Blood Gases: Continuous in vivo Recording of Partial Pressures by Mass Spectrography

Abstract. *Gases were sampled directly from circulating blood through a membrane at the tip of an intravascular cannula that was connected to the analyzing section of a mass spectrometer. Partial gas pressures and membrane permeability determine gas flow into the spectrometer. Arterial carbon dioxide and oxygen pressures were simultaneously recorded in an anesthetized animal subjected to various respiratory maneuvers.*

In the course of investigations of the regulation of breathing (*1*) it became desirable to make continuous measurements of gas pressures in circulating blood. Analyzers currently in use in respiratory research, such as the polarographic oxygen electrode introduced by Clark (*2*) or the pCO_2 meter described by Severinghaus (*3*), do not meet these requirements: their sensitivity is restricted to a single gas; bulkiness and inflexibility make intravascular application impossible; being erratic, for not always easily recognized reasons (*4, 5*), they cannot be expected to give reliable results unless repeatedly recalibrated and used exclusively for static measurements in samples of blood.

Comparatively few publications mention the use of the mass spectrometer in respiratory research (*6*). The mass spectrometer makes an instantaneous quantitative analysis, separating the gases by molecular weight. The analyzing section of the mass spectrometer, operating at near vacuum pressure, requires extremely minute quantities of gas for proper functioning. To achieve this, the sample to be analyzed is usually introduced into a fore vacuum that is connected to the analyzer through a micropore leak, thus providing a stepwise reduction of flow into the analyzer. In the present study a mass spectrometer has been modified by eliminating the fore vacuum and replacing the micropore leak with a cannula capped at the tip by a gas-permeable membrane. In this way diffusible gas molecules immediately enter the analyzer in quantities that are determined by partial gas pressures and by the diffusion properties of the membrane, including its thickness and surface area.

The mass spectrometer used in this study was a CEC model 21-610. Molecules entering the analyzing section of this instrument are ionized, accelerated in a variable electric field, deflected in a constant magnetic field, and projected to a target plate (collector), upon which they confer their charge. Different molecular masses are brought into focus by manipulation of the electrical parameters of the analyzer, as illustrated in Fig. 1*A*. In the early stages of our research we focused our attention and the analyzer on a single gas (usually carbon dioxide or oxygen) by fixing the parameters, but later on we decided to scan the various peaks of the molecular spectrum by varying the accelerating voltage with a sawtooth generator. This method enabled us to make repeated successive measurements of more than one gas. An added advantage was increased recording stability; each scan produced molecular peaks in their entirety, and this eliminated noise from peak and focus shifts which, due to slight irregularities in accelerating voltage or analyzer pressure, are bound to occur in a mass spectrometer that has been adjusted to continuously read a single peak maximum. The molecular spectrum, shown in Fig. 1*A*, covers only a small span of molecular weights between 20 and 50, sufficient for the study of most gases encountered in respiratory research. In most experiments this spectrum was scanned at a frequency of six per minute. As a further technical refinement, two of the spectral components (carbon dioxide and oxygen) were isolated by electronic gates and individually displayed on separate recorders, with results as shown in Fig. 2.

By far the most important parts in the present application were the membrane through which the gases were absorbed from the blood, and the cannula that conducted these gases into the spectrometer. Polyethylene tubing (inside diameter, 0.4 mm; outside diameter, 1.1 mm; length, approximately

