Breathing While Trotting

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Breathing is most easily studied while an animal is resting, and indeed we know most about breathing under these conditions. But breathing while resting is breathing at its least intense. To see breathing when the demands on it are greatest, we must study it in animals running fast.

Dennis Bramble and David Carrier did just that, in pioneering work at the University of Utah that was published in 1983 (*1*). They showed that galloping dogs and horses take one breath per stride, this breath always coming (at least in horses) at the same point in the stride. The ideas that this observation suggested have guided much subsequent research. Now Bramble, working this time with Farish Jenkins of Harvard University, is forcing us to think again in a report in this issue of *Science* (*2*). This time, the emphasis is neither on resting nor on galloping (the fastest gait) but on trotting, the most used gait of dogs and the one used for traveling at moderate speeds.

While research in breathing was confined to resting animals, the mechanism seemed straightforward. The thoracic cavity, which contains the heart and lungs, is separated from the abdomen by the diaphragm, a musculotendinous partition which is domed, concave toward the abdomen. Contraction of the muscle of the diaphragm flattens it, enlarging the thoracic cavity (so drawing air into the lungs) and displacing the abdominal viscera caudally. When the muscle of the diaphragm relaxes, the air is driven out of the lungs by their elastic recoil, aided if necessary by contraction of the abdominal muscles. The effect of contraction of the diaphragm is supplemented by enlargement of the rib cage, driven by the intercostal muscles.

In running, the observed synchrony of breathing with leg movements suggests a much more passive role for the diaphragm, with breathing being driven largely by the movements of locomotion. The synchronization is widespread: Quadrupeds ranging from gerbils to rhinoceros take one breath per galloping stride (*3*) and wallabies take one breath per hop (*4*). Bramble and Carrier (*1*) suggested several possible mechanisms, among which their visceral piston hypothesis seemed particularly attractive. The diaphragm has elastic properties (*5*), so the abdominal viscera can be thought of as a spring-mounted mass and must have a natural frequency of vibration. If this matches the stride frequency, the accelerations and decelerations of the body that occur in each stride will excite the resonance and drive breathing very effectively. Consistent with the hypothesis, galloping mammals and hopping wallabies use the same stride frequency at all speeds: To go faster, they take longer strides.

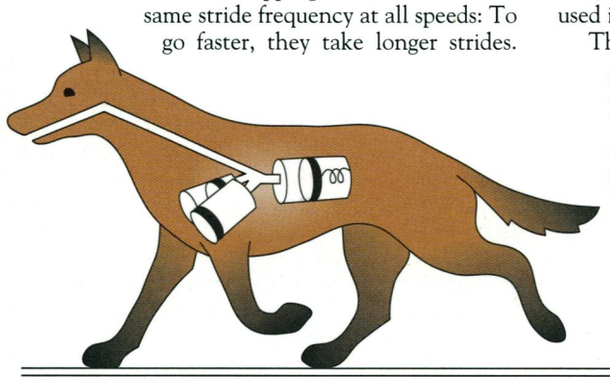

ILLUSTRATION: T. SMITH

Running on all cylinders. Three parts of the lungs of a trotting dog are ventilated asynchronously.

Mathematical modeling confirmed that the phase relation between breathing movements and the stride is consistent with the visceral piston hypothesis for wallabies (*6*) but not for horses (*7*). The breathing of horses seems more likely to be driven by the bellows action of the bending and extension of the back, a distinctive feature of galloping.

Both the visceral piston mechanism and the bellows mechanism assign passive roles to the diaphragm, but another recent observation suggests an active role (*8*). Bundles of muscle fibers from mouse, rat, and rabbit diaphragms were made to lengthen and shorten sinusoidally, while being stimulated at appropriate stages of the cycle. They delivered maximum power output when the frequency of the length changes matched the galloping stride frequency: Diaphragm muscle seems to be adapted to do work at the frequency of galloping.

Bramble and Jenkins (*2*) have now used cineradiography to observe the movements of the diaphragm of trotting dogs. (The position of the diaphragm is apparent in x-ray pictures because the lungs are highly transparent to x-rays.) They find that the diaphragm moves forward and back not once but twice in each stride cycle. This is consistent with a visceral piston mechanism because in trotting (unlike galloping) the trunk suffers two almost identical decelerations and re-accelerations in each stride. However, it requires the visceral piston to have a high resonant frequency. The stride frequencies of mammals at preferred trotting speeds are about 0.85 times the galloping frequency (*9*), so the frequency of breathing while trotting is 1.7 times the galloping frequency. That accords well with an observation that the resonant frequency of the thorax of anesthetized dogs, measured in experiments designed to simulate panting, is 1.7 times the galloping stride frequency (*10*). If breathing in galloping mammals is driven by the bellows mechanism (*7*), it seems reasonable for the visceral piston to be tuned to the frequency used in trotting.

The x-ray pictures show that, even in steady trotting, the diaphragm does not move forward and back through the same amplitude in successive cycles. It seems that its muscles become tense (this could be checked by electromyography) at intervals of one to four stride cycles. This apparently restricts the diaphragm's movements, making groups of a few large diaphragm oscillations alternate with groups of a few smaller ones. Records of airflow through a mask show, surprisingly, that this modulation of the oscillations of the diaphragm is not reflected by modulation of airflow. The explanation seems to be that both anterior-posterior and dorsoventral movements of the diaphragm and viscera affect the volume of the lungs. The periodic contractions of the diaphragm muscles may restrict only the anterior-posterior component of the movements driven by locomotion. The main effect of these periodic contractions may be to ensure that different parts of the lungs are ventilated in turn.

