The Nitrous Oxide Test
An Improved Method for the Detection of Left-to-Right Shunts

By ANDREW G. MORROW, M.D., F.A.C.S., RICHARD J. SANDERS, M.D.,
AND EUGENE BRAUNWALD, M.D.

The diagnosis of a left-to-right shunt generally depends upon the demonstration of significant differences in oxygen content among the venae cavae, the chambers of the right heart, and the pulmonary artery. The inconclusive or misleading results sometimes obtained with this method have stimulated interest in improved technics. The wide arteriovenous difference that normally exists during the inhalation of nitrous oxide has been studied in the development of a more accurate diagnostic approach. The superiority of the nitrous oxide test over the oxygen method is demonstrated.

IN THE past, the detection of intracardiac and extracardiac left-to-right shunts has been based upon differences in the oxygen content of blood obtained from the venae cavae, right atrium, right ventricle, and pulmonary artery. Since relatively large differences in oxygen content may exist in these areas in subjects without shunts, the oxygen method sometimes provides inconclusive or even misleading diagnostic information. The inhalation of nitrous oxide, an inert foreign gas, and the subsequent measurement of its concentration in arterial and right heart blood provides a new diagnostic approach in which the difficulties inherent in the oxygen method are largely obviated. This communication presents the theory, technic, and diagnostic applications of the nitrous oxide test, and a comparison of the nitrous oxide and oxygen methods in the detection of left-to-right shunts. The clinical study to be presented is an extension of the technic devised and experimentally evaluated by Callaway. 1,5

Kety and Schmidt 6 observed that immediately following nitrous oxide inhalation, cerebral tissue absorbed large quantities of the gas and a substantial arteriovenous difference existed until tissue saturation neared completion. Figure 1 demonstrates that a similar arteriovenous difference exists when pulmonary artery blood is used instead of jugular vein blood. During the first minute of inhalation the concentration of nitrous oxide in right heart blood is low, since its appearance is delayed by tissue absorption. In the presence of a left-to-right shunt, blood from the left heart, rich in nitrous oxide, raises the level in right heart blood thereby making the detection of shunts possible.

MATERIAL AND METHODS
A total of 208 satisfactory nitrous oxide tests in 184 patients in whom the diagnosis was confirmed, forms the basis of this report. Most of

\[ \text{Arterial } \text{N}_2\text{O Content} \]

\[ \text{P.A. } \text{N}_2\text{O Content} \]

\[ \text{VOL.\% } \text{N}_2\text{O} \]

\[ \text{TIME-MINUTES} \]

FIG. 1. Nitrous oxide levels in the femoral artery and pulmonary artery of a patient without a left-to-right shunt. Fifteen per cent nitrous oxide was inhaled for 10 minutes.
the patients were adults; 53 had left-to-right shunts, 43 at the atrial, 5 at the ventricular, and 5 at the pulmonary artery level. The diagnosis in the patients with shunts was established at operation in 29, by the passage of a catheter across the defect into the left heart in 19, and by retrograde thoracic aortography or dye-dilution studies with left heart injection in 5. The 131 control patients without shunts all had valvular rheumatic heart disease. This diagnosis was established by clinical examination as well as right and left heart catheterization. More than half of these patients were operated upon and the absence of a shunt was further confirmed. In all 53 patients with shunts and in 96 of the 131 patients without shunts right heart blood samples were analyzed for oxygen content.

**Technic.** Nitrous oxide tests were performed by placing the tip of a cardiac catheter in the right atrium, right ventricle, or pulmonary artery and a needle in a peripheral artery. Specimens for nitrogen blank determination were obtained before administration of nitrous oxide. The patient was instructed to breathe a mixture of 15 per cent nitrous oxide, 21 per cent oxygen, and 64 per cent nitrogen as deeply and as rapidly as possible. The gas was administered with a mask or mouthpiece and a Collins 3-way respiratory valve. Patients under general anesthesia were hyperventilated by manual compression of the breathing bag. Five or 10-ml. blood samples were then simultaneously drawn at a constant rate from the right heart and peripheral artery during the 60 seconds of gas inhalation. Early in the series another sample proximal to any shunt was collected from the vena cava or a peripheral vein. As will be indicated, this sample was found unnecessary. A 10-minute period for desaturation was allowed when the test was to be repeated in another chamber. A double-lumen catheter was sometimes employed to shorten the procedure by sampling from 2 chambers simultaneously. Blood samples for oxygen analysis were obtained after the nitrous oxide tests had been completed. In general, 3 samples each were drawn from the pulmonary artery, right ventricle, and right atrium and 2 from each vena cava. In 40 of the 149 patients in whom specimens for oxygen content were obtained, the inferior vena cava could not be entered and in 10 patients pulmonary artery blood was not sampled. The oxygen contents to be presented in figure 4 represent the mean values of all specimens obtained in each site. The mixed vena caval oxygen content in the 109 patients in whom both cavae were entered was calculated by weighting the inferior caval values as twice the superior caval values.

