zymatically inactive, dissociable, mixed complex:

$$\text{PPO-Cu} + n\,\text{MBT} \longrightarrow (\text{PPO-Cu})\,(\text{MBT})_n$$

Active enzyme with attached copper — Mixed complex of unknown composition

The exceptionally low concentrations of MBT made it difficult to obtain a conclusive explanation for the abrupt reversal of inhibition. The oxidation of dopamine to dopamine quinone probably proceeds slowly throughout the inhibitory period, catalyzed by trace concentrations of active enzyme in equilibrium with the inactive complex. The quinone formed then reacts with the free MBT to form the disulfide. We prepared MBT disulfide by chemical oxidation (*10*) and established that it is noninhibitory. Thus, the free MBT in the system is continually oxidized throughout the inhibition period and effectively removed from the system as the noninhibitory disulfide. Alternatively, the quinone could react with MBT to form a noninhibitory compound of thiazole and quinone (*7, 11*). In either case, as the free MBT concentration approaches zero, the equilibrium in the reaction shown will be displaced to the left, increasing the concentration of active enzyme. This further increases the rate of MBT oxidation (or reaction) and accelerates the dissociation of the inactive complex of enzyme and MBT, accounting for the abrupt reversal of inhibition. Failure to return entirely to the uninhibited rate at higher MBT concentrations may be explained by the partial inactivation of PPO by the quinone products. Phenoloxidases are notorious for such product inactivation.

Further studies of this unique inhibition, particularly the establishment of the precise structure and properties of the complex of enzyme and MBT could well provide the key to the mechanism of phenolase action.

JAMES K. PALMER*
JEREMY B. ROBERTS†

Central Research Laboratories, United Fruit Company, Norwood, Massachusetts

References and Notes

1. J. K. Palmer, in *Phenolics in Normal and Diseased Fruits and Vegetables*, V. C. Runeckles, Ed. (Plant Phenolics Group of North America, Montreal, 1965), pp. 7–12.
2. Nomenclature in this report: phenoloxidase, generic term to include all enzymes which catalyze the oxidation of phenols; tyrosinase, enzyme which catalyzes the oxidation of both mono- and diphenols; polyphenol oxidase, enzyme which catalyzes the oxidation of orthodiphenols only.
3. The stock solutions were supplied by R. T. Vanderbilt Co., New York, in approximately 50 percent solution (trademark *Nacap*). The exact concentration was determined by potentiometric titrations with 0.05*N* $AgNO_3$ (silver versus calomel reference electrode). This stock solution is stable for at least a year in the dark; dilute solutions tend to become cloudy 1 or 2 days after dilution.
4. J. K. Palmer, *Plant Physiol.* **38**, 508 (1963).
5. Crude mushroom tyrosinase was obtained from Nutritional Biochemicals Corporation.
6. K. Miyaki and J. Enomoto, *J. Pharm. Soc. Japan* **77**, 103 (1957).
7. W. S. Pierpont, *Biochem. J.* **98**, 567 (1966).
8. P. Job, *Ann. Chim. Paris* **9**, 113 (1928); S. Chakerek and A. E. Martell, *Organic Sequestering Agents* (Wiley, New York, 1959), p. 78.
9. J. Felber, T. L. Coombs, B. L. Vallee, *Biochemistry* **1**, 231 (1962).
10. A. J. Gracia, U.S. Patent 2,119,131 (1938).
11. J. L Webb, *Enzyme and Metabolic Inhibitors* (Academic, New York, 1966), vol. 3, p. 436.
12. We thank Miss Leslie Short for technical assistance.
* Present address: Division of Food Preservation, CSIRO, North Ryde, NSW, Australia.
† Present address: Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

18 May 1967

Photoperiodic Control of the Cloacal Gland of the Japanese Quail

Abstract. *Changes in photoperiod cause correlated changes in testes, cloacal glands, cloacal foam, and reproductive behavior of male Japanese quail. The cloacal protrusion may serve as a convenient external index of androgen, permitting repeated measurement without operation on or killing of the animal.*

The usefulness of Japanese quail (*Coturnix coturnix japonica*) for many types of biologic research has been demonstrated repeatedly; several studies have been made of their reproductive system and its response to various photoperiods (*1*). When males have been studied, the size of their testes has been the standard criterion of reproductive condition. However, the reproductive system of the quail has a distinctive feature, the cloacal gland, which has not been described in any other species or subspecies, or been systematically studied in this subspecies (*2*). This unique structure, present in both sexes of *C. c. japonica*, is especially noticeable in the male because in the reproductively active individual the gland swells, causing a large protrusion just posterior to the cloacal vent.

