cloacal gland as does transfer from long days to short days, and treatment by replacement of androgen stimulates the cloacal gland just as does exposure of the males to long days (5, 7).

These data indicate that activity of the cloacal gland is androgen-dependent, and that the cloacal protrusion is a reliable indicator of the male's gonadal response to changes in photoperiod. The size of the protrusion correlates highly not only with testicular indexes of reproductive condition but also with behavioral criteria such as copulation and crowing (7).

Thus the cloacal protrusion of a male quail can be used as a convenient external index of androgen; unlike measurement of the testes, the method requires no surgery; like the testes, the protrusion is closely related to behavioral and other physiological indicators of breeding condition. Such an index is especially useful in experiments requiring repeated measurements over time, as well as in studies (for example, in the field) in which surgery may interfere with the variables being observed.

BENJAMIN D. SACHS

Institute of Animal Behavior, Rutgers-The State University, Newark, New Jersey 07102

References and Notes

- C. A. Padgett and W. D. Ivey, Science 129, 267 (1959); J. R. Howes and W. D. Ivey, J. Amer. Vet. Med. Assoc. 140, 162 (1962); F. A. Beach and N. Inman, Proc. Nat. Acad. Sci. U.S. 54, 1426 (1965); H. Abplanalp, Na-ture 189, 942 (1961); L. Z. McFarland, B. Mather, W. O. Wilson, Amer. J. Vet. Res. 25, 1531 (1964); A. Oksche, W. O. Wilson, D. S. Farner, Z. Zellforsch. 61, 688 (1964); W. O. Wilson, H. Abplanalp, L. Arrington, Poultry Sci. 41, 17 (1962); B. K. Follett and D. S. Farner, Gen. Comp. Endocrinol. 7, 111, 125 (1966).
- 125 (1966). My inferences are necessarily limited to C. c.japonica, since the cloacal gland has been described only in this subspecies. However, it seems unlikely that the gland is a subspecific trait; as the reproductive systems of other quail are examined, the generality of my find-ings can be better assessed. A. Wolfson, Bull. Chicago Acad. Sci. 10, 1
- 3. (1954). W. H. Coil and D. K. Wetherbee, *Ohio J.*
- 4.
- W. H. Coil and D. K. Wetherbee, Ohio J. Sci. 59, 268 (1959).
 C. L. Nagra, R. K. Meyer, N. Bilstad, Anat. Record 133, 415 (1959).
 K. Ikeda and K. Taji, Sci. Rept. Matsuyama Agr. Coll. No. 13 (Nov. 1954), p. 1.
 B. D. Sachs, paper read before Western Psy-chol. Assoc., June 1965; dissertation, Univ. of Coliforcia Breakeday. 1965; dissertation, Univ. of
- choi. Assoc., June 1965; dissertation, Oniv. or California, Berkeley, 1966. Report based on part of a doctoral disserta-tion submitted to the Department of Psychol-ogy, University of California, Berkeley. Work supported by NIMH fellowship 21,586 to the supported by NIMH fellowship 21,886 to the author and by NIMH grant MH-04000 to F. A. Beach. Part of the work was done at the Berkeley Field Station for Research on Animal Behavior. I thank A. A. Blaker, Uniof California Scientific Photography tory, for the microphotographs, and versity Laboratory, R. J. Barfield for suggestions regarding the

manuscript. 18 May 1967

14 JULY 1967

Silicone Rubber: Oxygen, Carbon Dioxide, and Nitrous Oxide **Measurement in Gas Mixtures**

Abstract. Pressure changes, arising from counter diffusion of gases through a sealed silicone rubber tube, may be used to measure tension of gases in a mixture. When the tube is initially filled with one of the gases, and the mixture surrounds it, the pressure rise is related to the tension of the remaining gas.

