SOME METHODS OF ANALYSIS AND DETERMINATION OF ANESTHETIC AGENTS*

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The determination of the presence and concentration of an anesthetic drug from gaseous mixtures or from body fluids or tissue is not a routinely simple procedure. This is particularly true of quantitative measurement, when more than one drug is to be identified. The factors of expense, both in time and money, of obtaining and maintaining elaborate equipment necessary for some of the tests, is a big deterrent. The time factor likewise makes such testing clinically impractical to a great extent, especially since a good anesthetist must maintain minute to minute control over the patient and the anesthetic drugs he is receiving. Individual variations from the norm also should be judged by the individual's response to single or multiple anesthetic agents—this too must be evaluated clinically, regardless of the quantitative assay of the drugs. All in all, it may be stated that the best determination is made by the anesthesiologist who maintains surgical anesthesia as the level necessary for the operative procedure and at the same time keeps metabolism and bodily function as close as possible to normal.

In certain types of anesthetics the amount of agent offered the patient may be carefully ascertained. This is true of anesthetic liquids given orally, rectally, or parenterally as intravenous technics. With the use of calibrated flowmeters (15) it is true to a lesser extent of gaseous agents. However, variances in absorption, utilization, secretion and excretion, decomposition and detoxication of the drugs in the body completely overshadow the picture of the fate of the drug once it has been offered to the body. There are no hard and fast rules or formulas which allow us to estimate the amount of drug in the body from the amount which has been offered.

Such determinative procedures as we shall later mention may play a role in anesthetic recovery work, in overdosage and poisoning—this particularly from a medicolegal standpoint—in research problems. As tests are elaborated which are quickly and easily performed, they eventually may be of great value to the practicing anesthesiologist.

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Gas analysis was the subject of earliest investigation and, to date, there is more work in this field than with other agents. Determination of gases falls into one of two categories, chemical and physical. Within the chemical group there are in turn three classes; volumetric determinations; manometric determinations; and chemical reactions from which the end product is ultimately determined volumetrically, gravimetrically, or colorimetrically.

Most apparatus for volumetric gas analysis derives from the original Henderson-Haldane, of which the two most commonly used are the Orsat (11) and the Peters-Van Slyke (28). The former is used mainly for commercial and industrial purposes. The latter or variations of it is commonly used in hospital and research laboratories. Various gases may be absorbed by various chemical reagents, and the residue determined in volume percentage of gas. The displacing fluid is commonly mercury or a saturated metallic salt solution, such as calcium chloride. Some of the specific reactions will be mentioned later.

Manometric gas analysis is carried out similarly by absorption, but the determination is made on the basis of the pressure exerted on a manometer, with the volume and temperature kept constant or corrected. The apparatus used most often is the Van Slyke-Neill (30-21).

The third type of chemical gas determination is illustrated in the test commonly used for hydrocarbons, the "iodine pentoxide train." This consists of passing the gas through a tube heated between 200 and 300°C. The tube contains iodine pentoxide and asbestos and from the free end emerges carbon dioxide, water, free iodine and hydrogen iodide. The latter two are dissolved in a solution of potassium iodide and the amount determined volumetrically or colorimetrically, usually by titration against a thiosulfate solution. This method may be used for cyclopropane, ethylene or any of the ethers.

Physical methods of gas determination include the following: determination of the heat conductivity of differing gases by passing a constant current of electricity along a wire surrounded by the gas, measurement of sound conduction time in a gaseous medium by measuring the index of refraction of the gas, and calorimetric determinations in which the gas is oxidized in a combustion chamber and the heat produced measured by thermocouple or similar device (1). Some specific examples will be mentioned later.

Gases may be determined from blood, other body fluids and tissue extracts. Gases in simple solution may be extracted with the negative pressure of a partial vacuum and determined volumetrically or manometrically. Other gases may require chemical reaction to extract the gas (28). Blood, for example, if treated with saponin yields hemolyzed cells. Then, if lactic acid is added, the carbon dioxide is released from carbonates and carbamime compounds. If potassium ferricyanide is used the hemoglobin and oxyhemoglobin is converted to methemoglobin,
freeing oxygen. Similarly, ethyl ether may be extracted with a saturated solution of sodium chloride and glycerine.

