Upsala J Med Sci 85: 113–124, 1980

A Gas Chromatographic Technique for Determination of Blood Flow and Metabolism in Individual Organs (with Special Reference to the Heart)

Lars Gisselsson,[1] Martin Ericsson[2] and Lennart Johansson[1]

From the Department of Anaesthesia, Malmö General Hospital[1] and the Department of Medical Rehabilitation, University Hospital,[2] Uppsala, Sweden

ABSTRACT

A method for determining blood flow, oxygen uptake and carbon dioxide release in individual organs is presented. For blood flow measurement an inert gas (N_2O) technique was used. Blood contents of O_2, CO_2 and N_2O were measured by a gas chromatographic method with use of a special vacuum chamber for extracting the gases from blood. There was a strong correlation between the contents of O_2 determined by the gas chromatographic and a spectophotometric method (correlation coefficient 0.953). Good agreement was found between CO_2 in gas samples analysed by the Scholander technique and by the gas chromatographic method. A correlation coefficient of 0.998 was obtained between the N_2O content calculated theoretically and that determined by the gas chromatographic technique. The new technique presented makes it possible to calculate blood flow in ml/100 g tissue/min, O_2 uptake and CO_2 production in an individual organ, whereby the predominant type of metabolism in the organ can be ascertained.

INTRODUCTION

Three decades ago Kety et al. (7) published a method for determining regional blood flow with use of an inert gas as indicator. This technique has been employed for measurements of myocardial and cerebral blood flow in man. Different gases have been used as indicators, e.g. nitrous oxide (7), argon (3, 13, 17) and helium (9).

In all these studies arterio-venous differences of inert gas concentrations have been measured and the amount of gas in the tissue has been calculated.

The tissue concentration of inert elements has also been determined directly by different radioactive methods. For instance bolus injection of diffusable gamma-emitters such as ^{85}Kr and ^{133}Xe into a coronary artery has been reported (8, 16). This technique is limited by difficulties in separating cardiac from extracardiac radioactivity following systemic administration of the tracer. Positron emitters such as ^{84} Rb were introduced by Bing (1). This gave an opportunity of using coincidence counting as a method for isolation of the

heart on systemic tracer administration.

A new approach was introduced by Ganz et al. (4) in 1971, when they presented the thermodilution technique for measurement of coronary sinus blood flow.

However, all these methods only measure the myocardial blood flow (ml/100 g tissue/min) or the coronary sinus blood flow (ml/min) and do not relate the flow to the metabolic needs of the myocardium.

The aim of this study was to develop a method for simultaneous measurement of blood flow (ml/100 g tissue/min), oxygen uptake (mol/100 g tissue/min) and carbon dioxide release (mol/100 g tissue/min) in an individual organ. The present report describes the use of the method on the myocardium.

METHODS

Principle

Inert gases can be administered with the inspired air. The partial pressure of a gas in the alveoli will rapidly equal that in the inspired air if the gas has a low solubility in blood. This will also be the case in arterial blood, provided that the diffusion across the alveolar membrane is prompt. The concentration of an inert gas in the venous blood of different organs will, however, vary during the saturation period. The higher the solubility of a gas in the organ, the longer will be the time for saturation. Furthermore, the time for saturation will be longer the slower the blood flow in an organ (flow/g tissue/min). The time for saturation will be a measure of the perfusion of an organ if the solubility of a gas in a certain tissue is constant.

Blood flow was calculated according to the Fick principle (13):

$$\frac{F}{t} = \frac{\pm\,(C_{VA} - C_{VE} \times \lambda_0}{\dfrac{6}{(C_A - C_V \times dt}} \times 100$$

F/t = Organ flow/time

C_{VA} = Concentration of inert gas in venous blood at the beginning of the measurement

C_{VE} = Concentration of inert gas in venous blood at the end of the measurement

C_A = Concentration of inert gas in arterial blood during the measurement

C_V = Concentration of inert gas in venous blood during the measurement

λ_0 = Partition coefficient between blood and tissue for the inert gas

6 = Specific weight of the tissue

The arterio-venous difference of inert gas has to be integrated over time. This can be done by calculating the arterial and coronary venous saturation curves of the gas. The mean arterio-venous difference is obtained by planimetric integration, which can be achieved by taking single arterial and coronary

venous samples throughout the period of saturation or desaturation. The difference in inert gas content between these samples represents the mean arteriovenous difference in N_2O content during the sampling period. The N_2O concentration is assumed to be uniform throughout the left ventricle after 2 - 5 min of N_2O breathing (5). In order to calculate the change in tissue content of N_2O, the N_2O concentration in venous blood was determined before and after the measurement.