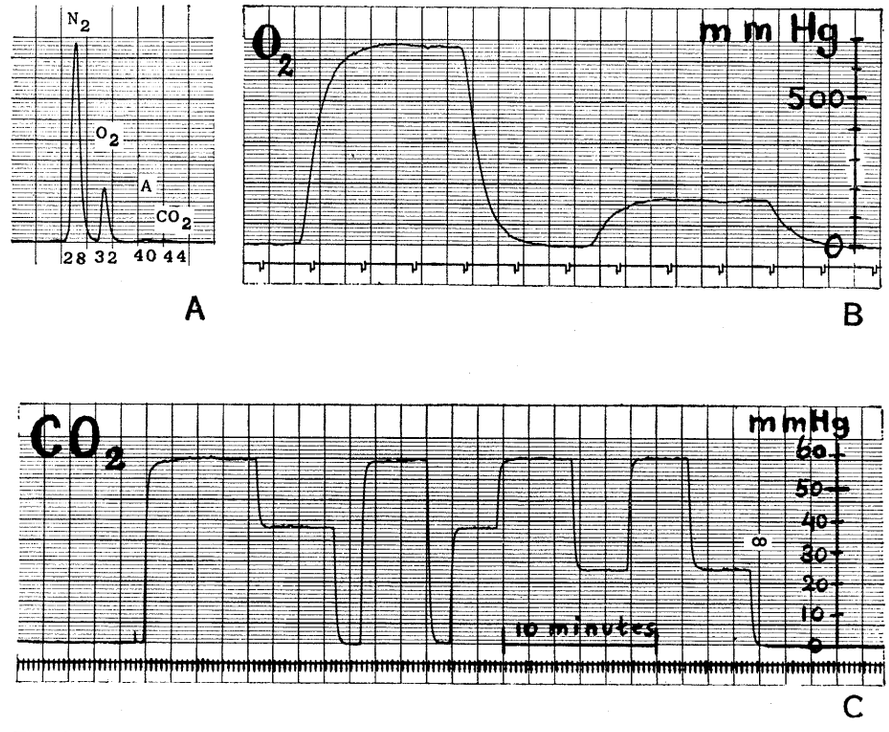

Fig. 1. (*A*) Mass spectrum of air. Molecular weights shown below the graph. (*B*) Mass spectrometer adjusted to give a continuous record of the partial pressure of oxygen; wet membrane, 20°C; zero line, 100 percent nitrogen; first response, 90 percent oxygen; second response, air (21 percent oxygen). Barometric pressure, 760 mm-Hg. Time, 20 seconds. Pressure calibration to the right in mm-Hg. (*C*) Continuous recording of carbon dioxide pressure; wet membrane, 20°C; zero line, 100 percent nitrogen; responses to gas mixtures containing, respectively, 8.2, 5.1, 0, 8.2, 0, 5.1, 8.2, 3.3, 8.2, 3.3, and 0 percent carbon dioxide. Barometric pressure, 748 mm-Hg. Time signal, 20 seconds; time bar, 10 minutes. Pressure calibration in mm-Hg.

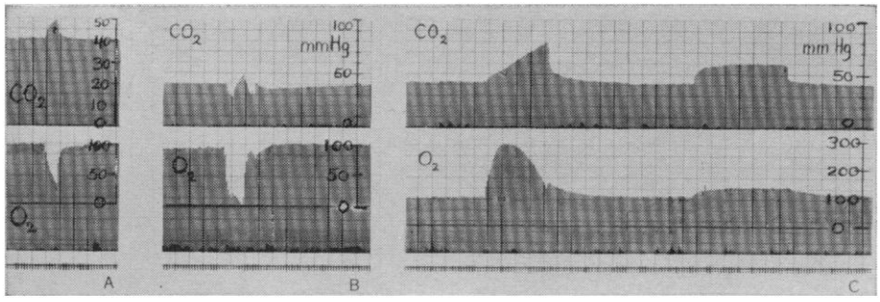

Fig. 2. Continuous recordings of arterial carbon dioxide and oxygen in anesthetized cats. (*A*) Response to asphyxiation by closing of the airway. (*B*) Asphyxiation by nitrogen inhalation. (*C*) First response: animal rebreathing from a bag containing 100 percent oxygen; second response: inhalation of 8.6 percent carbon dioxide in air. Time signal, 20 seconds; pressure calibrations to the right in mm-Hg.

1 m) was used as cannula, a choice determined mainly by its availability. Being permeable to air, the tubing produced a background reading in oxygen and nitrogen that would become serious when the gas flow through the membrane was of the same order of magnitude as the gas flow through the wall of the tubing. Since less-gas-permeable tubing of comparable size was not available at short notice, we present the data obtained with polyethylene tubing.