Although oxygen is not an anesthetic agent, oxygen determinations are of prime importance in any determinative procedures on gases as applied to anesthesia. Oxygen may be quantitatively analyzed either volumetrically or manometrically. This is commonly done by absorption with pyrogallic acid (1, 2, 3, trihydroxybenzene), and the difference between the original volume percentage and the residual volume percentage represents the amount of oxygen. The gas also can be absorbed in the oxidation of sulfites to sulfates, such as sodium hyposulfite, and also in the oxidation of copper and cuprous compounds. Hoechstetter (23) reported a method of oxygen analysis for tent therapy in which the oxygen is absorbed by metallic copper in the presence of ammonium hydroxide and ammonium chloride. Oxygen may also be determined by igniting with a known amount of hydrogen. Other tests for oxygen content may be made by physical methods. The Beckman Oxygen Analyzer works on the principle that oxygen has an unusually high magnetic susceptibility. When a gas containing oxygen is admitted into a magnetic chamber containing a glass dumbbell mounted on a vertical quartz fiber, the percentage concentration of the oxygen is shown on a scale reflecting the rotation of the dumbbell in the magnetic field as balanced by the torsional force of the quartz fiber.

Carbon dioxide is another important gas from the anesthesiologist’s viewpoint. It may be determined in the Van Slyke apparatus after absorption by an alkali such as sodium or potassium hydroxide, and the volume variances determined; or it may be freed again by acid and determination made of the pure carbon dioxide. The historical background of the work of Waters, Foregger, Adriani and others in the development of carbon dioxide absorption technics in anesthesia, although interesting, is out of the realm of this discussion.

Nitrous oxide gas may be determined from mixtures by using the Van Slyke-Neill manometric apparatus as previously mentioned. Orcutt and Waters (27) reported such a method for nitrous oxide, cyclopropane and ethylene. In their procedure the oxygen and carbon dioxide are absorbed (as previously referred to), and the anesthetic gas determined as the residual gas. Corrections for unextracted gas are given in a table to be added to the figures. Orcutt and Waters (26) have also reported another method for nitrous oxide analysis by a diazotization technic. A nitrous oxide and oxygen mixture, without any free nitrogen present, is passed through a heated porcelain tube which acts as a catalyst, producing higher oxides of nitrogen. These in turn are passed through a potassium hydroxide solution yielding potassium nitrite. Potassium nitrite plus alpha naphthylamine and hydrochloric acid and sulfanilic acid produce a red dye which may be compared colorimetrically. Mention might be made here of a physical test for nitrous oxide. Bell and Krantz (8) have devised an inter-
ferometer method for the assay of the gas; this is simply an application of the varying index of refraction of different concentrations of the gas.

Ethylene, in a manner similar for that of nitrous oxide, may be determined manometrically after first absorbing the oxygen and carbon dioxide. It may also be determined by a quantitative volumetric method in which the gas is mixed with sulfuric acid and ethyl hydrogen sulfate results from the reaction.

The concentration of cyclopropane also may be arrived at manometrically. More commonly, the iodine pentoxide train method is used. Robbins (29) gave a technic in which the gas, either in air, water or blood, is introduced into a tube containing iodine pentoxide and asbestos and heated to a temperature of 205 to 210 C. Thence the fumes of liberated iodine are collected in a potassium iodide solution and this is titrated against a standard solution of thiosulfate. Another test is the older Orsat-Henderson of absorption of cyclopropane with concentrated sulfuric acid, and volumetric or manometric measurements made therefrom.

All the ethers may be tested with the iodine pentoxide train method. This is true not only for ethyl ether and vinyl ether, but for the newer ethers such as cyprone and metopryl. Ethyl ether may also be converted to alcohol or acetic aldehyde by adding sulfuric acid and potassium dichromate. Then when tested iodometrically, as mentioned by Nicloux, Ronzoni and others (1), the test for the alcohol becomes a test for ether.

If blood samples are to be tested, the ether should first be extracted. This may be done as previously mentioned with a saturated solution of sodium chloride and glycerine. The collection of blood samples deserves a word here. It may be done rather technically as described by Peters and Van Slyke (28) using either mercury or oil as a displacing agent. Similarly, serum may be obtained by anaerobic centrifugation of blood. They also recommended that blood, if not used immediately, be chilled to 0 C. However, Adriani (2) mentioned a way that is much simpler clinically and in which the only equipment required is an intravenous needle, a three-way stopcock, a safety pin, a small amount of rubber tubing, a tight fitting syringe and plunger, and a small amount of mercury.

Ethyl ether can be determined from a blood sample by the method of Andrews and his co-workers (6). They deliver the blood sample under the surface of cold precipitating reagents, acid mercuric sulfate and sodium tungstate. The ether is then removed from this mixture with rapid aeration by heating and the vapors absorbed quantitatively in a beak tower containing a chromic acid-sulfuric acid mixture. The residual oxidizing agent is determined iodometrically.

Chloroform is usually determined by means of the Cole test (1). When pyridine and sodium hydroxide are added to chloroform a red
color results. Colorimetric determinations can be run against known colored concentrations.