The tissue-blood partition coefficient has to be known. For N_2O it is 0.94 at a blood haematocrit of 40 (9).

Blood sampling

Arterial blood was taken from a radial artery. For myocardial blood flow determination representative venous blood was sampled from a catheter in the coronary sinus.

Release of gases from blood

For evacuation of the gases from blood a special extraction chamber (Zeiss AG, Göttingen, BRD) was used (17). This was connected to a high vacuum pump (Edwards), which was able to produce a pressure of less than 13.3 Pa (10^{-1} mm Hg).

Release of oxygen from blood in the extraction chamber

Haemoglobin was denaturated by $K_3Fe\ CN_6$ and saponin was used for haemolysis of the red cells. 32 g of $K_3Fe\ CN_6$ and 8 g of saponin were mixed and transferred into a bottle. Distilled water was added to a volume of 100 ml (solution 1). For more complete haemolysis of red blood cells Oxidiluent (Instrumentation Laboratory, Padermo, Italy) was used (solution 2).

Release of carbon dioxide

Carbaminohaemoglobin was destroyed by $K_3Fe\ CN_6$. CO_2 in aqueous solution will form carbonic acid, which in turn will dissociate, forming hydrogen ions and bicarbonate ions. $H_2O + CO_2 \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$. In order to force the formula to the left an excess of acid was added. 8 ml of concentrated lactic acid was mixed with 92 ml of distilled water (solution 3). Solutions 1 and 3 were kept in a refrigerator and fresh solutions were mixed every week (6).

Release of inert gas

N_2O and other physically dissolved inert gases were released from blood by the vacuum.

Gas chromatographic equipment

In order to separate CO_2, N_2O, O_2 and N_2 extracted from the blood samples, and helium (He) or neon (Ne) used as tracer gas, a columns in series-across detector system was used (19). A Varian Aerograph model, series 1400 (Varian AB, Solna, Sweden), equipped with a two-filament thermal conductivity detector, was used. Hydrogen served as carrier gas. Fig 1 shows a complete block diagram of the gas chromatographic system. The system was equipped with two gas valves.

The sample loop of valve C was used for calibration purposes. Valve D connected the extraction chamber and the gas chromatographic system.

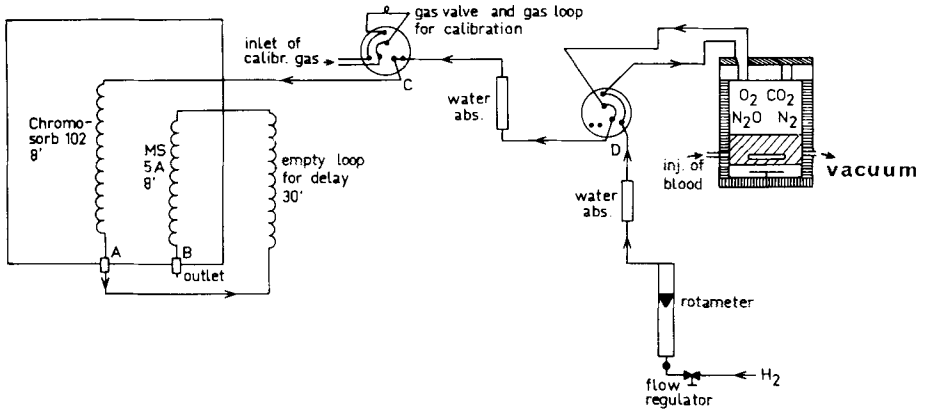

Fig 1 Block diagram of the gas chromatographic system.

