.9		55.3
	46.6		54.2
45.9	45.6	57.5	57.6
	45.6		57.8
	44.8		58.6
	45.8	43.6	43.3
			44.5
			44.1

* Root mean square difference ±1.95 per cent.

contamination of the sample of gases with ether, permits the calculation of the contribution of this gas to the total voltage as measured at peaks 32 and 44. In a similar manner the contribution of nitrous oxide to these peaks can be calculated by measuring peak 30.

VALIDATION OF THE METHOD OF ANALYSIS

Since for many years the Van Slyke-Neill (4) manometric method of analysis of blood for its carbon dioxide and oxygen contents has been generally accepted as one without peer, it was chosen as the most

suitable technic with which to compare the mass spectrometric method. The results given in tables 1 and 2 represent those obtained from the analysis of samples of blood for carbon dioxide and oxygen by both methods. The mass spectrometric results represent repeated analyses of individual samples of blood following the standardization of all procedures and the apparent solution of all technical problems. The Van Slyke-Neill analyses of the samples of the same blood were performed in duplicate by technicians thoroughly skilled in the use of this method. The recorded figure represents the average of the duplicate determination, which in this laboratory is the customary method of reporting results of the Van Slyke analysis. The mass spectrometric results subjected to statistical analysis reveal a root mean square difference of

TABLE 2

COMPARISON OF REPEATED MASS SPECTROMETRIC DETERMINATIONS OF THE OXYGEN CONTENT OF BLOOD WITH THOSE OBTAINED BY THE MANOMETRIC METHOD OF VAN SLYKE-NEILL*

Van Slyke-Neill, Vol. per 100 Ml.	Mass Spectrometer, Vol. per 100 Ml.
21.9	23.4
	21.5
	22.7
	21.5
23.2	23.3
	22.2
	22.2
	22.5
	22.4
21.7	21.7
	22.6
	22.0
	22.0
	23.0
10.9	10.9

* Root mean square difference ±3.4 per cent.

± 3.4 per cent for the determinations of oxygen and ± 1.95 per cent for those of carbon dioxide as compared to those obtained by Van Slyke analyses. This was considered to be sufficiently accurate for our purposes, and was well within the 5 per cent range which we had arbitrarily set as a goal.

SUMMARY

A method adapting the mass spectrometer so that it may be used in the determination of the carbon dioxide and oxygen in blood is described. This method requires the extraction of all the gases from the sample of blood to be analyzed. This technic requires release of chemically bound oxygen and carbon dioxide in the blood followed by vacuum extraction and drying of all dissolved gases. Argon is added as an internal standard, the resulting mixture of gases is analyzed on

the mass spectrometer and the original concentration of oxygen and carbon dioxide in the blood is then determined. This technic is shown to be adaptable to mixtures containing ether and nitrous oxide. Results of multiple analyses on the mass spectrometer of eight different samples of blood were compared with results of the Van Slyke-Neill determinations for carbon dioxide. The mass spectrometric results differed by a root mean square of ± 1.95 per cent. When a comparison was made between results of multiple analyses of four different samples of blood for oxygen with results of the Van Slyke determinations a root mean square difference of ± 3.4 per cent was found.

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