Oiled, heparinized syringes were employed and air contamination was prevented with mercury-filled syringe caps. Nitrous oxide content was determined by a modification of the Van Slyke manometric method. Oxygen contents were determined by the method of Van Slyke and McNeill.

**Results.** Nitrogen Blanks. Since the manometric method does not distinguish dissolved nitrogen from nitrous oxide, blank samples are necessary to determine the nitrogen content of the blood prior to each nitrous oxide test. No systematic difference was found among nitrogen blank values in systemic artery, peripheral vein, or right heart blood in any given patient. The average of the 2 or 3 blank values in each patient was therefore used in all calculations. This average value for all patients ranged from 0.50 to 1.97 volume per cent with a mean of 1.30 ± 0.18 volume per cent. No difference in blank values existed between control patients and those with shunts. Six patients in whom the difference between simultaneous blanks exceeded 0.40 volume per cent were excluded from the study, since this large difference made sampling or analytic technics suspect.

**Nitrous Oxide Sample.** The nitrous oxide sample, as used in this presentation, refers to the nitrous oxide content of the specimen less the blank value. In the control patients the range and scatter of nitrous oxide samples were widest in the peripheral vein and progressively narrowed as the pulmonary artery was reached (fig. 2).
Fig. 3. A, Top. Relation between right atrial and arterial nitrous oxide samples in patients with and without left-to-right shunts. Broken diagonal lines, RA/A ratios. B, Bottom. Relation between pulmonary artery or right ventricular and arterial nitrous oxide samples in patients with and without shunts.

As would be anticipated, the patients with left-to-right shunts had peripheral venous and vena caval samples similar to the control patients (fig. 2A and B). However, 39 of the 43 patients with shunts at the right atrial level had right atrial nitrous oxide samples between 1.06 and 3.39 volumes per cent. The highest right atrial sample among the control patients was 0.88 volume per cent. Thus, all but 4 of the patients with shunts had samples greater than the right atrial sample of any of the 83 control patients (fig. 2C). All 24 patients with shunts who had pulmonary artery or right ventricular tests had samples exceeding 0.64 volume per cent; the highest pulmonary artery or right ventricular sample of the 58 control patients was 0.47 volume per cent (fig. 2D). The systemic artery nitrous oxide samples ranged from 1.60 to 4.62 volumes per cent with a mean of 3.04 ± 0.59 volumes per cent. No systematic difference existed between the arterial nitrous oxide levels of patients with and without shunts (fig. 2E).

Relation between Arterial and Right Heart Nitrous Oxide Samples. In control patients high arterial nitrous oxide levels, reflecting better nitrous oxide uptake were, in general, associated with slightly higher right heart levels (fig. 3A and B). In patients with shunts the right heart nitrous oxide level distal to the shunt is determined to some extent by the venous level but primarily upon the quantity of shunted blood and its nitrous oxide content. Since the nitrous oxide content of shunted blood is identical to that of arterial blood, the ratio between right heart and arterial levels becomes meaningful and is therefore related to the magnitude of the shunt. In the 83 control patients, the ratio between right atrial and arterial samples (RA/A \times 100) was less than 30 per cent in all but 1, in whom it was 31 per cent. In 41 of the 43 patients with right atrial shunts the RA/A ratio was 30 per cent or more. The other 2 patients had ratios of 29 per cent (fig. 3A). In the 58 control patients with tests in the pulmonary artery or right ventricle the highest PA/A or RV/A ratio was 16 per cent. All 24 patients...
with shunts had a PA/A or RV/A ratio of 20 per cent or more (fig. 3B).