In order to separate CO_2 and N_2O from O_2, N_2 and He or Ne, a stainless steel column 2.40 m long and 0.3 mm in diameter, packed with chromosorb 102, was used. These gases were analysed on detector A. The first peak thus consists of O_2, N_2 and He or Ne, the second of CO_2 and the third of N_2O. In order to delay the arrival of the gas at detector B, an empty loop 9 m long and 0.3 mm in diameter was placed between detector A and the second separation column. For separation of He/Ne, N_2 and O_2 a stainless steel column, 2.40 m long and 0.3 mm in diameter, packed with molecular sieve 5A 30/60 mesh was employed. Both CO_2 and N_2O pass through this material very slowly and can be regarded as absorbed by the column (19). This means that the column has a short life span and has to be frequently reactivated. He/Ne, O_2 and N_2 were analysed on detector B.

As both detectors were used for analysis, the polarity had to be reversed after the N_2O peak. Both columns were activated at 250°C for 5 hours and were reactivated at least once a week. The two water absorbers contained Dehydrite (Arthur H Thomas Co., Philadelphia, Pa, USA).

The gas chromatograph was connected to a CDS 111 integrator (Varian AB, Solna, Sweden) for peak detection and for calculation of peak areas. The peak areas are expressed as counts (relative area) and as per cent of total peak area. The CDS integrator was connected to a Hewlett Packard writer in order to check peak separation and baseline drift. Gas chromatographic conditions were: carrier gas flow 51 ml/min, detector temperature 100°C, bridge current 200 mA and column temperature 40°C. All four gases could be detected within 6 minutes.

Determination of gas contents in blood

Before the extraction of gases, the vacuum chamber was evacuated by the pump. 1 ml each of solutions 1, 2 and 3 (see above) were mixed in a syringe and injected into the chamber. Gas was extracted from the mixed solution by the pump, and extraction continued for one minute. The pump connection was then closed. Samples of gas (0.1 ml) were taken from the chamber for analysis in order to check the vacuum. A gas sample was taken and if no peaks were detected the vacuum was considered adequate. 5-10 ml of the blood to be analysed was then injected into the chamber and the magnetic stirrer was started. After 5 minutes the haemoglobin was completely broken down, the blood corpuscules were haemolysed, the extraction of gases was complete and the partial pressures of gases were equal in all parts of the chamber. Gas samples were then taken from the chamber and the peaks were analysed by the integrator. In order to quantify the peaks, 4 ml of a test gas containing known amounts of CO_2, O_2 and N_2O was injected into the chamber. Five minutes later the gas concentrations in the chamber were again equal in all parts. Further gas samples were taken. An increase of the peak area after injection of the test gas was related to the peak area obtained at the first analysis of each gas. From these values the gas contents of the blood sample were calculated.

The gas contents of the blood had to be corrected for ambient temperature and barometric pressure and are presented as ml gas ($273^{\circ}K$ 101kPa) per litre blood or as mmol gas per litre blood.

Helium or neon as a tracer gas

A gas chromatographic system is never quite stable. Minimal changes of flow and temperature always occur. In the extraction chamber the pressure conditions will also alter as the test gas is injected and according to the amount of blood in the chamber. This necessitates the use of tracer gas. For these analyses He or Ne was used. The tracer gas was injected into the extraction chamber before the blood, and as the content of tracer gas in the chamber was constant throughout the procedure, each peak could be related to the actual peak area of the tracer gas. For calculations, the ratio gas area/ actual Ne area was used instead of the actual gas area in question alone.

RESULTS

Validity of the method

The O_2 and N_2O contents determined by the gas chromatographic method were compared with those obtained by other available methods for measurement of gas contents in blood. This was done by regression analysis. Regression equations, correlation coefficients and standard error of estimate were

calculated.

Oxygen chemically bound to haemoglobin constitutes the major part of blood oxygen. Only very small amounts are physically dissolved. The gas chromatographic method was compared both with the Eschweiler technique and with spectrophotometric determination of the oxygen content. For the latter determination the method of Holmgren et al. was used (6). The oxygen content in blood was calculated from the equation:

O_2 content (ml x litre^{-1}) = Hb (g x litre^{-1}) x 1.39 + P_{O_2} (kPa) x 4 x 10^{-1}

Blood samples for analysis by spectrophotometric and gas chromatographic methods were taken simultaneously into heparinized, siliconized glass syringes. Both arterial and venous samples were used. The results are presented in Fig 2. The regression equation was y = 1.039 x - 6.235; correlation coefficient = 0.953 and standard error of estimate = 7.54.