The choice of the membrane posed a number of problems, including availability, ease of handling and mounting, structural strength and need of support, exposure of maximum surface area with minimum membrane thickness, permeability to gases and loss of permeability upon immersion in water, and change of permeability with temperature. Of many materials tested, natural rubber (latex rubber from toy balloons) gave the best results. Its high elasticity and tensile strength made it easy to stretch over the tip of the cannula without tearing and to make a leakproof seal with a simple ligature of surgical silk. Sheets of neoprene and silicone rubber were usually thicker, less elastic, and less tear-resistant than natural rubber. The same applied to many plastics, specifically polyethylene and polyvinyl. Permeability of the various rubbers to carbon dioxide, oxygen, and nitrogen was good. They were equalled in this respect by polyethylene and polyvinyl. Polyvinylidene ("Saran wrap") and Mylar were almost impermeable. Teflon was highly permeable when used dry, but lost its permeability entirely as soon as it was dipped in water, confirming recent studies by Moran, Kettle, and Cugell (*4*) and Rhodes and Moser (*5*). This may be due to the presence of micropores in thin sheets of Teflon (personal communication from Dupont Research Labs., Wilmington, Delaware) that become plugged on contact with water. Reduction of permeability upon immersion of the membrane tip in water was a peculiarity of most membrane materials, although less pronounced than with Teflon. We made it standard procedure, therefore, to wet the membrane before use and to calibrate in wet environment. Membrane permeability was also observed to change with temperature; these effects were not consistent for gases of different molecular weight and were, moreover, strongly influenced by the conditions of the membrane; dry membranes, for example, showed an increase in permeability for oxygen and a decrease in permeability for carbon dioxide with a rise in temperature, whereas with wet membranes these effects were exactly reversed; in between there was a degree of humidity at which a change in temperature did not affect the permeability of the membrane. Further investigations are needed to clarify these findings.

An effort has been made to measure the gas uptake by the membrane tip. Because of the small amounts involved, we failed to make exact measurements. A rough estimate, based on air uptake from a bag in a volumetric device, and confirmed by measurements of oxygen depletion around a tip immersed in water saturated with air at 20°C, gave figures of the order of magnitude of 1 ml per 24 hours or 3×10^{14} molecules per second, which is ten times the number of molecules in 1 ml at 10^{-6} torr).

Calibration records for oxygen and carbon dioxide are shown in Fig. 1, *B* and *C*. Linearity and stability are adequate. Base-line noise was mainly due to the fact that these are continuous readings of the maximum value of a single peak in the molecular spectrum. The response delay, clearly visible in the higher-speed recording of Fig 1*B*, could be ascribed to membrane resistance to gas diffusion, considering the reduction of response time if more permeable membranes were used. The time constant of the rubber membranes used in the present study averaged 10 seconds, which means that it takes about 30 seconds before the response reaches its full value. It also means that, at a scanning rate of six per minute used in our experiments, virtually every part of the response is recorded.

The results of direct measurements of oxygen and carbon dioxide pressures in circulating blood are shown in Fig. 2. A cannula with a rubber membrane at its tip was inserted in the aorta of an anesthetized cat via the right brachial artery. Since native blood caused a rapid deterioration of membrane permeability, presumably due to coagulation at the tip, it was necessary to inject an anticoagulent (heparin). Calibrations shown to the right of each trace in Fig. 2 were verified by analysis of blood samples taken from the femoral artery, by use of an Epsco blood parameter analyzer, an instrument that is equipped with a Severinghaus pCO_2 electrode, a Clark polarographic oxygen electrode, and a *p*H electrode. Figure 2 shows responses to various respiratory maneuvers. The records contain information that should be of interest to the respiratory physiologist. Figure 2, *A* and *B* both show a response to asphyxiation; in *A*, resulting from closing the trachea of the animal, and in *B* resulting from inhalation of 100-percent nitrogen. Closing the trachea resulted in a gradual depletion of oxygen and a gradual buildup of carbon dioxide at rates that differed due to the difference in storage capacity of blood and tissues for oxygen and carbon dioxide.

Inhalation of nitrogen caused an initial hyperventilation due to anoxic stimulation of chemoreceptors; both oxygen and carbon dioxide were washed out, which led to depression of respiration and a subsequent respiratory standstill; during respiratory arrest arterial carbon dioxide tension is seen to rise again to a level where it caused enough stimulation to restore breathing. At this moment nitrogen administration was stopped and the animal was allowed to recover without artificial ventilation. Figure 2*C* shows an hour-long record

of arterial oxygen and carbon dioxide pressures in another cat. In the first experiment (response to the left) the animal rebreathed from a bag filled with approximately 300 ml of pure oxygen. If the changes in bag volume had been recorded during this experiment, these tracings should have given information on oxygen consumption, carbon dioxide production, lung volume, and distribution of carbon dioxide in the body. The second response in Fig 2*C* (to the right) was produced by administration of a breathing mixture containing 8.6 percent carbon dioxide in air. Alveolar carbon dioxide pressure is seen to rise to 60 mm-Hg, a figure that comes close to the carbon dioxide pressure of the inspired air saturated with water at 37°C. Due to hyperventilation caused by increased carbon dioxide pressure, arterial oxygen pressure rose from 100 mm-Hg to 130 mm-Hg which, considering the reduced oxygen pressures in another cat. In the first indicates a more than tenfold increase in alveolar ventilation.

