of heat-exchanger bath temperature and flow rate showed a heat-sink capacity of the four-pronged fork averaging 0.2 cal/sec. The average temperature difference between the input and output lines measured from the surface of the fork was 3°C. Unfortunately, this value is subject to the errors produced by the thermal gradient across the top surface of the fork. Because of the high pressures (intermittent pressures of over 100 lb/in.^{2} at flow rates of 70 cc/min occur) and the small diameter of the flow lines, thermistors could not be placed in the liquid system. The heat-source capacity of any area of brain varies with the blood flow, and the blood flow is quite temperature-dependent. The feedback aspects of this dynamic system and the requirements of relatively steady states for accurate calorimetry necessitate separate measurements of the heat-sink capabilities of the fork and the thermal gradients produced in brain. Therefore, the value given above (0.2 cal/sec) may not be a true representation of the heat-source properties of the brain.

The relationship between low temperature and nervous activity has been extensively investigated. Temperatures below 20°C abolish electroencephalographic activity in mammalian brains (*10*), reduce cerebral metabolic rate to less than one-fourth of normal (*11*), and decrease acetylcholine production by choline acetylase to one eighth of normal (*12*). The freezing point of brain is about −1.5°C and temperatures for permanent damage of peripheral nerve and of brain appear to be in the range of 0° to 8°C (*13*).

Suitable cooling forks and other devices can be designed to block off almost any mass of tissue within the brain. Further extensions of the principle of cooling in two and three dimensions in the depths of the brain should allow analysis of the behavioral effects of ablations and of sections in the (relatively) intact animal. The technique shows promise of usefulness in the analysis of brain mechanisms of learning and memory, in the determination of sites of action of behaviorally active drugs, and in providing the potential for reversible "trial" neurosurgery of mass lesions in man (*14*).

ROBERT BYCK*
PAUL DIRLIK

Clinical Neuropharmacology Research Center, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, D.C.

References and Notes

1. G. S. Dawes, J. C. Mott, J. G. Widdicombe, *J. Physiol. London* **115**, 258 (1951).
2. E. Balthasar, *Deut. Z. Nervenheilk.* **176**, 173 (1957); G. F. Rowbotham, A. L. Haigh, W. G. Leslie, *Lancet* **1**, 12 (1959); L. Reis, J. Tytus, *Bull. Mason Clin.* **14**, 20 (1960); M. Dondey, L. Rey, J. LeBeau, *Rev. Neurol.* **103**, 220 (1960); M. Tanche, J. Chatonnet, M. Cabanac, *J. Med. Lyon* **42**, 13 (1961); I. S. Cooper, *J. Am. Med. Assoc.* **181**, 600 (1962).
3. A. Ommaya and M. Baldwin, *Neurology* **12**, 882 (1962).
4. H. T. Hammel, J. D. Hardy, M. M. Fusco, *Am. J. Physiol.* **198**, 481 (1960); B. Andersson and B. Larsson, *Acta Physiol. Scand.* **52**, 75 (1961).
5. V. H. Mark, J. C. Chato, F. G. Eastman, S. Aronow, F. R. Ervin, *Science* **134**, 1520 (1961); W. Trendelenburg, *Arch. Ges. Physiol.* **133**, 305 (1910); **137**, 515 (1911). (Translation on file at the library of the National Institutes of Health).
6. The requirements for a refrigerant in this system are (i) low freezing point, (ii) boiling point above 37°C, (iii) low viscosity at low temperatures, (iv) high thermal conductivity and specific heat, (v) compatibility with Millipore filters, (vi) availability of an inexpensive anhydrous preparation.
7. P. Dirlik and R. Byck, *Rev. Sci. Instr.* **33**, 1472 (1962).
8. The temperatures on the surface of the fork tines within the brain are higher by about 10°C, as seen in Fig. 2. This puts them in the "safe" range (compare *12*).
9. A 16-mm silent motion picture film of a typical experiment and the equipment used is available on special request from the authors.
10. B. Woodhall and D. H. Reynolds, *Proc. Soc. Exptl. Biol. Med.* **97**, 194 (1958); J. C. Callaghan, D. A. McQueen, J. W. Scott, W. G. Bigelow, *Arch. Surg.* **68**, 208 (1954).
11. J. Field, F. A. Fuhrman, A. W. Martin, *J. Neurophysiol.* **7**, 117 (1944).
12. A. S. Milton, *J. Physiol. London* **142**, 25P (1958).
13. J. D. McQueen, in *Physiology of Induced Hypothermia*, R. Dripps, Ed. (Natl. Acad. Sci. Publ. 451, 1956), p. 243; D. Denny-Brown, R. D. Adams, C. Brenner, M. M. Doherty, *J. Neuropath. Exptl. Neurol.* **4**, 305 (1945).
14. We thank William Wright, John Buchanan, and Harry Marshall for their assistance. The temperature measurement data were obtained with the expert advice and assistance of John K. Cullen, Jr., who is investigating the thermodynamic properties of this cooling system in the brain. This is a preliminary report.