Oxygen Samples. Figure 4 presents the differences in oxygen content between contiguous sites of sampling. The oxygen content of inferior caval blood exceeded that of superior caval blood in 83 of 109 patients (fig. 4A). In control patients the range and scatter of oxygen content between consecutive cardiac chambers fell progressively as the pulmonary artery was reached. This range of difference was greatest between vena cavae and right atrium (fig. 4B). It diminished slightly between right atrium and right ventricle (fig. 4C), and was smallest between right ventricle and pulmonary artery (fig. 4D). These data confirm previous observations indicating incomplete mixing of blood in the cavae and right atrium.1-3

In all but 1 of the 43 patients with left-to-right shunts at the atrial level the oxygen content of right atrial blood exceeded that of vena caval blood. However, in 11 of these 43 patients the oxygen content of right atrial blood did not exceed that of caval blood by 1.5 volumes per cent or more. Among 93 control patients 6 had a right atrial blood oxygen content exceeding caval content by 1.5 volumes per cent or more (fig. 4B). Two of the 5 patients with left-to-right shunts at the right ventricular level and 1 of the 5 patients with shunts into the pulmonary artery did not have oxygen differences exceeding 1.0 volume per cent (fig. 4C). Among 94 control patients, 11 had increases of 1.0 volume per cent or more between the right atrium and right ventricle and 4 of 86 controls had increases of this magnitude between the right ventricle and pulmonary artery.

Comparison between the Oxygen and Nitrous Oxide Methods. In figure 5A the differences in oxygen content between vena caval and right atrial blood have been plotted against the nitrous oxide RA/A ratios in the 122 patients in whom both tests were performed. For purposes of comparison, an oxygen increase of 1.5 volumes per cent between the caval and right atrial blood and an RA/A
ratio of 30 per cent have been employed as standards for the diagnosis of a shunt at the atrial level. With these criteria there were 5 false positive tests with the oxygen method and 1 false positive test with the nitrous oxide method in the 79 control patients. In the 43 patients with proved shunts at the atrial level there were 11 false negative tests with the oxygen method and 2 false negative nitrous oxide tests. Thus, the oxygen method resulted in 16 diagnostic errors and the nitrous oxide method in 3.

It is also apparent from figure 5A that all 3 diagnostic errors in the nitrous oxide tests occurred in patients with RA/A ratios between 29 and 31 per cent, close to the diagnostic level set at 30 per cent. In contrast, 9 of the 15 diagnostic errors in the oxygen method occurred in patients in whom the oxygen differences were more than 0.5 volume per cent from the somewhat arbitrary diagnostic line. A difference of 1.5 volumes per cent was selected because it is the standard generally accepted and represented a judicious balance between false positive and false negative oxygen results. It may be noted that if an oxygen increase of 1.0 volume per cent had been employed as the diagnostic criterion there would be a total of 13 diagnostic errors, and if 2.0 volumes per cent was chosen, there would be 22 errors.

In figure 5B the differences in oxygen content between right atrial and right ventricular blood and between right ventricular and pulmonary artery blood are plotted against the nitrous oxide ratios in the 27 patients who had both tests. Diagnostic criteria selected for these areas were an oxygen increase of 1.0 volume per cent or more and an RV/A or PA/A ratio of 20 per cent or more. In the 17 control patients there were 3 false positive tests with the oxygen method and none with nitrous oxide. In the 10 patients with shunts there were 3 false negative tests with the oxygen method and none with nitrous oxide. Thus, in these 27 patients the oxygen method resulted in 6 diagnostic errors and the nitrous oxide test in none.

Discussion

Errors in the performance of the nitrous oxide test relate to the administration and uptake of the gas as well as to the collection and chemical analysis of the blood samples. An adequate uptake of nitrous oxide is essential but may be prevented by a large leak around the mouthpiece, hypoventilation, or severe pulmonary parenchymal disease. When the test is performed in an anesthetized patient, the anesthesiologist must provide hyperventilation and of course cannot employ nitrous oxide as the anesthetic agent prior to the test.

