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   There have been several reports of stimulus storage at low temperature. G. E. Fogg [in *The Growth of Plants* (Penguin Books, Baltimore, Md., 1963)] states that if Mimosa leaflets are mechanically stimulated at 10°C, they do not fold up. When, however, the plant is placed at room temperature, the leaflets fold. Similarly Brauner and Hager [*Planta* **51**, 115 (1958)] report that bean plants, when placed on their side in the cold and later placed upright at room temperature, show a delayed geotropic response. Indeed, it might be speculated that stimulus storage at low temperature can be observed in all sensory systems. However, the rapidity of some responses to a sensory stimulus may make detection of the responses to some types of stored stimuli difficult.
3. D. Dennison, thesis, California Institute of Technology (1958).
4. M. Delbrück and W. Reichardt, in *Cellular Mechanisms in Growth and Development*, D. Rudnik, Ed. (Princeton Univ. Press, Princeton, N.J., 1956). By the system of Delbrück and Reichardt $I = +1$ and $t = (2)$.

5. An account of this work was given at the 12th Biophysical Society Meeting at Pittsburgh (1968). We thank R. A. Cellarius (University of Michigan) for his suggestion of the story of Baron Münchausen and M. Delbrück (California Institute of Technology) and J. R. Platt (University of Michigan) for advice. Much of this work was done at the Cold Spring Harbor Laboratory of Quantitative Biology in 1965 during Prof. Delbrück's course on *Phycomyces*. Supported by NIH grant GM-14035-02 and NSF grant GB-3149.

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Octopamine: Normal Occurrence in Sympathetic Nerves of Rats

Abstract. *Octopamine has been identified in several organs of normal rats by means of a sensitive enzymatic assay. It is localized within the sympathetic nerve endings.*

Octopamine, first identified in the posterior salivary glands of *Octopus vulgaris* (*1*), has been demonstrated in mammalian organs and urine after inhibition of monoamine oxidase (MAO), and in small amounts in urine from normal humans (*2*). It has not been identified previously in any tissue of normal animals. We developed an enzymatic assay which measures 0.5 ng of octopamine in a 0.5-ml tissue extract. Using this method, we found octopamine in several sympathetically innervated organs of the rat.

Male and female Sprague-Dawley rats (100 to 225 g) were killed by a blow to the head, and the organs were removed and homogenized in 0.01 *M* tris (hydroxymethyl)aminomethane buffer, *p*H 8.6. The homogenate was heated to denature proteins, and, after centrifugation, the supernatant was assayed for octopamine. Bovine adrenal phenylethanolamine *N*-methyltransferase was used to transfer the methyl group from C^{14}-*S*-adenosylmethionine to the nitrogen of octopamine to form C^{14}-*N*-methyloctopamine (synephrine). This enzyme is specific for those phenylethylamines having a β-hydroxyl group (*3*). The synephrine formed was extracted into a mixture of toluene and isoamyl alcohol (3:2) at *p*H 10. Under these conditions, epinephrine formed from norepinephrine was not extracted.

Octopamine assays were performed on several organs of the normal rat. The apparent concentration of octopamine varied from 5 ng/g in the brain to about 500 ng/g in the adrenal gland (Table 1).

To identify the radioactive product extracted, samples of the extraction mixture were subjected to thin-layer chromatography; *N*-butanol saturated with 1*N* HCl or a mixture of isopropyl alcohol, NH_4OH, and H_2O (80 : 10 : 19) were used as developing agents. Similar results were obtained with both solvents.

In the heart, synephrine and dimethyloctopamine, formed by the further methylation of synephrine, were the only radioactive compounds present (Fig. 1, solid lines). In the salivary gland and spleen, however, synephrine was only one of several C^{14}-methylated compounds extracted. From such chromatographic separations, the percentage of total radioactivity represented by C^{14}-*N*-methyloctopamine (synephrine) was estimated for six sympathetically innervated organs (Table 1). Authentic octopamine calculated from these percentages varied from 2.4 ng/g in the brain to 461 ng/g in the adrenal gland. Inhibition of MAO with catron (β-phenylisopropylhydrazine) resulted in a five- to tenfold increase in the concentration of octopamine in most organs examined. Under these conditions, synephrine comprised at least 90 percent of the radioactivity extracted.