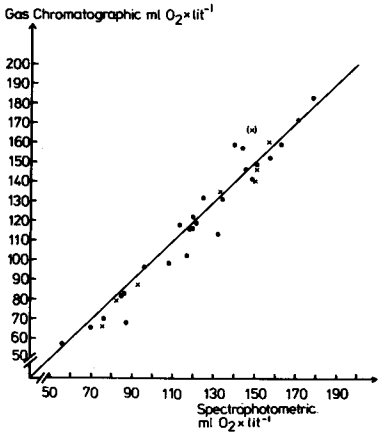

Fig 2 Correlation between O_2 content determined by the gas chromatographic method and that obtained spectrophotometrically.

For direct determination of the blood oxygen content, the Eschweiler technique, a method described by Laver et al. and Borgström et al. (11,2) was used. For these analyses a commercially available apparatus for polarographic determination of total oxygen content (Eschweiler and Co., Kiel) was used. The oxygen contents of blood samples taken from the patient at the same time were also determined by the gas chromatographic technique. Both arterial and venous samples were taken. The results are presented in Fig 3. The regression equation was y = 0.956 x + 1.915; correlation coefficient = 0.988 and standard error of estimate = 5.18.

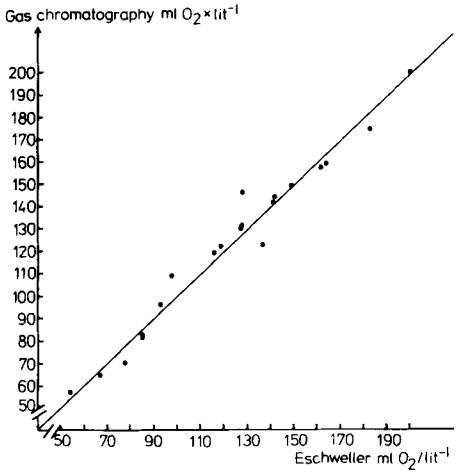

Fig 3 Correlation between oxygen content determined by the gas chromatographic method and that obtained polarographically.

The reproducibility of the method was tested by repeated analysis. A total of 26 single determinations on 13 duplicate samples taken in different syringes but at the same time were made. The values are presented in Table 1. The standard deviation of total oxygen content was 5.4 ml O_2 x litre $^{-1}$.

Table 1 .

Results of gaschromatographic determination of oxygen content		Oxygen content determined by spectrophotometric technique
ml blood	**ml O_2 x litre^{-1}**	**Theoretical values**
1	11	
94	98	96
81	83	85
86	80	86
118	114	119
98	97	118
143	148	146
146	136	149
177	171	172
136	128	134
185	182	179
67	72	76
159	158	140
158	159	163
146	146	133

The largest fraction of CO_2 in the blood is in the form of bicarbonate ion. CO_2 is also bound to haemoglobin, forming carbaminohaemoglobin. The rest is physically dissolved.

The method described by van Slyke (20) is the only generally accepted method for determination of CO_2 in blood. Other techniques have been described but these have never been widely approved (21,22). The van Slyke technique is

difficult to perform, however, and is no longer available in our hospital. Thus, we were unable to compare our method for determination of the CO_2 content in blood with another technique. On the other hand, the gas chromatographic measurement of CO_2 in gas samples gave reliable results as compared with the Scholander technique (Fig. 4), and for extraction of gas from the blood samples the same technique as described by van Slyke was used.