Small differences in the nitrous oxide content of superior caval, inferior caval, and coronary sinus blood and and incomplete mixing of these streams in the right atrium introduce an element of variability into the results of the test. However, this source of error is not so great as in the oxygen method, since the differences in the oxygen content of the right atrial tributaries are greater than the variations in their nitrous oxide content.

An important potential source of error is improper positioning of the catheter. When the test is performed in the right atrium, the catheter tip must be placed close to the tricuspid valve to insure that the sample will be drawn from an area distal to the entrance of any shunt at this level. Should the catheter inadvertently be passed through a defect into the left atrium, a nitrous oxide sample equal to the arterial will be obtained. Another test in the pulmonary artery or right ventricle will indicate whether a true atrial septal defect exists or whether the catheter passed through a nonshunting patent foramen ovale.

Contamination of the blood samples by air and excessive suction on the sampling syringe should be avoided, since either can falsely lower the nitrous oxide content of the sample. Blood must be drawn at a constant rate during the sampling period, since both the arterial and venous nitrous oxide contents are changing throughout this period (fig. 1). The difference between duplicate Van Slyke nitrous oxide determinations in this laboratory
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is ordinarily less than 0.10 volume per cent.

Since the errors inherent in the technics of sampling and analysis are independent of the arterial nitrous oxide level, they assume greater significance when the ratio is calculated with a low arterial sample. For example, an error of 0.20 volume per cent in a right heart sample represents 10 per cent of an arterial level of 2.00 volumes per cent, but only 5 per cent of an arterial level of 4.00 volumes per cent. Fifteen nitrous oxide tests were eliminated from the series because of arterial levels less than 1.50 volumes per cent. Seven of these unsatisfactory tests were attributed to hypoventilation. The other 8 tests were performed in cyanotic patients with right-to-left shunts. In these patients pulmonary venous blood, with a high nitrous oxide level, was presumably diluted by right heart blood with a low level and thereby lowered the arterial content. Thus, in the presence of a large right-to-left shunt, the arterial nitrous oxide content may not accurately reflect the content of blood that has been shunted from left to right. Therefore, the right heart arterial nitrous oxide ratio (fig. 3) can no longer be relied upon. However, in such circumstances, the absolute level of nitrous oxide in right heart blood (fig. 2) may still be useful in the detection of left-to-right shunt.

The vena caval and peripheral venous nitrous oxide samples were originally obtained for comparison with the sample from the right heart, much as caval and right atrial oxygen contents are compared. However, it was apparent that in the absence of a shunt the nitrous oxide content of right atrial blood was relatively constant and less than 30 per cent of the arterial content (fig. 3). When this maximum value had been established, it was no longer necessary to sample the cava or peripheral vein. Furthermore, the inclusion of caval and peripheral venous samples did not improve the diagnostic accuracy of the method.

The superiority of the nitrous oxide test over the oxygen method, as demonstrated in figure 5, is related to several factors. The oxygen content of right heart blood varies markedly among different individuals and in the same subject at different times. Therefore, blood proximal to any shunt must always be sampled. This may lead to errors in the diagnosis of atrial septal defects because caval blood is poorly mixed and laminar flow is present, particularly from the renal veins.12 The sampling of renal vein blood with its high oxygen content may mask the presence of a shunt into the right atrium. In some patients the catheter cannot be passed into the inferior vena cava. It has been demonstrated (fig. 4A) that the oxygen content of superior caval blood is generally lower than inferior caval blood. Hence, a comparison of superior caval and atrial blood could lead to the false diagnosis of an atrial shunt. In 3 of the 5 false positive oxygen tests only the superior cava could be sampled (fig. 5A). Further error in the oxygen method is introduced by the variations in the patient's physiologic state that occur during the time required for complete sampling. This source of error is also obviated with the nitrous oxide tests, since the 2 samples required are drawn simultaneously. An added practical advantage of the nitrous oxide over the oxygen method is that fewer samples and analyses are necessary.

Modifications of the nitrous oxide test involving different gas concentrations, sampling periods, and ventilatory states are being investigated. The test may be further improved by the use of other inert gases that are presently being evaluated. Since it is apparent that the nitrous oxide ratio is a function of the contribution of the shunt to total pulmonary flow, consideration is being given to the application of nitrous oxide in the quantitation as well as the detection of left-to-right shunts.