* Present address: Department of Pharmacology, Albert Einstein College of Medicine, New York 61.

30 January 1963

Cyanogenic Glandular Apparatus of a Millipede

Abstract. *The cyanogenic secretion of the polydesmoid millipede* Apheloria corrugata *(Wood) is discharged from paired, serially arranged glands, each consisting of two compartments. In one compartment is stored an undissociated cyanogenic compound, and in the other, a chemical factor that triggers cyanogenesis. The gland is constructed in such a way that the contents of the two compartments are mixed, and cyanogenesis is initiated, at the very instant of discharge. The stored cyanide precursor may be mandelonitrile.*

Certain polydesmoid millipedes produce a cyanogenic secretion (*1–3*). The glands are unquestionably defensive in function. A discharge occurs only in response to traumatic stimuli, and the secretion is strongly repellent to ants and some other predators (*2, 4*). Neither the structure of the glands nor the mechanism controlling cyanide emission has been properly understood. The purpose of this note is to present experimental evidence for the hypothesis (*2*) that the glands store an undissociated cyanogenic precursor, and that cyanide emission is triggered at the moment of discharge by addition of a catalyst (*5*).

Apheloria corrugata (Wood) is an aposematically colored polydesmoid millipede. The glands, like those of other polydesmoids (*2, 6*), are serially arranged in pairs, one pair to each of most body segments. Their openings are visible as tiny pores on the notal lobes. Each gland has two compartments: an inner relatively large membranous reservoir, and an outer rigid vestibule (Figs. 1–4). The reservoir leads into the vestibule by way of a narrow duct, the terminal portion of which is tightly occluded by a spring-like valvular infolding of the duct wall (see *d*, Figs. 3 and 4). A muscle, originating on the body wall, inserts on this infolding (see *e*, Figs. 2 and 4) and serves to open the valve, thus clearing the duct lumen when the contents of the reservoir are discharged. The vestibule leads directly to the outside through a permanently opened orifice (see *c*, Figs. 1, 3, and 4). The entire glandular apparatus is lined with cuticle. The cuticle is overlaid with a variously modified secretory epithelium. There are no compressor muscles around the reservoir, so that the discharge must be effected by other means (*7*).

To test the supposition that the reservoir holds a stored undissociated cyanogenic compound, and the vestibule supplies an agent that initiates cyanogenesis at discharge, a series of qualitative tests were made on the contents of individual excised glands which had been spotted on filter paper. Several millipedes were killed by freezing, and while frozen, the cuticle of the body wall was chipped away, to expose

the glands. After severing the muscle that operates the prevestibular valve, each gland was lifted with fine glass rods and exposed to air until the fluid adhering to its outsides dried. This brief desiccation did not noticeably decrease the volume of the fluid contents of the gland. However, the narrow prevestibular duct shriveled and collapsed, thereby providing a leak-proof juncture at which both compartments could be separated without loss of fluid. The isolated reservoirs and vestibules were then transferred singly to filter paper; they were punctured and drained, and the emptied sacs were removed.

Spot-testing for free hydrogen cyanide by means of benzidine acetate–copper acetate reagent (*8*) gave a weak positive test (blue coloration) for the contents of both the vestibule and reservoir if the tests were made within seconds after the compartments were drained on the paper. If the secretion spots were first briefly exposed to air, a positive test was no longer obtainable, indicating that the initial free hydrogen cyanide had dissipated and was not being replaced at a detectable rate. If the aired secretion sites were subsequently spot-tested with a dilute solution of emulsin in the benzidine reagent, then the contents of reservoirs, but not those of vestibules, yielded an instantaneous strong positive reaction. The reservoirs evidently contain an undissociated, emulsin-sensitive, cyanogenic compound (*9*).