The cloacal protrusion of many male passerines, in breeding condition, appears similar in relative size and external appearance, but its cause is quite different. In passerines the protrusion results from increased convolution of the sperm ducts (*3*).

In Japanese quail there is no such increase; rather there is considerable growth of the glandular tissue lining the wall of the cloaca just posterior to the cloacal opening. Microscopically the gland appears as columnar epithelial cells lining a network of tubules or lumina. When the enlarged cloacal protrusion (Fig. 1, left) is gently compressed, a white foam, having the appearance and consistency of meringue, is expressed from the cloaca (Fig. 1, right (*4, 5*). During copulation the foam is transferred into the female's cloaca, but little else is known about it; there is still no clear evidence showing that the

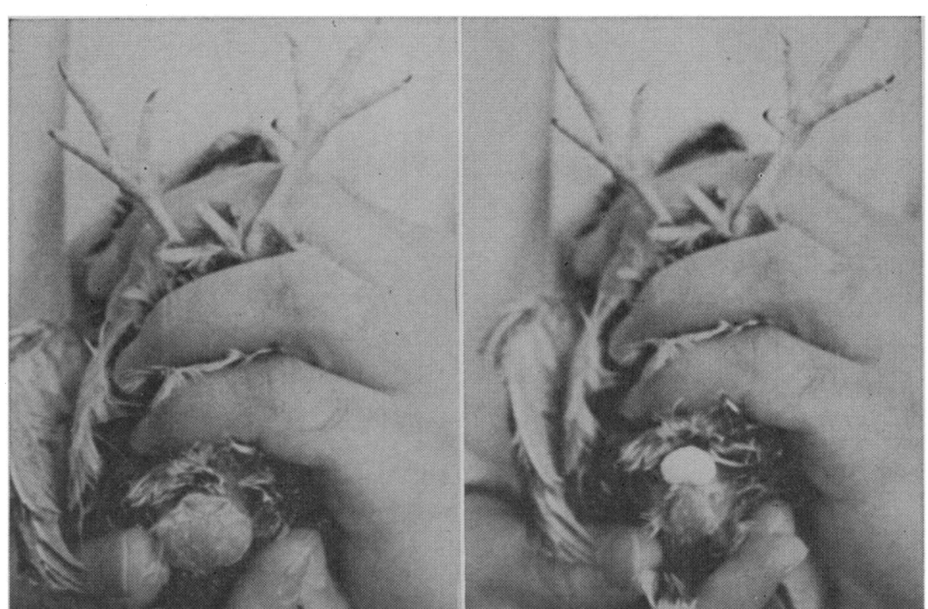

Fig. 1. (Left) External appearance of the plucked cloacal protrusion. (Right) Squeezing of the protrusion expresses the cloacal foam.

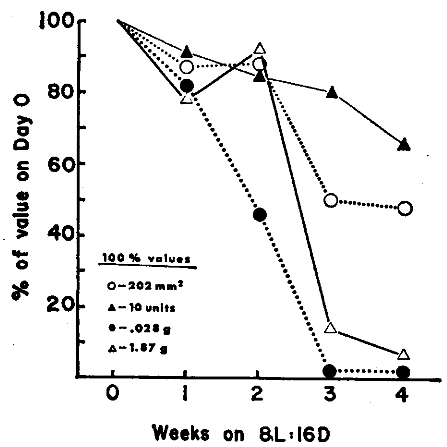

Fig. 2. Percentage decline in median area of the cloacal protrusion (○), height of columnar cells of the cloacal gland (▲), weight of foam (●), and weight of the left testis (△) after 0 to 4 weeks in an 8L:16D cycle. Inset are absolute values of the variables on day 0.

cloacal gland is really the source of the foam. No sperm are found in foam obtained by squeezing the cloacal protrusion, but foam taken from the female's cloaca after copulation is well mixed with sperm (*6*). I now report the response of the cloacal gland to changes in photoperiod; I relate this response to change in size of the testes, the common criterion of the reproductive condition of males.

