In the Laboratory

A Microanalyzer for Very Small Gas Samples (0.4-1 mm.^{3})

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The apparatus to be described was developed to determine the composition of small bubbles formed in animals decompressed to simulated high altitudes (*1*). It is, however, suitable for more general use in biological research or in any situation requiring analysis for CO_2 and O_2 in very small samples of gas (0.4–1 mm.^{3}). Modification of the procedure might adapt it to other gases as well. The constant-pressure, capillary-tube method is used, following Krogh (*3*), Scholander (*4*), and others (see review by Hartridge, *2*), but modifications in construction and technique permit more rapid analyses of smaller volumes of gas. The apparatus has the advantages of simple construction and rapid operation.

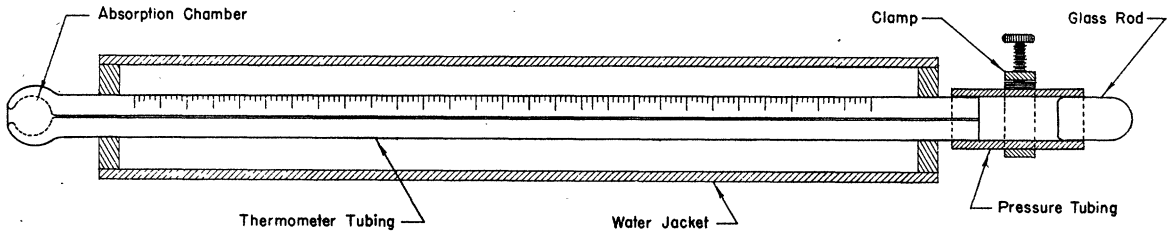

Fig. 1

Construction

The analyzer (Fig. 1) consists of a 6-inch length of capillary thermometer tubing (0.11-mm. bore) with an open, bell-shaped absorption chamber blown at one end. A short piece of rubber pressure tubing, plugged with a short glass rod, fits over the other end and is provided with a screw clamp. The capillary tubing is surrounded by a water jacket to minimize temperature fluctuations.

Operation

To prepare for an analysis, the rubber tube is fitted over the end of the capillary of the analyzer and is filled with a saturated aqueous solution of LiCl.[1] The glass rod is then pushed into the open end of the rubber tube and LiCl solution is forced through the capillary to fill the absorption chamber, in which it is retained by surface tension. The analyzer is held vertically with the absorption chamber downward, and the bubble of gas to be analyzed is introduced into the chamber from a small pipette containing LiCl solution. If extraneous fluids (such as blood) must be removed from the bubble, it is first washed in a small dish of LiCl solution, from which it is transferred into the absorption chamber. The analyzer is held vertically, and the bubble rises to the constriction of the tube at the top of the absorption chamber and is slowly and carefully drawn into the capillary by opening the screw clamp. It is necessary to standardize the rate at which the bubble is drawn into the capillary in order to avoid variations in the amount of liquid retained on the capillary wall. With practice, the gas sample can be transferred into the analyzer in a few seconds.

The length of the column of gas, which is proportional to its volume, is then measured on the graduated scale of the thermometer tubing. It varies about 1 per cent, depending on whether it is moved toward or away from the absorption chamber. By averaging several readings the error of this measurement is reduced to 0.1–0.2 per cent.

Having measured the length of the entire gas sample, the next step is to remove the CO_2 and measure the length of the remainder. This is accomplished by substituting a solution of LiCl + KOH for the LiCl solution in the absorption chamber and forcing the sample of gas out into this new solution. The KOH absorbs the CO_2 while the LiCl impedes the exchange of other gases by diffusion. Absorption of the CO_2 is completed in a few seconds. The bubble is again drawn into the capillary and measured. If the O_2 content of the sample is to be determined, alkaline pyrogallol solution (which absorbs O_2) is substituted in the absorption chamber, and the procedure for absorption is repeated with rotation of the analyzer for 20 seconds, in this case, to facilitate absorption. The operation should be repeated a second time to be sure that the absorption of O_2 is complete. The percentages

[1] Gases are relatively insoluble in saturated LiCl solution, which therefore reduces errors due to loss by diffusion.

of CO_2 and O_2 in the original sample are calculated from the changes in length of the gas column, and the volume of the bubble is calculated from the length of the gas column (40–100 mm.) and the cross-section area of the capillary (.0095 mm.^{2}). A complete analysis can be performed in 10–15 minutes.