Experiments were performed to determine whether octopamine was present within the sympathetic nerve endings. 6-Hydroxydopamine, a compound which selectively destroys the sympathetic nerve endings in most tissues of the rat (*4*), was administered, and the tissues were examined for octopamine 2 days later. Almost all of the octopamine disappeared from the heart, spleen, and salivary gland (Fig. 1, dashed lines). Similar results were ob-

Table 1. Endogenous octopamine in rat tissues. Tissues were obtained from six male Sprague-Dawley rats (100 to 125 g). The percentage of apparent octopamine has been estimated from four separate chromatograms in which *N*-butanol saturated with 1*N* HCl or a mixture of isopropyl alcohol, NH_4OH, and H_2O (80:10:19) were used as developing agents.

Tissue	Octopamine		
	Apparent (ng/g)	Authentic (% of apparent)	Authentic (ng/g)
Adrenal gland	461 ± 49	100	461
Brain	4.7 ± 0.93	50	2.4
Heart	49.6 ± 2.8	100	50
Salivary gland	95.7 ± 8.8	50	48
Spleen	23.7 ± 3.8	60	14
Vas deferens	75.6 ± 2.9	40	30

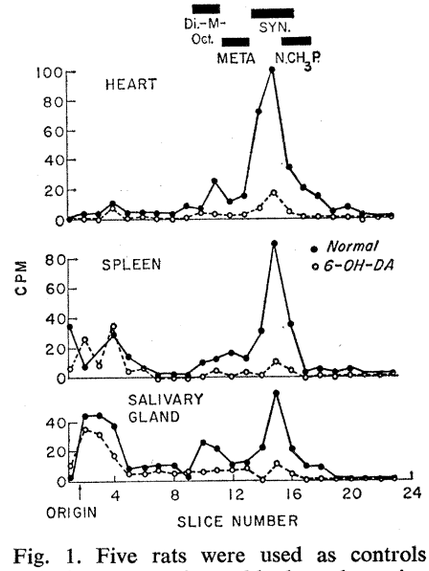

Fig. 1. Five rats were used as controls and five were given 6-hydroxydopamine (100 mg/kg, intravenously) 24 and 48 hours before death. Assay for octopamine was performed as described in the text. A sample of the extract was evaporated to dryness and subjected to thin-layer chromatography (silica gel), butanol saturated with 1*N* HCl being used as developing agent. Nonradioactive dimethyloctopamine (Di-M-Oct), metanephrine (Meta), synephrine (Syn), and *N*-methylphenylethanolamine (NCH_3P) were cochromatographed with each sample. The compounds were made visible by staining with diazotized *p*-nitroaniline followed by ninhydrin. After staining, sections (1.5 by 0.6 cm) were transferred to vials for scintillation spectroscopy.

tained when salivary glands, denervated by removal of ipsilateral superior cervical ganglia, were compared with their contralateral controls. On chromotography, octopamine was present only in the innervated glands.

Kopin and his collaborators have shown that H^3-octopamine is formed after the administration of H^3-tyramine (*5*). The H^3-octopamine is retained by the same granules which store norepinephrine (*6*), and it can be released from the isolated perfused cat spleen by nerve stimulation (*7*). These observations and our demonstration that octopamine is a normal constituent of sympathetically innervated organs suggest that it is normally released, along with norepinephrine, from sympathetic nerves.

The physiological significance of octopamine is unclear. It has weak sympathomimetic activity, about 1 percent that of norepinephrine, and its concentration in the heart, for example, is only about 5 percent that of norepinephrine. However, the urinary excretion of its major metabolites is quantitatively comparable to those of norepinephrine (*2*, *8*), suggesting that octopamine turns over rapidly.

