Trace amounts of amino sugar were present in all the samples. In the fossil oolites, the nonprotein amino acid alloisoleucine was present. The ratio of alloisoleucine to isoleucine is about 0.05 which is approximately the same ratio as found in fossil shells of comparable age (*8*).

The Great Salt Lake oolites were serially dissolved to sample the outer and inner portions of the organic matrix. No significant differences in composition were detected. The second acid treatment of Great Salt Lake oolites exposed some nuclei, but they were composed entirely of mineral grains, mainly quartz.

In addition to natural oolites, artificial oolites, obtained from a water-processing plant in Homestead, Florida, were analyzed. In cross section they do not exhibit the concentric pattern of natural oolites and they do not contain any amino acids at the 10^{-10} mole level, either free or combined. Only natural oolites, therefore, appear to contain a proteinaceous matrix.

Several attempts have been made to grow oolites in the laboratory under a variety of conditions (*9*). Evaporation of seawater, addition of excess carbonate, and bacterial cultures caused precipitation of $CaCO_3$. These precipitates, however, did not resemble natural oolites. Bacteria have also served as nuclei for precipitation of $CaCO_3$ by concentrating calcium ions on their cell surface (*10*). There is no evidence that natural oolites formed in this manner.

Most of the organic matrix in oolites consists of uncharacterized organic matter which appears to be distributed throughout the carbonate phase. Most investigators, whether supporting a physicochemical or biochemical origin, consider the matrix to be an algal product (*4*, *5*), probably a cellular secretion which forms a coating on the oolite grain or nucleus. The presence of calcareous algae as one of the very few proliferating organisms, and the only carbonate-precipitating organism, in the Great Salt Lake supports the view that algae are the source of the organic matter in oolites. Proponents of an inorganic origin consider this organic matter to be simply occluded or trapped in the growing oolite (*11*). As Chave (*2*) has noted, organic coatings on mineral grains may be a common occurrence in the sedimentary environment.

The composition of the organic matrix in oolites, however, suggests that it may influence the deposition of $CaCO_3$. Molluscan shells, bones, and other biological hard parts contain protein as the substrate upon which the inorganic phase is formed (*12*). The organic matrix in these biochemical systems causes formation of the mineral either by concentrating the appropriate ions on charged sites on the protein or by providing a set of specific templates upon which the mineral nucleates. It has been suggested that the acidic amino acids in the protein of mineralized tissues are important in concentrating calcium because of their polar side groups (*12*).

Artificial oolites, formed by physicochemical precipitation, do not have an organic matrix. The presence in natural oolites of a proteinaceous matrix with a high concentration of acidic amino acids strongly suggests that the organic matter may influence the precipitation of $CaCO_3$ by concentrating calcium ions and it may be a prerequisite to natural oolite formation. Calcareous algae are a possible source of the protein. Natural oolites do not appear to form simply as physicochemical precipitates.

RICHARD M. MITTERER
Geosciences Division,
Southwest Center for Advanced Studies,
Dallas, Texas 75230