To demonstrate the role of the vestibule, excised reservoirs and vestibules were drained on filter paper as before, and the spots were exposed to air until free hydrogen cyanide could no longer be demonstrated by the benzidine test. The spotted sites were then individually cut out of the paper, pressed together in pairs one over the other, and each double disc was tested with benzidine reagent. The test was negative when the reservoir contents or vestibular contents had been paired with their own kind, but intensely positive where the match was between the contents of a vestibule and a reservoir. Thus, contact of the contents of the two chambers causes the liberation of hydrogen cyanide. Since the reservoir is the demonstrated source of undissociated cyanogenic compound, the factor that causes the emission of hydrogen cyanide must arise from the vestibule.

The discharged secretion of *Apheloria* contains benzaldehyde and hydrogen cyanide, but no free sugar (*3*); this suggests that the cyanide precursor in the reservoir is mandelonitrile (the cyanohydrin of benzaldehyde), rather than a glycoside. By thin layer chromatography (*10*) it was shown that mandelonitrile is indeed present in the secretion, and that after discharge it dissociates, since there was a progressive increase of free benzaldehyde in the exposed secretion. The four samples chromatographed were: fresh secretion (plated within a minute after discharge), aged secretion (stored in capillary tubing for one hour after discharge), authentic mandelonitrile, and authentic benzaldehyde. Both samples of secretion showed a pair of spots, corresponding precisely to those of the two authentic samples. In the fresh sample, the mandelonitrile spot was by far the more intense; in the aged sample the predominance was reversed. Authentic mandelonitrile showed no comparable dissociation on storage (*11*).

The hydrogen cyanide is released from the secretion gradually, rather than only at the moment of discharge. Solutions of silver nitrate, renewed every 5 minutes, were used to trap the vapors from a flask containing the freshly discharged secretion of four millipedes. White precipitate of silver cyanide (identified by treating with acid, and categorizing the liberated gas as hydrogen cyanide by the benzidine test) was still being formed when the experiment was discontinued after 30 minutes.

The sensitivity of the contents of the reservoir to emulsin, which is a mixture of glycosidases, does not mean that the stored precursor must be a glycoside, since mandelonitrile is itself highly sensitive to emulsin, as indicated by the immediate intense blue color that developed at the periphery of paper-spotted droplets of authentic mandelonitrile treated with emulsin in benzidine reagent. Amygdalin (the glucoside of mandelonitrile) is far more resistant to emulsin than mandelonitrile or reservoir contents, and required repeated reapplication of the reagent