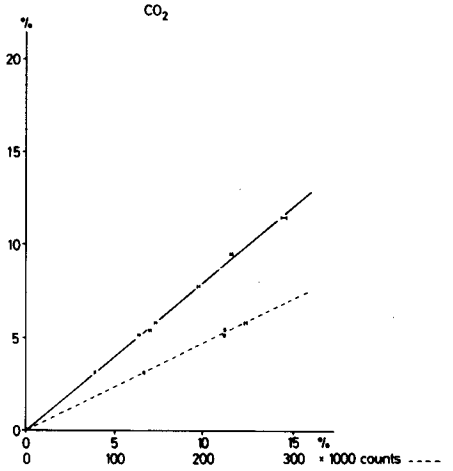

Fig 4 Relationship between CO_2 content in gas samples measured by the Scholander technique and by the gas chromatographic method.

As the determinations of the contents of O_2 and N_2O (see below) in blood proved reliable, there is no reason why the CO_2 values should have diverged. Results of determinations of the CO_2 content in blood are presented in Fig 5 together with CO_2 dissociation curves published by other authors (12,10). The agreement is as good as can be expected, as none of the values were corrected for differences in pH or Hb. CO_2 was also released from $NaHCO_3$ and this amount was determined by the present method.

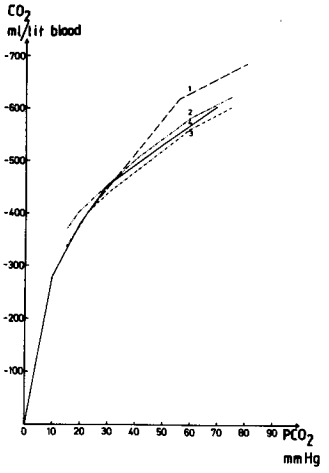

Fig 5 Analysis of CO_2 content in blood. Line 1: Values obtained with the gas chromatograpic method on fresh blood equilibrated at different CO_2 tensions. Lines 2 and 3: Analysis made with the van Slyke technique, where 2 represents values from fully oxygenated blood and 3 values from blood with low oxygen tension (Nunn, J, 12). Line 4: Analysis with the van Slyke technique presented by Knowles (10). The values presented by Nunn and Knowles represent the mean of values reported from a large number of studies.

As can be seen in Fig 6, there was a linear relationship between the amount of $NaHCO_3$ injected and the CO_2 peak area.

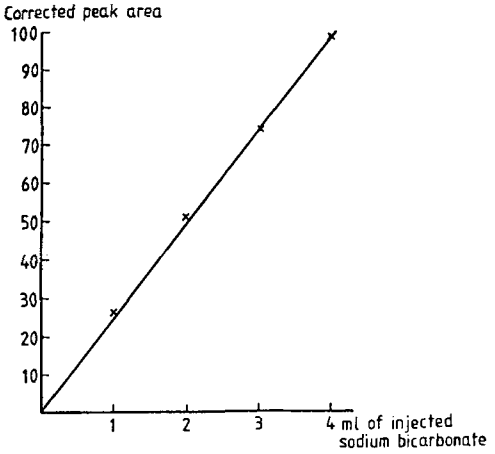

Fig 6 CO_2 released from $NaHCO_3$ and determined by the gas chromatografic technique.

As N_2O is only present in the physically dissolved form in blood, its content in the blood can be calculated, if the partial pressure of the gas in blood in known, from the formula: Content N_2O (ml x litre^{-1}) = P_{N_2O} (kPa) x S (ml N_2O x litre^{-1} x kPa^{-1}), where S is the solubility coefficient for N_2O in the solvent (all gases as STPD). Samples of human blood were equilibrated at 37°C in tonometers with N_2O tensions ranging from 20 to 100 kPa. The N_2O content of the samples was determined by the gas chromatographic technique and also calculated from the above formula. The results are plotted in Fig 7. If y = theoretically calculated N_2O content and x = N_2O content determined by the gas chromatographic technique, the regression equation is: y = 1.12 + 1.0 x; correlation coefficient = 0.998 and standard error of estimate = 7.60.

The N_2O peak must not be quantified, but the corrected value for peak area can be used directly in the equation for calculating blood flow.