DIAGNOSTIC CRITERIA

The diagnostic criteria currently employed in the application of the nitrous oxide test may be summarized. In the right atrium,
an RA/A ratio above 30 per cent is considered diagnostic of a shunt. An RA/A ratio below 25 per cent indicates the absence of a shunt; ratios between 25 per cent and 30 per cent are considered inconclusive. A PA/A or RV/A ratio above 20 per cent is diagnostic of a shunt, while the absence of a shunt is indicated by a ratio below 15 per cent. Pulmonary artery or right ventricular tests with ratios between 15 per cent and 20 per cent are considered inconclusive. An arterial level of at least 1.5 volumes per cent is necessary for a valid test.

**Summary**

An improved method for the detection of left-to-right cardiac shunts is presented. Fifteen per cent nitrous oxide is inhaled for 1 minute while integrated blood samples are drawn simultaneously from the right heart and a systemic artery. The technic, diagnostic criteria, and sources of error of this method are presented in detail.

In 41 of 43 patients with proved shunts at the atrial level, the ratio of right atrial to arterial nitrous oxide content (RA/A) exceeded 31 per cent, the highest value observed in 83 control patients. In all 24 patients with proved shunts the ratio PA/A or RV/A exceeded 20 per cent. The highest PA/A or RV/A ratio in 58 patients without shunts was 16 per cent.

The nitrous oxide test was demonstrated to be distinctly superior to the oxygen method. In 149 patients in whom both tests were performed, there were 22 diagnostic errors on the basis of oxygen differences and 3 errors with the nitrous oxide test.

**Summario in Interlingua**

Es presentate un meliorate methodo pro le detection de derivation cardiace sinistro-dextere. Oxygen nitrose in un concentration de 15 pro cento es inhalate durante 1 minuta, e simultaneemente specimens de sanguine integrate es obtenite ab le corde dextere e ab un arteria systemic. Le technica, le criterios diagnostic, e le causas de error in iste methodo es presentate in detalio.

In 41 ex 43 patientes con demonstrate derivationes al nivello atrial, le proportion inter le oxydo nitrose dextero-atrial e le oxydo nitrose arterial exceedeva 31 pro cento (le valor maximal observate in 83 patientes de controlo). In 24 ex 24 patientes con demonstrate derivationes le proportion inter le oxydo nitrose pulmono-arterial e le oxydo nitrose arterial o inter le oxydo nitrose dextero-ventricular e le oxydo nitrose arterial exceedeva 20 pro cento. (Le valor maximal observate in 58 patientes sin derivationes eseva 16 pro cento.)

Le test a oxydo nitrose se mostrava claramente superior al methodo a oxygenu. In 149 patientes in qui ambe tests eseva executate, 22 errores diagnostic haberea resultate del test a oxygeno, 3 del test a oxydo nitrose.

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The frequency of signs and symptoms of hypopotassemia was investigated in relationship to plasma potassium levels with evaluation of the clinical status of the patients before and after administration of potassium salts. During a 7 month period 557 cases manifesting hypokalemia were encountered in 2786 potassium determinations. The incidence of plasma potassium levels below 3.5 mEq per liter was higher in females (22 per cent) than in males (18.3 per cent). The mortality of patients with hypopotassemia was 49 per cent compared to that of the total hospital fatality rate of 15 per cent. Fifty patients were selected for detailed study of serum electrolytes, causes of depletion and other clinical features. Among the disorders associated with potassium depletion, hepatic cirrhosis headed the list. Inadequate diet, infusion of potassium-free solutions, vomiting or gastrointestinal suction, diarrhea and renal disease were factors operating to lower the plasma potassium levels. Patients with the lowest potassium levels also had the lowest concentrations of chlorides and calcium and were more often alkalotic. The clinical abnormalities encountered in these patients were of the type seen in seriously ill patients with or without hypopotassemia. In order to determine which signs and symptoms were attributable to potassium deficiency, a rapid infusion of potassium was employed. The most significant changes accompanying infusion were improved mental status and increased peristaltic activity. A decrease in activity of deep tendon reflexes showed some correlation with the decrease in plasma potassium levels. The nonspecific nature of the clinical aspects of hypopotassemia in seriously ill patients is emphasized indicating the importance of electrocardiographic and electrolyte determinations in the diagnosis of this disorder.

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