References and Notes

1. R. A. Berner, *Science* **159**, 1965 (1968).
2. K. E. Chave, *ibid.* **148**, 1723 (1965).
3. J. W. Graham and S. C. Cooper, *Nature* **183**, 1050 (1959); J. W. Graham, *Science* **129**, 1428 (1959).
4. W. D. Nesterhoff, *C. R. Hebd. Seances Acad. Sci. Paris* **242**, 1047 (1956); A. Rothpletz, *Amer. Geol.* **10**, 279 (1892).
5. H. A. Lowenstam and S. Epstein, *J. Geol.* **65**, 364 (1957); N. D. Newell, E. G. Purdy, J. Imbrie, *ibid.* **68**, 481 (1960); R. N. Ginsburg, R. M. Lloyd, K. W. Stockman, in *The Sea*, M. N. Hill, Ed. (Wiley, New York, 1963), p. 554.
6. The oolites were collected off the coast of North Carolina and are similar to oolites dated at about 26,000 years described by O. H. Pilkey, D. Schnitker, D. R. Pevear, *J. Sediment. Petrology* **36**, 462 (1966).
7. The instrument is a custom-made amino acid analyzer based on modifications by P. E. Hare [*Fed. Proc.* **25**, 709 (1966)] of the system described by D. H. Spackman, W. H. Stein, S. Moore, *Anal. Chem.* **30**, 1190 (1958).
8. P. E. Hare and R. M. Mitterer, *Carnegie Inst. Wash. Year B.* **65**, 362 (1966).
9. P. H. Monaghan and M. L. Lytle, *J. Sediment. Petrology* **26**, 111 (1956); C. H. Oppenheimer, *Geochim. Cosmochim. Acta* **23**, 295 (1961).
10. L. J. Greenfield, *Ann. N.Y. Acad. Sci.* **109**, 23 (1963).
11. A. J. Eardley, *Amer. Ass. Petrol. Geol. Bull.* **22**, 1305 (1938).
12. P. E. Hare, *Science* **139**, 216 (1963); K. M. Wilbur, in *Physiology of Mollusca*, K. Wilbur and C. Yonge, Eds. (Academic Press, New York, 1964), vol. 1, p. 243; E. T. Degens, D. W. Spencer, R. H. Parker, *Comp. Biochem. Physiol.* **20**, 553 (1967).
13. Supported by NASA grant NSG 269-62. I thank M. L. Jensen, R. D. Perkins, O. H. Pilkey, and H. F. Nelson for providing samples; I thank S. E. Moiola and M. Bilelo for technical assistance. Contribution No. 80, Geosciences Division, Southwest Center for Advanced Studies.

23 September 1968; revised 12 November 1968

Receptors Sensitive to Carbon Dioxide in Lungs of Chicken

Abstract. *Receptors responsive to the removal of carbon dioxide from ventilatory gas were demonstrated in the lungs of the chicken. Apnea was induced in 0.64 second after rapid lowering of respired carbon dioxide in the absence of blood flow through the lungs. Afferent impulses from the receptors are conducted centrally in the vagi.*

Exact location of functional peripheral chemoreceptors that influence respiration in the bird is unknown despite the presence of tissue that appears to be similar to that in the mammalian carotid body (*1*). Chickens are, however, acutely sensitive to changes in ventilatory CO_2 (*2*), which suggests the presence of CO_2-sensitive chemoreceptive tissue. Furthermore, speed of the response suggests that the receptor site may be near or in the lungs. We, therefore, attempted to locate the rapidly responding CO_2-sensitive receptors.

Mature, male, White Leghorn chickens, secured in dorsal recumbency, were

Table 1. Respiratory response to rapid reduction of P_{CO_2} in the respiratory system. The response time is a measure of the time between entry of CO_2-free gas into the trachea and the first indication of an alteration in sternal movements.

Conditions	Response time (sec)	Birds (No.)	Trials (No.)
Control	0.50 ± 0.20*	34	276
Extracorporeal loops in brachiocephalic arteries	0.58 ± 0.22	6	127
Extracorporeal loops in aorta	0.52 ± 0.22	3	18
Lung circulation occluded	0.64 ± 0.22	10	27
Bronchi cannulated at lung hilus	0.47 ± 0.13	2	17
Unilateral thoracic vagotomy	0.78 ± 0.32	15	51
Bilateral thoracic vagotomy	4.5 ± 1.3	15	71

* Mean ± standard deviation.

anesthetized with sodium pentobarbital. Thoracic and abdominal cavities were exposed midventrally, and unidirectional, artificial respiration was begun with 94 percent air and 6 percent CO_2. The unidirectional respirator and water-spray humidifier were described previously (*3*).

Carbon dioxide was added to the gas stream at the tracheal cannula. The respiratory gas entered the trachea as a steady stream, passed through the lungs, and exited to the atmosphere via the ruptured air sacs. This ventilating procedure allowed the bird to alter its respiratory movements without altering the gas flow through the lungs. Because of the unique structure of the avian respiratory system, there is essentially no anatomical dead space with this ventilating procedure. Thus, the preparation afforded control of CO_2 tension (P_{CO_2}) at gas exchange surfaces of the lungs and, hence, complete regulation of blood P_{CO_2} (*4*). Five to six percent CO_2 was added to the ventilating gas to produce normal blood P_{CO_2} levels and, hence, normal respiratory rate and amplitude. The rate of gas flow used was 4000 ml/min, approximately seven times normal minute volume (*5*). The increased flow insured that endogenous CO_2 would not appreciably alter the CO_2 content in the air passageways of the lungs.