After each analysis the apparatus is cleaned by drawing tap water and then cleaning solution through the capillary, rinsing with tap and distilled water.

Accuracy

The accuracy of analysis depends primarily on the amounts and kinds of gases in the sample. Other important factors, subject to a considerable degree of control, are (1) alteration of the sample through gaseous exchange with the analyzer fluid by diffusion; (2) changes in the water vapor tension in the bubble when in contact with solution of different osmotic pressure; (3) rate at which the bubble is drawn into the capillary; and (4) reading error. In actual practice, diffusion of gases is found to be the greatest source of error. If the composition of the bubble diverges only slightly from the gas tensions in the analyzer fluid, which is equilibrated with air, the error due to diffusion is negligible, but it increases as the composition of the sample diverges from that of air. This error was found to be considerably reduced by (1) using saturated aqueous LiCl for the analyzer and transference fluids; (2) using alkaline LiCl solution as the CO_2 absorbent; and (3) introducing the gas sample into the analyzer as rapidly as possible.

TABLE 1

Sample No.	Vol. of bubble	Known composition of gas mixture*			Composition as measured in analyzer		
		CO_2	O_2	N_2	CO_2	O_2	N_2 (by difference)
	(mm.^{3})	(%)	(%)	(%)	(%)	(%)	(%)
1	1.43				...	20.7	79.3
2	0.42	0.03	20.95	79.02	...	21.0	79.0
3	1.41				...	20.9	79.1
4	0.78		(air)		...	21.1	78.9
1	0.55				5.0	91.1	3.9
2	0.45	5.29	93.63	1.08	4.9	89.6	5.5
3	1.03				5.4	92.6	2.0
4	0.40				5.4	92.1	2.5
1	0.50				14.5	8.1	77.4
2	0.57	14.90	7.65	77.45	14.7	8.0	77.3
3	0.45				14.4	7.9	77.7

* Determined by means of the Haldane gas analysis apparatus.

Greater accuracy could be obtained by equilibrating the solutions with a gas mixture of composition approximating that of the sample to be analyzed. Under most favorable conditions the error of analysis is less than 0.3 per cent. Under less favorable conditions it increases but is not unreasonable considering the extremely small volumes of gases analyzed.

Typical analyses of air and known gas mixtures by this method are given in Table 1.

References

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A New Method for the Purification of Arginase

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Many and varied studies on arginase can be and have been made on very crude solutions. With two exceptions, most preparations reported from 1924 (*3*) to 1944 (*2*) were essentially extractions in glycerol, with reduction in large volume of acetone. The exceptions were Edlbacher and Simons (*1*), who tried adsorption on alumina C and Willstätter kaolin, and Richards and Hellerman (*5*), who made repeated fractionations in acetone, ammonium sulfate, sodium salicylate, and sodium alizarin sulfonate, with frequent dialyses. These methods gave products which, although much purer than the usual solutions, were neither comparatively very active nor very pure.

The first reported systematic study of preparative methods is that of Mohamed and Greenberg (*4*). The procedure finally adopted by them consisted in extractions and fractional precipitations in sodium acetate, lead acetate, ammonium sulfate, adjustment to pH 8,

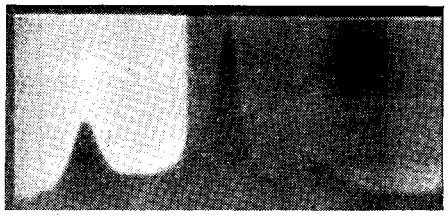

Fig. 1. Tiselius curve of purified arginase in pH 6.2 phosphate buffer μ0.10; 205 minutes at 5.8 volts/cm.; $U_P = 6.3 \times 10^{-5}$; $U_S = 2.3 \times 10^{-5}$. (Courtesy of Dr. C. H. Li, Institute of Biological Research, University of California.)

reduction in acetone, and solution in pH 7 phosphate buffer. The final product, a green-brown solution, showed by electrophoresis in the Tiselius apparatus the presence of three or four constituents. Catalase was a definite contaminant of the mixture. The problem remaining, therefore, was that of freeing the preparation of catalase and other proteins. It was

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