Fifteen adult male quail were randomly assigned to five groups of three after having been housed for at least 2 months on a cycle of 16 hours of light and 8 hours of darkness (16L : 8D). On day 0 one group of three males were killed, and the others were transferred to 8L : 16D. Each week thereafter another group were killed; just before death each male's cloacal protrusion was measured and the foam was collected in an airtight bottle for weighing. The protrusion was measured, with an ordinary millimeter ruler, from the cloacal vent to the posterior edge and laterally at the widest point; the product of these measurements yielded the area of the protrusion. To facilitate measurement, all protrusions were plucked before the start of the experiment. After death the testes were weighed, measured, and, together with the cloacal protrusion, prepared for histology. Sagittal 10-μ sections of the protrusion, stained with hematoxylin and eosin, were examined at ×240 with an ocular micrometer. Forty columnar epithelial cells were measured from each male's gland, the cells being selected randomly except for the following constraints: (i) the cell's nucleus had to be visible, (ii) the cell's upper and lower borders had to be clear, (iii) the neighboring cells had to be approximately the same height, (iv) the cell had to be approximately perpendicular to the edge of the lumen it bordered, and (v) no two cells bordering the same lumen were measured. The heights of the cells were recorded and are reported in terms of the number of micrometer units subtended; at the magnification used, each unit equaled 1 to 2 μ.

Figure 2 shows that the amount of foam and the sizes of the cloacal protrusion, cells in the cloacal gland, and the testes all start to decrease during the 1st week and, with the possible exception of the gland cells, reach or approach low asymptote within 3 to 4 weeks of transfer from long days (16L : 8D) to short days (8L : 16D). The close relations among the several variables were confirmed by rank-order correlations: area of cloacal protrusion correlated +.83 with weight of the left testis ($p < .005$), +.73 with the size of epithelial cells ($p < .005$), and +.54 with weight of foam ($p < .05$).

Figure 3 shows histologic changes in the cloacal gland during decline in the reproductive condition. As the testes' tubules shrink and spermatogenesis stops, the cloacal gland's epithelial cells get shorter and the lumina shrink (Fig. 3, a and b), causing the decline in size of the cloacal gland and cloacal protrusion (Fig. 3, c and d). The same changes occur in reverse order, and at about the same rate, when the males regain breeding condition after transfer from short days to long days (*7*). Castration has the same effects on the