It became clearer that different parts of the lungs must be considered separately when Bramble and Jenkins (*2*) took dorsoventral x-ray films. These showed that, whenever a forepaw is on the ground, the same side of the thorax is compressed where the scapula presses on it. In trotting, the forepaws are set down alternately, so that the two sides of the thorax are compressed alternately. They are each compressed once in each stride, but the diaphragm makes two cycles of movement per stride. Thus, we must now think of the breathing apparatus not as one piston in a cylinder but as three interconnected cylinders, each with its own piston (see figure). Two of these cylinders are the left and right

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anterior (apical) lobes of the lungs, driven by the forces on the forelegs. These two cylinders work in antiphase to each other, both at the stride frequency. The third cylinder represents the posterior (diaphragmatic) lobes of both lungs, driven by the oscillations of the abdominal viscera at twice the stride frequency. The whole complicated system is driven by the movements of trotting.

This complex pattern of ventilation may be an almost inevitable consequence of the dynamics of trotting. It may, however, bring positive benefits. If all the lobes of the lungs filled and emptied simultaneously in a simple tidal flow, the bronchi would be dead space: Air that was still in a bronchus at the end of inspiration would be breathed out again without entering the alveoli where gas exchange occurs. Asynchronous ventilation of the lobes must make air move between them, flushing out the connecting bronchi. The angles at which the bronchi meet (schematically indicated in the diagram) must tend to promote air movement between lobes. These angles have been thought to be important in ensuring one-way circulation of air through the lungs of birds (*11*).

The most pressing question now seems to be: What are the physiological consequences of these complex patterns of ventilation? The modulation of diaphragm oscillations presumably confers an advantage, for there is nothing inevitable about the contractions of the diaphragm. The asynchronous ventilation of the lobes of the lungs may be unavoidable in a trotting animal, but its consequences should also be explored. We need to know about the flow of air and the fluctuations of oxygen concentration in the major airways, phenomena that will not be easy to investigate.

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To Be^{2+} or Not To Be^{2+}: Immunogenetics and Occupational Exposure

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The lungs are engaged in a Sisyphean struggle with the environment. With every breath, we inhale unwanted particles and gases, most of which are innocuous but others of which injure the lungs through their effects on the immune system. Scavenger phagocytes in the lungs can engulf the foreign particles and present them as antigens to T lymphocytes, triggering an antigen-specific cellular immune response and lung inflammation. Nowhere is this confrontation between the environment and human immunologic defense as apparent as in occupational situations in which workers are exposed to immunotoxins. Only a subset of these exposed workers develop disease, likely because environmentally induced lung disease results from a complex interaction of the toxin with the genetic constitution of the individual. But proof of this hypothesis has been thwarted by an inability to identify the genetic features that help account for an individual's risk.

In this issue of *Science* (p. 242), Richeldi and co-workers break the stalemate by describing a genetic marker in workers who have an immunologic lung disease resulting from inhalation of the metal beryllium (*1*). They observed that 32 of 33 occupationally exposed workers with chronic beryllium disease (CBD) exhibit the amino acid glutamate in a potentially critical location (position 69) in a cell-surface glycoprotein that participates in antigen recognition. Of those beryllium-exposed workers who did not have CBD, only 30% have glutamate in this position. The authors present convincing evidence that this small difference in the genetic sequence in the major histocompatibility complex (MHC) allele HLA-DPβ1 identifies humans who, if exposed to beryllium, are at increased risk of developing CBD.

CBD is a 20th-century, man-made disorder that continues to occur in diverse industries—high-technology ceramics, electronics, dental alloy preparation, nuclear weapons, metal extraction, and aerospace (*2*). Exposed individuals become sensitized to beryllium and accumulate pathologic clusters of immune cells called granulomas around beryllium particles in the walls of alveoli (*3*). Blood lymphocytes from individuals sensitive to beryllium proliferate in vitro when cultured in the presence of beryllium salts. This reaction is the basis of the beryllium lymphocyte proliferation test (BeLT) that is now used to make a specific diagnosis of CBD (*4*) and for detecting early CBD in industrial screening programs (*5*).

What does the association of CBD with the MHC allele HLA-DPβ1-Glu^{69} tell us about the mechanism of the disease? We now know that if a person is exposed to beryllium, he or she has an increased risk of developing CBD, but we do not yet know how this occurs. Thus far the HLA-DPβ1-Glu^{69} marker is only guilty by association. But its location in a key region of an immunologically essential locus suggests that it may directly contribute to the unusually strong immunological response of these individuals to beryllium.

As shown in the figure, the human MHC locus (HLA), includes a class II D region that is subdivided into multiple subregions, at least three of which participate in the presentation of antigens to T lymphocytes—HLA-DR, -DQ, and -DP. Each D subregion contains genes encoding the α and β chains of heterodimeric, cell-surface glycoproteins that present antigens to CD4+ (helper) T lymphocytes. These HLA class II molecules are highly polymorphic so that they can embrace a wide variety of antigens in their antigen binding groove and present them to diverse T lymphocyte antigen receptors, triggering antigen recognition (*6*, *7*). Amino acids located at key positions along the α-helical portions of these HLA heterodimers dictate which peptide antigens can bind. Even single amino acid substitutions in these regions may alter the shape of the HLA-peptide binding pocket sufficiently to change its specificity. In patients with CBD, Richeldi and co-workers found a glutamate (instead of a positively charged lysine) at position 69, likely one of the hypervariable sites of the HLA-DP allele (*7*). This glutamate could allow the presentation of beryllium, likely bound to an intracellular peptide, and the generation of an antigenic response. Perhaps the other common allele—containing the lysine—cannot accommodate this hypothetical complex. Thus, the HLA-DPβ1-Glu^{69} molecule may not only be a genetic marker for CBD, but may par-

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