Flow of CO_2 was stopped suddenly by closing a solenoid valve in the CO_2 line, which deflected the time line of the recorder. Due to the residual volume of the tracheal cannula (7 ml) beyond the point where CO_2 entered the gas stream, gas free of CO_2 was delayed from reaching the lungs by about 0.11 second after the CO_2 line was closed, so this time was subtracted from the respiratory response times observed after the solenoid valve closed.

Respiratory response to the removal of CO_2 was measured from changes in sternal movements with a strain gauge attached to the tip of the sternum (*6*). Time between CO_2 removal and beginning of the response, defined as the point on the respiratory curve where the slope was unmistakably altered from its normal pattern, was taken as an indication of the rapidity with which the bird sensed and responded to the changed stimulus. Blood pressure was recorded from the right sciatic artery.

When CO_2 was abruptly removed from the ventilatory gas mixture, apnea began within 0.50 second (Fig. 1A and Table 1). When CO_2 was returned to the ventilatory gas, respiratory response was delayed, and the return to normal respiratory amplitude was gradual. Blood pressure showed a transient rise within 5 seconds after removal of CO_2 (*7*).

Circulation time in the chicken has been estimated as 2.8 seconds (*8*), suggesting blood flow might be fast enough to enable blood deficient in CO_2 to reach extrapulmonary chemoreceptors and account for the rapid response. To test this idea, we inserted extracorporeal loops of tubing (Clay Adams P.E. 320, 1.6 m in length) into both brachiocephalic arteries to effectively delay circulation time to all cranial structures by from 4.5 to 13.5 seconds. If the receptors in question were located cranial to the brachiocephalic arteries, in the region of the proposed carotid bodies, and if they were stimulated by chemical changes in the blood, the respiratory response to removal of CO_2 should be delayed the same as the increased circulation time. Placing such a delay circuit in the arterial system at that location did not retard respiratory response to abrupt removal of ventilatory CO_2 (Table 1). The respiratory response was therefore not caused by stimulation of structures cranial to the brachiocephalic arteries.

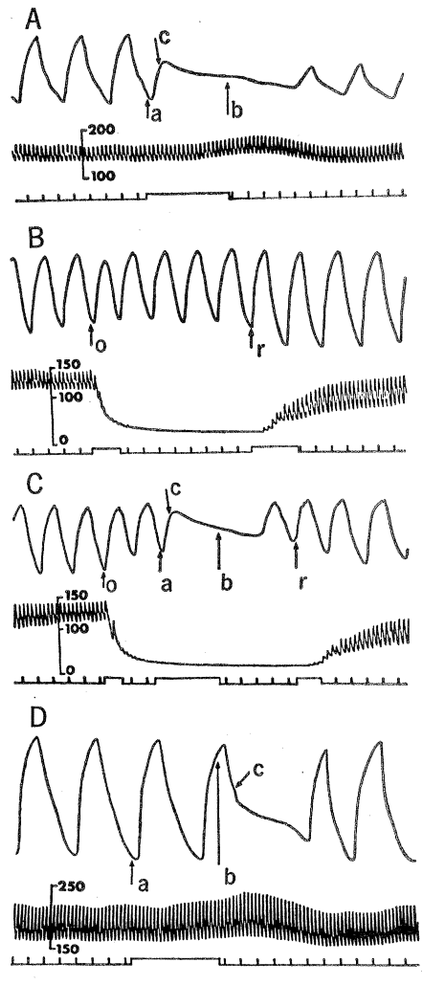

Fig. 1. Respiratory responses to elimination of CO_2 from ventilatory gas and to occlusion of pulmonary blood flow. Top trace in each record represents respiratory movements (inspiration up, expiration down); middle trace is blood pressure; and lower trace is time line in seconds. (A) Control response to CO_2 elimination. At *a*, CO_2 was removed from the ventilatory gas; *b*, CO_2 was restored to the ventilatory gas; and *c*, the respiratory response began. (B) Control response to brief occlusion of the pulmonary vessels. The right pulmonary vessels were ligated. At *o*, the left pulmonary artery and vein were occluded, and at *r*, the occlusion was removed. (C) Response to elimination of CO_2 from the respiratory gas following occlusion of pulmonary circulation. Symbols are the same as in parts A and B. (D) Response to elimination of CO_2 from the ventilatory gas after bilateral thoracic vagotomy. Symbols are the same as in part A. In this case the response occurred in 5.9 seconds after CO_2-free gas reached the trachea.