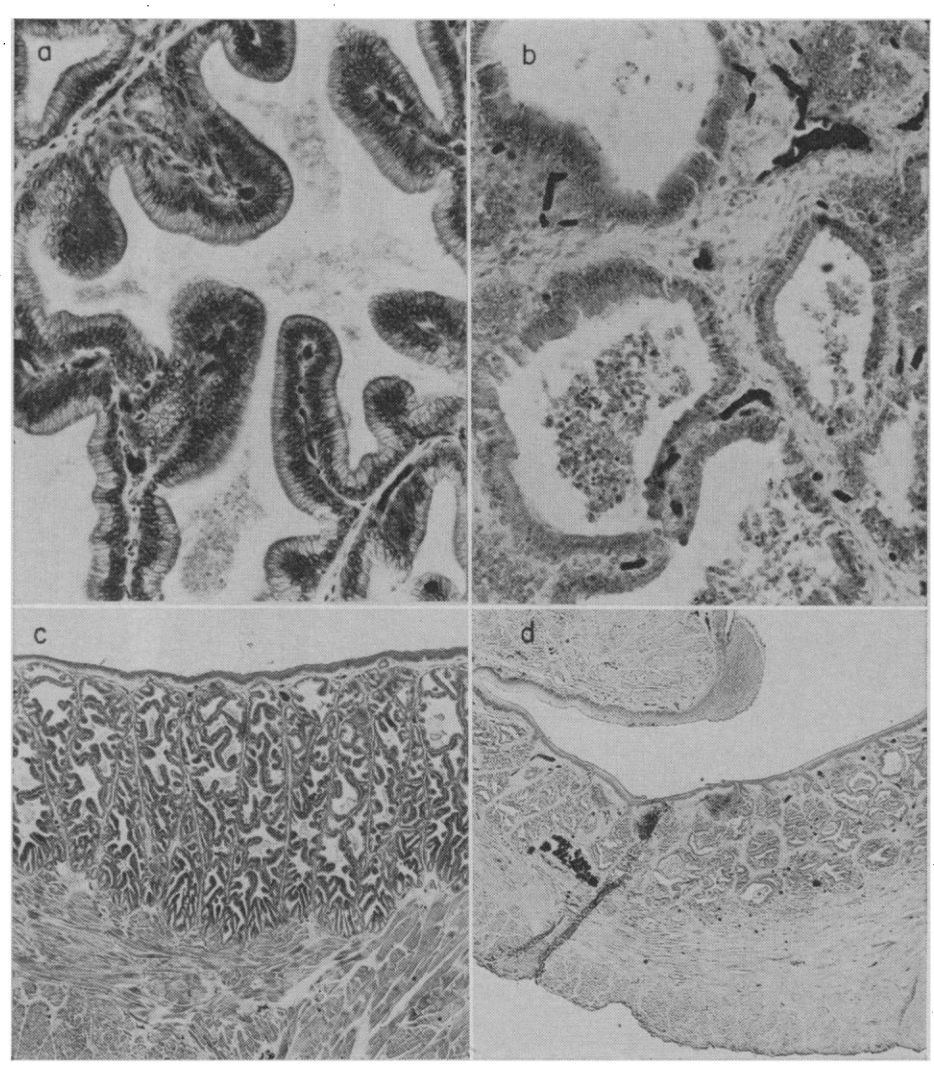

Fig. 3. Histologic changes in the cloacal glands of male Japanese quail before (a) and 4 weeks after (b) transfer from 16L:8D to 8L:16D (× 235). Cross section of cloacal gland, showing organization before transfer (c) and gross changes after transfer (d) (× 23).

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

1. 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, *Nature* **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).
2. 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 findings can be better assessed.
3. A. Wolfson, *Bull. Chicago Acad. Sci.* **10**, 1 (1954).
4. W. H. Coil and D. K. Wetherbee, *Ohio J. Sci.* **59**, 268 (1959).
5. C. L. Nagra, R. K. Meyer, N. Bilstad, *Anat. Record* **133**, 415 (1959).
6. K. Ikeda and K. Taji, *Sci. Rept. Matsuyama Agr. Coll. No. 13* (Nov. 1954), p. 1.
7. B. D. Sachs, paper read before Western Psychol. Assoc., June 1965; dissertation, Univ. of California, Berkeley, 1966.
8. Report based on part of a doctoral dissertation submitted to the Department of Psychology, University of California, Berkeley. Work supported by NIMH fellowship 21,586 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, University of California Scientific Photography Laboratory, for the microphotographs, and R. J. Barfield for suggestions regarding the manuscript.

18 May 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 outside 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

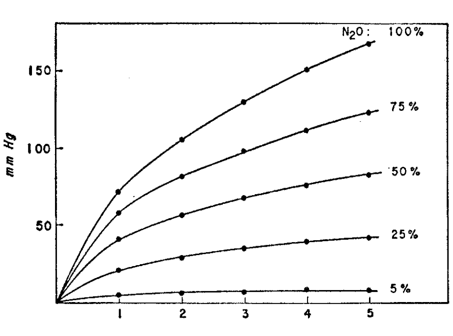

Fig. 1. Pressure within the sealed silicone rubber tube for varying concentrations of N_2O in O_2, plotted against time.