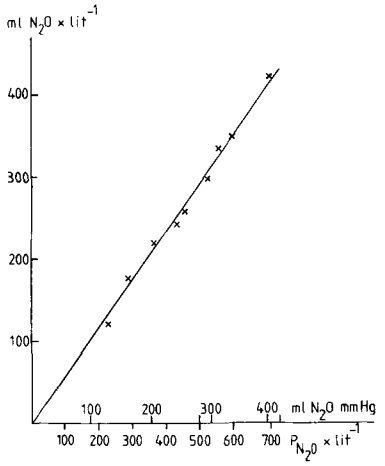

Fig 7 N_2O content in whole blood measured with the gas chromatografic technique in relation to the content calculated from the formula:
Content N_2O = P_{N_2O} x S = 4.375 ml N_2O x litre^{-1} x kPa^{-1}

DISCUSSION

The oxygen content measured with the gas chromatographic technique showed very good agreement with that obtained by other methods available in our hospital. Because of the shape of the oxygen dissociation curve, the direct method for determining the oxygen content is superior to the PO_2 technique, especially if the analysis is made on blood with a very low oxygen tension. Thus, the correlation tends to be better between the two methods for direct determination of oxygen content than it is between these methods and the indirect spectrophotometric technique in which haemoglobin analyses have to be combined with determination of oxygen partial pressure. As mentioned previously it was not possible for us to compare the gas chromatographic determination of carbon dioxide with other methods. On the other hand, the carbon dioxide dissociation curve obtained with this method shows very good agreement with the results of other authors. Moreover, in experiments in vitro with release of carbon dioxide from bicarbonate a linear relationship was found between bicarbonate content and CO_2 peak area.

Nitrous oxide was used as the inert gas in this study in spite of the fact that argon and helium are less soluble in blood. The solubility of the latter two gases is so low, however, that it is difficult to determine the exact concentration of gas with the gas chromatograph used in the present study. Moreover, nitrous oxide is easily available commercially for medical use.

It is also possible to separate helium and neon. These gases were used as an internal standard, but they could be used as inert gases. The air contains about 0.9% argon. As the present technique was used for determination of gas contents in the blood and argon is poorly soluble in blood, this gas was not separated, as this is a very difficult gas chromatographic procedure. Nitrogen is also sparingly soluble in blood, but as air contains about 80% nitrogen, the content in blood would have been large enough to disturb the analysis of other gases if nitrogen had not been separated. Nitrogen peaks were seen during the analyses, and too high nitrogen peaks were a sign of leakage in the system.

As myocardial metabolism is strictly aerobic, changes in oxygen consumption would seen to be a much better parameter of changes in the myocardial work than any cardial index. This assumption is supported by the fact that the oxygen consumption in relation to freed energy is fairly constant irrespective of whether the oxidated substratum is carbohydrate or fat. One litre of the oxygen used for oxidation of carbohydrate yields 21 kJ (5kcal). When used for oxidation of fat, it yields 20 kJ (4.8 kcal) (9). This means that the metabolism produces between 448 and 465 kJ for each mol of oxygen consumed.

The relation between carbohydrate and fat metabolism can be estimated by calculating the respiratory quotient (RQ), i.e. the ratio in mol of carbon dioxide released from the organ to oxygen taken up by the organ in one minute. When pure carbohydrate is metabolized, the quotient is 1, and metabolism of fatty acids derived from food fat gives an RQ of about 0.7 (9). Thus, if the arterio-venous differences of oxygen and carbon dioxide are known, an idea can be obtained of which is the predominant substratum for myocardial metabolism at a particular moment. As the method presented here permits calculation of the blood flow in ml/100 g tissue/min and oxygen uptake and carbon dioxide production as mol of gas/ml blood or mol of gas taken up per 100 g tissue/min, it is possible to determine the predominating type of metabolism in the organ in question, the amount of substratum oxidated and the energy yielded by the metabolism expressed per 100 g tissue/min.

ACKNOWLEDGEMENT

This work was supported by grants from King Gustaf V:s 80 years foundation.