To determine if the receptors might be in the circulatory system caudal to the heart, we placed an extracorporeal loop in the aortic arch which increased circulation time to caudal structures by 2.5 to 6 seconds. Rapid respiratory response to abrupt elimination of CO_2 was still unaffected (Table 1). These two experiments indicated that the response was being mediated by chemosensitive tissue located somewhere between the trachea and the vessels cranial and caudal to the heart.

A more precise localization was accomplished by determining the respiratory response time to removal of ventilatory CO_2 after temporary, complete occlusion of the pulmonary arteries and veins of both lungs. Two methods were used to occlude the vessels. (i) Strings were looped around each pulmonary artery and vein, and tension was exerted until the decrease in blood pressure indicated complete vascular occlusion. This had very little effect on respiratory movements for several respiratory cycles in many birds. In other birds, however, the procedure induced apnea. (ii) The vessels of the right lung were ligated and the circulation to the left lung was temporarily occluded with hemostats. This only slightly reduced respiratory amplitude (Fig. 1B), which usually increased shortly after the occlusion.

After establishing that transient prevention of entry and exit of blood from the lung did not markedly alter respiratory movements, we abruptly removed ventilatory CO_2 2 to 4 seconds after vascular occlusion. This caused rapid

apnea (Fig. 1C and Table 1). When 6 percent CO_2 was returned to the respiratory gas mixture before pulmonary blood flow was reestablished, respiratory movements began to return to the normal cyclic pattern. These results indicated that the receptors sensitive to CO_2 were either in the air passageways below the tracheal cannula or in the lungs.

We eliminated the possibility that the receptors were in the extrapulmonary air passageways by cannulating the bronchi at their points of entry into the lungs, thereby eliminating the trachea and extrapulmonary bronchi from the ventilating gas stream. After this, response to CO_2 change remained rapid (Table 1), indicating that the receptors were not in the extrapulmonary air passageways. It thus appears likely that the receptors are in the lungs.

The nerve supply to the CO_2-sensitive receptors was also investigated. Unilateral thoracic vagotomy on either the right or left side at the point of bifurcation of the recurrent nerve only slightly delayed rapid respiratory response time to removal of CO_2 from the ventilating gas (Table 1). Bilateral vagotomy at the same level, however, markedly delayed the response time (Fig. 1D and Table 1).

In these tests, the vagi were sectioned well below the carotid bodies so their nerve supply supposedly remained intact. Elimination of the rapid response to CO_2 removal after bilateral vagotomy indicates that these nerves contain afferent fibers that innervate the CO_2-sensitive receptors in the lungs. That apnea is greatly delayed, but not eliminated, by bilateral vagotomy suggests additional CO_2-sensitive areas in the chicken.

Our results support recent evidence that respiratory amplitude greatly increases after administering 15 percent CO_2 to the separately ventilated, vascularly isolated left lung of the chicken (*9*). Receptors located in the lungs of mammals are sensitive to veratridine and associated alkaloids but have been identified as stretch receptors (*10*). These receptors are not sensitive to changes in carbon dioxide or oxygen tensions in blood (*11*). Attempts to establish the existence of stretch receptors in lungs of the chicken have so far been unsuccessful (*12*).

D. F. PETERSON
M. R. FEDDE

Neuromuscular Laboratory,
Department of Physiological Sciences,
College of Veterinary Medicine,
Kansas State University, Manhattan