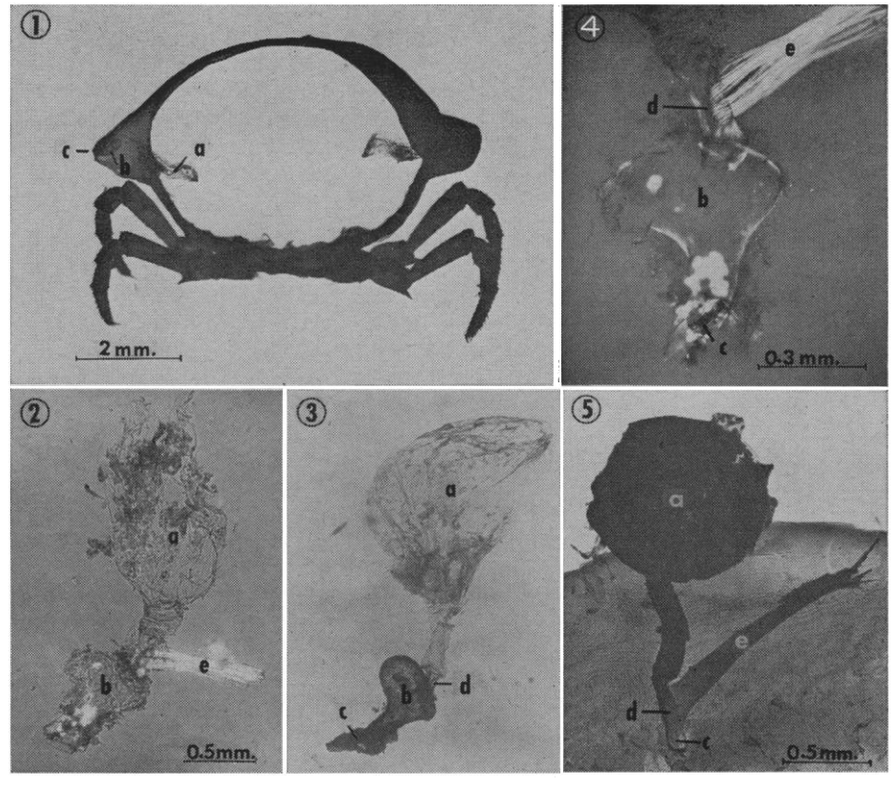

Fig. 1. *Apheloria corrugata* (Wood): isolated segment, KOH-treated and consisting of cuticle alone, with the complete left gland exposed. Fig. 2. Same: freshly isolated gland (partly polarized light). Fig. 3. Same: cuticular framework of isolated gland (KOH-treated). Fig 4. Same: close-up of vestibule and of the muscle-operated prevestibular valve (partly polarized light). Fig. 5. *Narceus gordanus* (Chamberlain): single gland (notice absence of vestibule in glandular apparatus of this quinone-secreting spiroboloid millipede). *a*, reservoir; *b*, vestibule; *c*, external opening; *d*, valvular cuticular infolding; *e*, muscle that operates the valve.

mixture to induce even a faint blue color.

The vestibular contents have an immediate dissociating effect on mandelonitrile, but not on amygdalin. No blue color developed when droplets of benzidine reagent, applied to filter paper, were allowed to spread toward adjacent droplets of mandelonitrile, or of amygdalin in concentrated or dilute aqueous solution. But when drained vestibule contents were interposed between reagent and mandelonitrile, so that the reagent as it spread eluted the secretion and carried it toward the mandelonitrile; then a blue color developed instantly on contact. Under the same circumstances, amygdalin showed no signs of dissociation. In its proven susceptibility to immediate dissociation by the contents of the vestibule, the precursor in the reservoir again resembles mandelonitrile rather than the glucoside.

The discharged secretion is an emulsion. The continuous phase is miscible with water, turns blue cobaltous chloride paper pink, and stains blue with benzidine reagent. It is probably an aqueous solution of hydrogen cyanide. The clear droplets of the discontinuous phase presumably contain benzaldehyde (pink stain with Schiff's reagent) together with residual undissociated mandelonitrile and some hydrogen cyanide. The liquid in the reservoir is also an emulsion, the discontinuous phase of which presumably contains the stored mandelonitrile. If the cyanogenic compound were a glycoside, one would expect a single aqueous phase.

The nature of the vestibular catalyst remains unknown. However, the paper-spotted vestibular contents, even after exposure to air at room temperature for 4 hours, liberate hydrogen cyanide from mandelonitrile. Heating to 130°C for 20 minutes destroys this property.

The defensive gland of *Apheloria* is an admirably refined weapon. The storage of mandelonitrile provides the organism with a convenient way of retaining relatively large quantities of hydrogen cyanide in a stable form. In the absence of catalyst, and with no opportunity for the hydrogen cyanide to escape, but little dissociation would be expected (*12*). At discharge, the addition of the vestibular catalyst forces the gradual liberation of hydrogen cyanide. The essentials of the mechanism may be no different in other polydesmoid species, but in some the precursor might be a glycoside rather than a cyanohydrin (*2*).

Among millipedes, a two-compartmented gland may be an exclusive feature of the order Polydesmida. The glands of other forms studied, including spiroboloids, spirostreptoids, juloids, and chordeumoids (*4, 13, 14*), have a typical reservoir and an efferent duct with a terminal muscle-operated valvular infolding, but they lack a vestibule (Fig. 5). None of these species are cyanogenic, and the absence of a second compartment in the gland may signify that the compounds they secrete—quinones and phenols (*14, 15*)—are stored as such in the reservoirs and do not require chemical activation when they are discharged.

T. EISNER, H. E. EISNER, J. J. HURST
F. C. KAFATOS,* J. MEINWALD
Departments of Entomology, Chemistry, and *Animal Husbandry, Cornell University, Ithaca, New York*