REFERENCES

1. Bing, R.J., Bennish, A., Blumenchen, G. et al: The determination of coronary flow equivalent with coincidence counting technique. Circulation 29: 833-846. 1964.
2. Borgström, S., Hägerdal, M., Lewis, L. and Pontén, U.: Polarographic determination of total oxygen convenient in small blood samples. Scand J Clin Lab Invest 34:375-380, 1974.
3. Bretschneider, H.J., Cott, L., Hilgert, G., Probst, R. and Rau, G.: Gaschromatographische Trennung und Analyse von Argon als Basis einer neuen Fremdgasmethode zur Durchblutungsmessung von Organen. Verhandl. Deutsch ges. Kreislaufforsch., 32:267-273, 1966.
4. Ganz, W., Kohji, T., Harold, S.M., Roberto, D., Shinji, Y. and Swan, H.J. C.: Measurement of coronary sinus blood flow by continuous thermodilution in man. Circulation 44:181-195. 1971.
5. Goodale, W.T. and Hackel, D.B.: Myocardial carbohydrate metabolism in normal dogs with effects of hyperglycemia and starvation. Circulat Res 1: 509-517, 1953.
6. Holmgren, A., Hagsand, U. and Hollstein-Ströberg.: Gas and blood gas analyses. Almqvist och Wicksell, Stockholm, 1968.
7. Kety, S.S. and Schmidt, C.F.: The nitrous oxide method for the quantitative determination of cerebral blood flow in man. Theory, procedure and normal values. J. Clin. Invest. 27:476-483. 1948.
8. Klein, M.D., Cohen, L.S. and Gorlin, R.: Krypton -85 myocardial blood flow. Precordial scintillation versus coronary sinus sampling. Amer J Physiol 209:705-710, 1965.
9. Klocke, F.J.: Coronary blood flow in man, Progress in cardiovascular diseases. 19, No 2:117-166, 1976.
10. Knowles, John, H.: Respiratory physiology and its clinical application. Harvard university press, Cambridge, Mass., 1959.
11. Laver, M.B., Murphy, A.J., Seifen, A. and Radford, E.P.: Blood O_2 content measurements using the oxygen electrode. J Appl Phys 20 (5): 1063-1069, 1965.

12. Nunn, J.E.: Applied respiratory physiology, 2nd edition. Butterworsters, London, 1977.
13. Rau, G.: Messung der Koronardurchblutung mit der Argon-Fremdgasmethode. Arch Kreislaufforsch 58:322-398, 1969.
14. Wright, S.: Applied Physiology. 11th ed. Oxford Univ Press., London, 1965.
15. Scholander, P.F.: Analyzer for accurate estimation of respiratory gases in one half cubic centimetre samples. J Biol Chem 167:235-250, 1947.
16. Shaw, D.J., Pitt, A. and Friesinger, G.C.: Autoradiographic study of the ^{133}Xe disappearance method for measurement of myocardial blood flow. Cardiovasc Res 6:268-276, 1971.
17. Tauchert, V.M., Koshsiek, K., Heiss, H.W., Rau, C. and Bretschneider, H. J.: Technic der Organdurchblutungsmessung mit der Argonmethode. Z Kreislaufforsch 10:836-841, 1971.
18. Tauchert, V.M., Heiss, H.W. and Probst, R.: Extraktionskammer mit Dosierhahn für die Gaschromatographische Bestimmung des Gehaltes von Blut und farbrigen Lösungen. Z Kreislaufforsch 60:836-841, 1971.
19. Thompson, B.: Fundamentals of gas analysis by gas chromatography. Varian, Hansen way, Palo Alto, California 94303, 1977.
20. Van Slyke, D.D. and Neill, J.U.: The determination of gases in blood and other solutions by vacuum extraction and manometric measurements. J. Biol Chem 61:523-526, 1924.
21. Van Stekelenburg, G.J. and Koorevaar, G.: A gas chromatographic method for the determination of the total CO_2 content on biological fluids, by means of the head space sampling technique, based on the methan conversion principles. Clin Chem Acta 44:367-375, 1973.
22. Van Stekelenburg, G.J., de Kayzer, M.H. and Donkerwolcke, R.A.M.C.: A simple micromethod for the direct determination of the total carbon dioxide content in biological fluids. Clin Chem Acta 76 (2):161-169, 1977.

Received March 15, 1980.

Address for reprints:

Martin Ericsson, M.D.
Dept of Rehab. Med.
University Hospital
S-750 14 Uppsala 14
Sweden