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27 December 1968

Brood Care in Halictine Bees

Abstract. *Four species of the halictine bee genus* Evylaeus *keep their brood cells open during most or part of the development of the larvae. In the colonial summer phase, house bees care for the young and keep brood cells clean from feces and exuviae. Progressive feeding of nector is present at least in* Evylaeus malachurus, *whose fully fed larvae are, on an average, 60 percent heavier than the egg-and-pollen stage. Interactions between the two generations of social Halictinae are of general occurrence, and their intensity corresponds to the level of social behavior attained.*

Societies of ants, bees, and wasps usually consist of a queen and her daughters, and parental care of the young appears to be the main preadaptation for social behavior (*1*). Growing up in the same nest leads to a degree of mutual tolerance which forms the basis for further social interactions among nest mates. The peculiar haploid-diploid mode of hymenopteran sex determination seems to have supplied a genetic mechanism which gives selective advantage to societies made up of closely related individuals (*2*).

Michener (*3*), while accepting the importance of parental care in the emergence of such societies as that of the termites, ants, and wasps, believes that a different evolutionary path was followed by most bees; he assumes that social organization of bees was established among groups of adults. His conclusions are based mainly on studies of Halictinae, a worldwide subfamily of primitive bees with such a wide range of solitary and social members that they can be regarded as an illustration of how insect societies may have originated.

Honey bees, ants, and some wasps show a variety of interactions between larvae and adults, including the exchange of food (trophallaxis) and social grooming. Both were thought to be characteristically lacking in the mass-provisioning halictines, which seal each cell after it is supplied with pollen and nectar and an egg is laid. Females of solitary species usually die before the emergence of their progeny, and contact with the immature stages is generally prevented by the location of the cells at the end of long, soil-filled laterals (*4*). However, the social species investigated construct cells which are easily accessible from the main burrow. Several of these halictines maintain at least occasional contact with their brood after the cells have been sealed. The reopening of closed cells for inspection has been observed in *Dialictus versatus* and *D. zephyrus*, two Nearctic species with a primitive social organization (*5*).

The evolutionary importance of this phenomenon can best be evaluated through studies of the many species of the genus *Evylaeus* which together cover a remarkably wide spectrum of behavior. Strictly solitary species have relatives with a fairly complicated social behavior and a nest structure suitable for complex societies. The socially most advanced *E. marginatus*, *E. linearis*, *E. malachurus*, and *E. cinctipes* deliberately leave their cells open or reopen them during the development of the larvae (*6*). The nature and intensity of interaction between the two generations vary widely in these species, but all are concerned with aspects of cell sanitation and progressive feeding of the brood. The Mediterranean *E. marginatus* forms the only perennial society known among the Halictinae. Queens live up to 5 years, producing annual clutches of physiologically distinct workers (*7*). On excavation, cells are open and free of larval exuviae and feces. Because *E. marginatus* has not been studied in the laboratory, it is excluded from this discussion.

The overwintered queens of the three annual species provision a few cells in early spring and await the emergence of their workers within the closed nests. During this phase, only *E. cinctipes* removes exuviae and feces from the brood cells, which remain open throughout the development of the young bees. *Evylaeus linearis* and *E. malachurus*, in sharp contrast, do not remove the feces prior to the closure (capping) of the cells. All three species respond unambiguously to the contents in the cells, since they recognize dead and introduced larvae, which they bury in the cell or remove. Cells without contents or feeding instars are never capped by *E. malachurus* or *E. linearis*. The trait to remove dead or "abnormal" larvae from their cells is an important social innovation in these species. As societies increase in size with higher social development, epizootics become a greater threat under the more crowded conditions. A mechanism for the elimination of the contagion is therefore clearly adaptive. In the honey bees, for example, only strains efficient in removing sick or perished larvae can check the spread of infectious diseases, and often achieve spontaneous recovery (*8*).