References and Notes

1. W. M. Wheeler, *Psyche* **5**, 422 (1890); L. M. Roth and T. Eisner, *Ann. Rev. Entomol.* **7**, 107 (1962).
2. M. S. Blum and J. P. Woodring, *Science* **138**, 512 (1962).
3. H. E. Eisner, T. Eisner, J. J. Hurst, *Chem. Ind. London* **1963**, 124 (1963).
4. F. C. Kafatos, Honor's Thesis, Cornell University (1961).
5. Supported by grant AI-02908-04 of the U.S. Public Health Service. We thank Prof. John Law, Department of Biochemistry, Harvard University, for helpful criticisms.
6. M. Weber, *Arch. Mikroskop. Anat.* **21**, 468 (1882).
7. Compression of the reservoir must be sudden, and sometimes almost complete, since the secretion droplet that appears at the gland opening emerges instantly, and sometimes reaches a volume (0.8 μl) closely approximating the estimated capacity of the reservoir. The reservoir is not maintained under sustained intrinsic pressure; cutting the prevestibular duct of an exposed gland does not cause a sudden emptying.
8. F. Feigl, *Spot Tests in Organic Analysis* (Elsevier, New York, 1960), p. 365.
9. The initial traces of hydrogen cyanide detected in vestibular contents might reflect slight contamination from the reservoirs during excision of the glands.
10. Chromatoplates coated with Silica Gel G, with benzene:chloroform (5:1) as solvent system, and 2,4-dinitrophenylhydrazine in phosphoric acid as spray reagent after development.
11. Mandelonitrile was undetected in our earlier work (*3*) because it probably dissociated to benzaldehyde and hydrogen cyanide during storage in vitro. In vapor phase chromatography it would have remained undetected even in fresh samples, since at the column temperature used (126°C), it also dissociates.
12. J. W. Baker, G. F. C. Barrett, W. T. Tweed, *J. Chem. Soc.* **2**, 831 (1952); C. K. Ingold, *Structure and Mechanism in Organic Chemistry* (Cornell University Press, Ithaca, N.Y., 1953), p. 676.
13. T. Eisner, unpublished observations on *Orthoporus;* P. Rossi, *Z. Wiss. Zool.* **74**, 64 (1903).
14. T. Eisner, J. J. Hurst, J. Meinwald, *Psyche,* in press.
15. A. Béhal and M. Phisalix, *Bull Muséum Hist. Nat. Paris* **6**, 388 (1900); A. Monro, M. Chadha, J. Meinwald, T. Eisner, *Ann. Entomol. Soc. Am.* **55**, 261 (1962); R. Trave, L. Garanti, M. Pavan, *Chim. Ind.* **41**, 19 (1959).

* Present address: Biological Laboratories, Harvard University, Cambridge, Mass.

26 December 1962

Hydrogen-Water Vapor Mixtures: Control of Hydrothermal Atmospheres by Hydrogen Osmosis

Abstract. *Experiments at 700°C and 800 bars total pressure demonstrate positive deviations from ideality for mixtures of hydrogen and H_2O gases. The deviations are greater than predicted with Stockmayer's method. The composition of the mixture and the fugacity of hydrogen are controlled by diffusing hydrogen through metallic membranes. The results give the fugacities of both H_2O and oxygen.*

An experimental technique has been devised which gives simple and continuously variable control of the fugacities of hydrogen, oxygen, and H_2O in hydrothermal experiments by means of controlled diffusion of hydrogen through metallic membranes. The pressure of pure hydrogen gas is an independent variable in addition to temperature and total pressure.

Consider a homogeneous gas phase containing only the constituents H_2, H_2O, and O_2, which is separated from an external source of H_2 by means of a membrane permeable only to H_2. The equilibrium constant for the reaction

$$H_2\,(g) + \tfrac{1}{2}\,O_2\,(g) \rightleftarrows H_2O\,(g)$$

$$K_f = \frac{f_{H_2O}}{f_{H_2} \cdot f_{O_2}^{1/2}} \qquad (1)$$

where g is a gas is well known for temperatures to 1500°K (*1*).

In this reaction it is evident from the equilibrium constant that at a given temperature if the fugacities of two of the constituents are specified, the third is determined and the total pressure of the gas mixture must be fixed. Conversely, if the fugacity (f) of hydrogen is arbitrarily controlled by means of osmotic equilibrium with the external reservoir and the total pressure of the gas mixture is also controlled, then f_{H_2O} and f_{O_2} are fixed. Unless the mixture is ideal, however, values cannot be assigned to f_{H_2O} and f_{O_2} without additional information. Several procedures might be followed to evaluate f_{H_2O} and f_{O_2}, but one stands out in simplicity. For nearly all conditions of interest in hydrothermal work f_{O_2} is numerically small and the gas phase can be assumed to be essentially a mixture of H_2 and H_2O. On this assumption it is necessary only to determine the molar proportion of H_2 and H_2O at any given temperature and total pressure. With this data and f_{H_2}, values of f_{H_2O} can be determined