References and Notes

1. W. Kose, *Arch. Mikr. Anat.* **69**, 563 (1907); G. Muratori, *Arch. Ital. Anat. Embriol.* **30**, 573 (1933); J. F. Nonidez, *Anat. Rec.* **62**, 47 (1935); D. S. Chowdary, thesis, University of Edinburgh (1953).
2. W. A. Heistand and W. C. Randall, *J. Cell. Comp. Physiol.* **17**, 333 (1941).
3. R. E. Burger and F. W. Lorenz, *Poultry Sci.* **39**, 236 (1960); M. R. Fedde and R. E. Burger, *ibid.* **41**, 679 (1962).
4. P. J. Ray, thesis, Kansas State University (1966).
5. H. S. Weiss, H. Frankel, K. G. Hollands, *Can. J. Biochem. Physiol.* **41**, 805 (1963).
6. M. R. Fedde, R. E. Burger, R. L. Kitchell, *Poultry Sci.* **42**, 1212 (1963).
7. Blood pressure was not studied; however, when absence of CO_2 was maintained for 30 seconds or longer, transient blood pressure rise accompanied both the removal and the readdition of CO_2 to the respiratory gas.
8. S. Rodbard and A. Fink, *Amer. J. Physiol.* **152**, 383 (1948).
9. R. E. Burger, *Fed. Proc.* **27**, 328 (1968).
10. G. S. Dawes and J. H. Comroe, Jr., *Physiol. Rev.* **34**, 167 (1954).
11. D. M. Aviado, Jr., T. H. Li, W. Kalow, C. F. Schmidt, G. L. Turnbull, G. W. Peskin, M. E. Hess, A. J. Weiss, *Amer. J. Physiol.* **165**, 261 (1951).
12. M. R. Fedde, R. E. Burger, R. L. Kitchell, *Poultry Sci.* **40**, 1401 (1961); P. D. Sturkie, *Avian Physiology* (Cornell Univ. Press, Ithaca, N.Y., ed. 2, 1965), pp. 170-172.
13. Contribution No. 49, Department of Physiological Sciences, Kansas Agricultural Experimental Station. Supported by PHS grant GM-01362-04 and NSF grant GB-3594.

3 September 1968; revised 23 October 1968

Separation of Cellular from Extracellular Controls of Drinking in Rats by Frontal Brain Damage

Abstract. *Rats that had undergone removal of the frontal portion of the brain, including the olfactory bulbs, did not drink, or drank less than controls, when subjected to cellular dehydration. These same animals drank normally in response to extracellular volume reduction. Rats from which only the olfactory bulbs had been removed drank normally in both tests. An essential part of the neurological system mediating drinking produced by cellular dehydration, but not by volume reduction, therefore lies within the frontal cortex or immediate subcortical tissue.*

Recent studies of thirst have provided substantial evidence for two components of regulatory drinking, or drinking related directly to body-water deficits. This may be contrasted with nonregulatory drinking which is independent of an animal's hydrational state, for example, prandial drinking, schedule-induced drinking, and some aspects of solution drinking (*1*). One component of regulatory drinking is cellular dehydration. The other is extracellular hypovolemia or a reduction in serum volume (*2*). These components operate independently and when activated simultaneously produce a simple additive effect (*3*).

This independence makes it unlikely that regulatory drinking is subserved by a single neuroanatomical substrate. There may be a neural system for each component. This report shows an anatomical separation of these systems in the rat and it demonstrates that the integrity of the frontal pole area (*4*) is necessary for precise control of drinking induced by cellular dehydration but not for drinking produced by a reduction of serum volume.

Fourteen adult female Sherman albino rats, seven of which had their frontal poles bilaterally ablated by subdural suction (*4*), served as subjects in these experiments. The operated rats were not tested for at least 2 weeks after surgery; at test time their body weights and food and water intakes did not differ from normals. All rats were maintained with free access to Purina lab pellets and distilled water except for the hour before testing (when the pellets were removed in an attempt to insure water balance at the inception of the experiment) and during the test period.

Cellular dehydration was produced by an intraperitoneal injection of 1*M* NaCl (2 percent of body weight). Distilled water was available immediately and the amount drunk was measured at half-hour intervals for the ensuing 6 hours.

The course of drinking in response to cellular dehydration is presented in Fig. 1. All of the normal rats responded

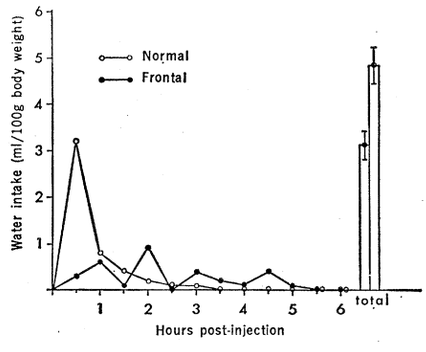

Fig. 1. Mean half-hourly water intake (ml/100 g of body weight) for normal rats and rats with removal of frontal cortex and subcortical tissue. Cumulative intakes (± 1 S.E.) are shown at the right.