In conclusion, it may be stated that (i) a technique of gas sampling through a membrane at the tip of an evacuated cannula enables the investigator to collect gases for analysis from locations in the living body that were inaccessible to existing equipment, (ii) a complete, instantaneous, and continuous analysis of the collected gases can be obtained by connecting the cannula to a mass spectrometer, and (iii) the quantities of gas needed by the mass spectrometer are so small that gas pressures can be measured in fluids such as circulating blood, and presumably in tissues. The recordings show excellent stability and reproducibility. The membrane itself is the most critical part of the application. Further studies are needed to clarify such problems as change in permeability upon immersion of the membrane in water, effect of temperature on permeability, and the impairment of permeability in native blood which necessitated the use of anticoagulants. Attempts to use biological membranes such as frog skin, omentum, pericardium, or dura mater, have so far failed due to high water permeability.

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Antibody Formation in Nonimmune Mouse Peritoneal Cells after Incubation in Gum Containing Antigen

Abstract. *Peritoneal cells from normal mice in a semisolid medium containing sheep erythrocytes were incubated at 37°C for 2 or 3 days. During this period, hemolytic antibodies developed spontaneously. Arguments are presented that true* de novo *synthesis of antibody has taken place in previously uncommitted cells.*

Stimulation in vitro of normal cells by an antigen, in order to induce antibody production in vitro by these cells is a traditional aim of immunologists. Attempts in this direction have been more or less successful (*1*) but the best positive results have yielded only a very small amount of antibody. New methods for the detection of antibody formation by single cells (local hemolysis in gum) (*2*) provide a tool well-suited to the study of the aforementioned problem.

We tried first to stimulate peritoneal cells by incubating these cells with sheep erythrocytes (SE) in liquid medium prior to their incorporation in a gum of carboxymethylcellulose (CMC) containing SE as a detecting medium for hemolysin production. It soon became apparent that prior incubation in vitro of the peritoneal cells resulted in a rapid decrease of their viability index and in a poor yield of plaque formation by the mixed population (peritoneal cells and spleen cells) in the gum. Thus we have investigated the behavior of mixed (either spleen or lymph node cells added to peritoneal cells) or pure (spleen, lymph node, or peritoneal cells alone) cells immediately incorporated, after their collection, in the test system (CMC + SE + complement); we followed the events in the system for 3 days at 37°C (*3*).

We used CBA mice, 8 weeks old. Peritoneal cells were collected either immediately or 5 minutes after the injection of 0.2 ml of Hanks medium. Peritoneal cells were collected by washing the peritoneum with 3 ml of Hanks medium; cells from five mice were pooled for each experiment. Cells were counted and differential counts were established. Macrophage-type cells (highly polymorphic) amounted to about 40 percent of the population, the 60 percent remaining being of the lymphocytic type.

These cells, after one washing in an Eagle buffer, were incorporated, usually at a concentration of 5×10^6 cells/ml, in a gum of 2.5 percent CMC in Eagle-tris buffer containing 5×10^8 sheep erythrocytes per milliliter and 10 percent fresh guinea pig serum (*3*). A known amount (usually 0.022 ml) of this mixture was placed on microscope slides under a coverslip sealed with vaseline, and the preparation was incubated at 37°C. Ten preparations were examined for each experiment. At intervals the plaque-forming cells (PFC) were counted under low-power, dark-field microscopy, the results being ex-

Table 1. Spontaneous formation of antibody (expressed as PFC) by normal peritoneal cells in carboxymethylcellulose (CMC) containing sheep erythrocytes (SE).

Mode of cell collection		No. PFC/10^6 cells after		
Time after injection of Hanks solution (min)	Treatment of cells	3 hr	18 hr	45 hr
CMC with SE and complement				
0	None	0.9	23	205
5	None	0.9	32	133
5	None	0.9	3.6	6.4
5	None	0.9	94	566
5	None	0	71	328
5	Heat	0	0	0
CMC containing 10^{-3}M DNP				
5	None	0	0	0
Incubation in CMC at 47°C				
5	None	0	0	0
CMC containing actinomycin (2μg/ml)				
5	None	0	0	0
CMC with SE without complement				
5	None	0	0	0