Ethyl alcohol, although not generally accepted as an anesthetic agent, should be mentioned because tests can be of clinical significance as well as medicolegal importance. Haggard and Greenberg have tested for ethyl alcohol by means of the iodine pentoxide train. Friedemann and Klass devised a method of oxidizing the alcohol with potassium permanganate and the excess permanganate is then determined iodometrically. This test has been the basis for both blood and exhaled air tests.

Brief mention might be made of some tests for other sedative, analgesic, and anesthetic agents. Paraldehyde, for example, may be tested in the manner of Bodansky, iodometrically, using potassium dichromate and sulfuric acid. Nitzescu, Giorgescu and Timus depolymerize the paraldehyde with sulfuric acid to acetaldehyde which combines with sodium bisulfite and an iodometric determination is done. Bromides may be tested in the way of Behr, Palmer and Clarke (7) which requires oxidation by permanganates in phosphoric acid and transference to carbon tetrachloride; this is too complicated for clinical use. No good tests for tribromethanol or trichlorethanol are reported, but there are tests for their decomposition products. Trichlorethylene may be tested in the iodine pentoxide train.

The barbiturates may be determined by a cobalt color reaction. Koppanyi (24) described a method in which the extract either of blood, urine or tissue is acidulated, then chloroform added and finally cobalt acetate, producing a blue color supposedly due to malonyl urea. Delmonico and Adams (12) enlarged on this and found that variances of the barbituric acid structure might produce a color variance from pink violet to green, the latter for the thiobarbiturates. To the chloroform extract these workers added isopropyl amine as well as cobalt acetate and then compared the colors colorimetrically. Since the malonyl urea ring is the color producing portion, actual differentiation depends on further studies, such as; crystal micrography, determinations of melting point, tests for solubility and other physical chemical tests. Hellman, Shettles and Stran (22) have devised a very interesting chemical physical test for sodium pentothal in blood. The pentothal is extracted in ether and allowed to absorb ultraviolet light at 2880 Angstrom units and numerical values of the absorption, or actually the concentration of the pentothal, read off on an ultraviolet sensitive cell and galvanometer. Bollman (10) and others have used radioactive isotopes of sodium or sulfur for such determinations and to follow the fate of the drug in the body. The cyclic disappearance and reappearance of barbiturates in the blood following intravenous administration, as shown by Anderson and Essex (5), is still unexplained. For procaine, the diazo reaction may be used on blood or tissue extracts. With an alcoholic solution of vanillin and sulfuric acid a yellow solution is produced.
This plus potassium mercuric iodide makes a white precipitate to which, when sodium phosphate is added, there results another yellow solution which may be compared colorimetrically. Gibb and Dehn (18) modify this by adding sodium nitrite, acetic acid, and ammonia to urine or tissue extracts and comparing the yellow or red color obtained. It must be remembered that this test is not specific for procaine or any other of the conduction blocking drugs, but only for para-aminobenzoic acid, which is a hydrolytic product of procaine.

- The acoustic gas analyzer as devised at the Mayo Clinic is worthy of mention. In 1940 Dublin, Boothby, Brown and Williams (13) found that mixtures of helium with oxygen and nitrogen could be analyzed by means of determining the velocity of sound within the gaseous mixture. This could be done by hooking up a tuning fork and a sound sensitive membrane in an electrical circuit with current measuring devices. Following this, Faulconer, Clarke and Osterberg (14) devised their apparatus. The principle of the varying velocity of sound in different gases is incorporated in a system with an audio frequency oscillator circuit and this is calibrated for percentage on graphs which they have worked out. It may be compared to a miniature radio circuit with a sound transmitter and receiver and when the sound passes from one to the other the circuit is made. They claim also that by varying different factors they can set the machine to function with a gas anesthesia delivery machine and so positively control the flow of gases to make any anesthetic mixture desired.

Curare as a recent adjuvant to anesthesia in the form of the alkaloid, d-tubocurarine, may be mentioned only to state that there are no reported chemical tests for its assay. This is still done by physiologic test on animals.

**Summary**

Some methods for the determination of anesthetic agents from gaseous mixtures and body fluids have been presented. Many of these tests for determinative analysis of certain anesthetic agents are time-consuming, expensive and elaborate. Also, since there are no good tests at all for some of the drugs in the anesthesiologist's armamentarium, it must be said that for the present all the determinative analyses of a practicing anesthetist must be done by him alone, by clinical evaluation and judgment within the operating room.

**References**

5. Anderson, B. M., and Essex, H. E.: Studies on Barbiturates Especially Their Cyclic Di-