The properties of silicone rubber include both a high permeability to all gases and a significant differential permeability between these gases (1-3). Recently it has been shown that these properties also hold true for anesthetic vapors and gases (4). While pursuing our studies on diffusion of gases and anesthetic agents through silicone rubber, we observed that when a sealed silicone rubber tube filled with a gas to which it is relatively impermeable is surrounded by a gas to which it is highly permeable, changes occur in pressure within the tube. Initially there is a rapid rise, followed by a short plateau before its slow exponential decline to the base line. These changes in pressure can be explained by the differing rates of permeation of the gases on either side of the membrane. The rapid rise would represent the inward diffusion of the highly permeating gas from without, while the plateau and slow fall would indicate progressive loss by outward diffusion of the less permeating gas. Consequently, the partial pressure of the highly permeating gas in the external mixture should be a function of both the rate and the height of the initial rise in pressure within the tube, thus providing a method for measuring the partial pressure of that gas.

To test this hypothesis, a silicone rubber tube of 50 cm length, 3 mm internal diameter, and 5 mm external diameter was attached to a sensitive pressure transducer at one end. A long inlet and a short outlet tube at the other end allowed a known gaseous mixture to be introduced and sealed off. This tube was then placed in a glass cylinder through which the atmosphere to be measured was circulated. In the first experiment, mixtures of varying concentrations of highly permeating nitrous oxide and less permeating oxygen were used as external atmospheres, while the tube was filled with 100 percent oxygen.

In order to establish an accurate base line, continuous flow of gases was maintained both inside and out-

side the tube until it had been clamped; thereafter, readings of pressure were taken at 60-second intervals. Five different concentrations of nitrous oxide in oxygen were tested, namely, 100 percent, 75, 50, 25, and 5 percent. Each was run three times (Fig. 1). We decided that the rise in pressure between the 1st and 2nd minutes after the tube was sealed would be the most accurate indicator of nitrous oxide permeation, because during this period a quasi-steady-state flux inward of nitrous oxide had been reached, following an initial unstable period during which saturation of the membrane occurs. When this reading for each gaseous mixture was plotted against the percentage of nitrous oxide in that mixture, a linear relationship was discovered (Fig. 2).

We further found that this held true for other gas mixtures. In the second experiment, nitrogen filled the tube while varying concentrations of oxygen and nitrogen were used as the external atmosphere. This was achieved by adding either of these gases to room air, which was considered, for the purposes of the experiment, as essentially a two-gas mixture. Oxygen is known to be approximately twice as permeating as nitrogen (1). The recorded rise in pressure in the tube could therefore be related to the oxygen tension in the external atmosphere. The procedure described in the first experiment

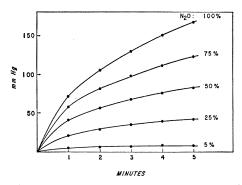


Fig. 1. Pressure within the sealed silicone rubber tube for varying concentrations of N₂O in O₂, plotted against time.

was used. In addition, the P_{0_2} of the external atmosphere was determined, by use of a standard oxygen electrode, from a sample withdrawn while the pressure changes were being recorded. Ten mixtures with varying oxygen tensions were examined, and again a linear relationship resulted when pressure rise between the 1st and 2nd minutes was plotted against the P_{O_2} (Fig. 3).

Carbon dioxide is five times as permeating as oxygen. Therefore, in the third experiment, air filled the sealed tube, while varying concentrations of carbon dioxide in air formed the external atmosphere. The $P_{\rm CO_2}$ of this external atmosphere was measured by means of a potentiometric analyzer, the method of sampling being the same as in the second experiment. Again the $P_{\rm CO_2}$ of each of ten mixtures was plotted against the pressure rise between the 1st and 2nd minutes and a straight line was obtained (Fig. 4).

Since previous investigations have already demonstrated the relative rate of permeation of gases through silicone rubber (1-3) many possible uses for this membrane have been suggested. Among these are: a membrane oxygenator in extracorporeal circulation (3), an artificial gill, for extracting oxygen from water (5), and the fractionation and purification of gases. However, we cannot find any reference in the literature to silicone rubber being used to measure tension in gaseous mixtures directly.

The method described depends upon a sealed silicone rubber tube of constant volume. To insure that this volume was not altered by changes in pressure, a relatively thick tube was chosen. At the start of each run it was necessary to have the internal gas at atmospheric pressure and 100 percent pure. This was achieved by flushing the tube with gas continuously for 30 seconds immediately prior to sealing the tube. The composition of the external gaseous mixture must remain constant throughout. This required a large volume flowing continuously over the tube. When the less permeating fraction of a given gaseous mixture is used as the internal atmosphere the whole mixture is used externally, and the rate in rise of pressure within the tube is a function of the partial pressure of the more permeating gas. This method may be modified by reversing the gases, in which case the fall of pressure is recorded, or by the use of

constant volumes separated by a silicone rubber membrane.

Certain precautions must be taken to insure accuracy. Dead space between the pressure transducer and the active part of the tube must be brought to a

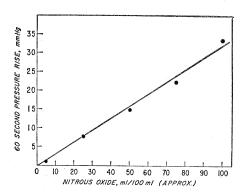


Fig. 2. Pressure rise in a 60-second period between 1st and 2nd minutes after closure, plotted against percentage of N₂O in a N₂O and O₂ mixture.

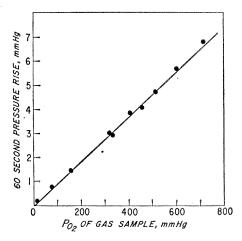


Fig. 3. Pressure rise in 60-second period between 1st and 2nd minutes after closure, plotted against the P_{0_2} of a sample taken at the time of the reading.

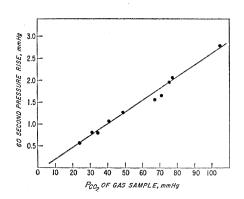


Fig. 4. Pressure rise in a 60-second period between 1st and 2nd minutes after closure. plotted against the P_{co_2} of a sample taken at time of the reading.

minimum and kept constant throughout. Minimum pressure must be used consistent with adequate flushing of the tube before closure; even under optimum conditions this flushing may interfere with readings during the 1st minute. The influence of temperature is obviously a possible source of error. However, the permeation rates of gases of various temperatures are known (1) and a correction factor could therefore be applied. Our experiments were all conducted at temperatures within the range of 22° to 27°C, and no significant differences were noted within this range. Changes in water vapor pressure in the mixture could lead to error; however, we could detect no effect when the readings were taken between the 1st and 2nd minutes.

This method, therefore, appears to offer a sturdy, portable apparatus for the rapid estimation of the partial pressure of a gas. It also has the virtue of being inexpensive. While our experiments have been limited to oxygen, carbon dioxide, and nitrous oxide, the method should be applicable to most gases. Possible applications of this method include (i) gas analysis in clinical anesthesia, (ii) respiratory care, and (iii) the estimation of oxygen and carbon dioxide in atmospheres of enclosed spaces, such as mines, submarines, or even space capsules. Recently we have modified our method to allow the measurement of partial pressure of oxygen, carbon dioxide, and nitrous oxide within the blood stream (6), thus increasing the clinical potentialities of the technique.

> H. STUART WINSEY JUDAH FOLKMAN

Sears Surgical Laboratory, Harvard (Fifth) Surgical Service, Boston City Hospital, Boston, Massachusetts 02118

References and Notes

- 1. R. M. Barrer and H. T. Chio, J. Polymer Sci. 10, 111 (1965).
- 2. S
- S. A. Reitlinger et al., Soviet Phys.-Tech. Phys. English Transl. 1, 2467 (1956).
 R. G. Buckles, thesis, Massachusetts Institute of Technology (1966).
 J. Folkman, D. M. Long, R. Rosenbaum, 3. R.
- J. Folkman, D. M. Long, R. Rosenbaum, Science 154, 148 (1966).
 B. R. Bodell, Amer. J. Med. Elec. 4, 170
- (1965) 6. J. Folkman, S. Winsey, B. Cohen, Trans.
- Amer. Soc. Artificial Internal Organs, in press Amer. Soc. Artificial Internal Organs, in press.
 Supported by NCI grant CA 08185-02 and by American Cancer Society, Massachusetts Di-vision, grant 1211-Cl. Dr. Folkman has a career development award from NCI. We thank Professor E. W. Merrill, Massachusetts Institute of Technology for reviewing the Institute of Technology, for reviewing the manuscript, and Shirley Zimmerman for permanuscript, and Shirley Zimmerman for forming the analyses of the gas samples 31 March 1967