achenes is less open and lacks the fine central hairs.

In natural populations the seeds of both *Orthocarpus* and *Hypochoeris* mature at approximately the same time. The *Hypochoeris* achenes fall to the ground with the upwardly directed pappus forming a sea of bristles. The entanglement of these achenes is so dense at times that falling *Orthocarpus* seeds often become attached to the pappus—either the net of the *Orthocarpus* seed is pierced by a pappus bristle or the seed becomes entangled by the fine hairs of the outer achene (Fig. 1). The percentage of *Orthocarpus* seeds which become attached to achenes is, of course, highly variable; and I have no good data for the general frequency of this event except that examination of the *Hypochoeris* achenes surrounding *Orthocarpus* individuals usually reveals several achenes with one or more attached seeds.

Several handfuls of *Hypochoeris* achenes were scraped from around mature *Orthocarpus* plants, placed in a large paper bag, and taken to the laboratory. The total numbers of both types of achenes were estimated to be about 5000, and the sample was carefully examined for attached *Orthocarpus* seeds. Only 11 (0.3 percent) of the 4000 inner achenes retained single seeds. However, 7 percent of the 1000 outer achenes carried one or more firmly attached seeds; 57 achenes each retained a single seed, 10 achenes carried 2 seeds, and one achene held 3 seeds. The outer achenes with the central mass of fine hairs are far more efficient than the inner achenes in retaining *Orthocarpus* seeds. It is important that several of the outer achenes carried two or more such seeds.

An interesting aspect of this interaction is the accuracy with which its origin can be dated. While *O. densiflorus* is restricted to western regions of California, *H. glabra* is a native of western Eurasia and North Africa and has only recently been introduced into the range of *Orthocarpus*. *Hypochoeris glabra* was common in the San Francisco area by 1870 but apparently did not reach Southern California until 1900 (*3*). While other species native to the California area have and still do serve as hosts for *Orthocarpus*, the *Orthocarpus–Hypochoeris* system is less than 100 years old. Intricate and complex interactions do not necessarily indicate a long evolutionary development but can be a fortuitous combination of features evolved independently.

Hypochoeris glabra is not only ecologically and physiologically a suitable host for *O. densiflorus* but also the seeds of host and parasite fit together so that the seeds may be clustered and the dispersal coordinated. Clustering is an important factor in all outbreeding populations, though it has primarily been discussed in specialized cases of founder populations. Baker (*4*) has pointed out the difficulty of long-range dispersal in self-incompatible plants, and Raven (*5*) has suggested a selective advantage in a self-incompatible plant of a multi-seeded unit but he noted that known examples are relatively heavy and unlikely to be dispersed. The attachment of more than one *Orthocarpus* seed to a single *Hypochoeris* achene not only provides the potential for long-range migration and establishment, but at the same time produces a degree of clustering within populations of these self-incompatible annuals.

The advantage of a coordinated dispersal of host and parasite is readily visualized, but perhaps the less obvious phenomenon of seed clustering in self-incompatible plants is of greater general significance, and may be an important aspect of plant-population interactions where parasitism is not a factor.

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References and Notes

1. Details of root grafting in *Orthocarpus* are similar to those for *Castilleja* reported by L. R. Heckard in *Bot. Gaz.* **124**, 21 (1962).
2. The term "obligate" is used because it describes the dependency of the natural population on the presence of a host. The fact that *individuals* of some species will complete their life cycle without a host should not be confused with the fact that natural populations growing without a host do not exist and, therefore, presumably cannot exist.
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28 June 1965

Prostaglandin: Release from the Rat Phrenic Nerve–Diaphragm Preparation

Abstract. *Release of a substance that stimulates smooth muscle was detected from a phrenic nerve–diaphragm preparation from the rat upon direct and indirect stimulation. On thin-layer chromatography most of the active material behaved as a mixture of prostaglandins. The effect of electrical stimulation was mimicked by catecholamines but not by acetylcholine or eserine. The effect of nerve stimulation was not antagonized by* d-*tubocurarine.*

There is a spontaneous and evoked release not only of acetylcholine (*1*) but also of unsaturated hydroxycarboxylic acids from the somatosensory cortex of the anesthetized cat (*2*). These acidic substances, which are also released from the perfused spinal cord of the frog on stimulation (*3*), produced a slow contraction of the isolated uterus of the rat and have now been identified with a mixture of prostaglandins (*4*), a class of substances whose chemical structure and pharmacological properties have now been elucidated (*5*). In order to study the mechanism of release and the possible function of these lipid acids, we attempted to detect their release from a simple nerve structure, namely the phrenic nerve–diaphragm of the rat. This isolated preparation was first introduced by Bulbring (*6*) and is very useful for studying the effects of drugs on cholinergic synapses. Despite the comment that the "behaviour of this preparation is sufficiently predictable to allow observations to be repeated and therefore confirmed" (*7*), there is no agreement concerning the release of acetylcholine from the nerve terminals (*8*).

Hemidiaphragms were dissected from female Wistar rats (150 g), suspended in a 2-ml bath of Tyrode solution at 37°C, and aerated with O_2 containing 5 percent CO_2. The costal margin of the diaphragm was attached to one side of the bath, and contractions of the muscle were recorded on a polygraph by connecting the central tendinous part of the diaphragm to a strain gauge. A pair of flexible platinum electrodes mounted on the lucite frame of the tissue bath served to stimulate the nerve or muscle directly. The bath fluid was changed every 15 minutes, and at least two resting samples were collected before maximum stimulation of either the nerve or the muscle at 25 stimuli per

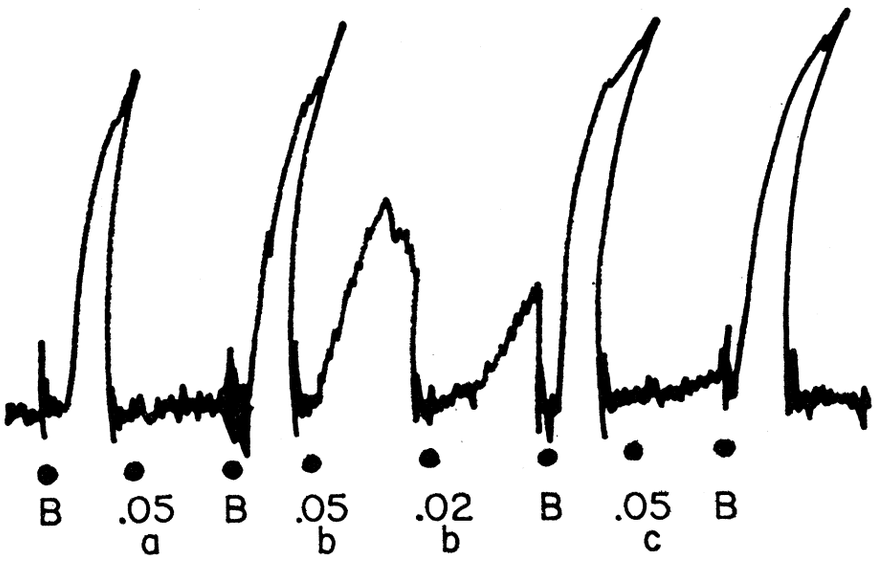

Fig. 1. Recorder trace showing the isometric response of an isolated rat uterus. The uterus responded by contraction to 0.25 ng/ml of bradykinin (*B*) and to samples of an extract of the bath fluid collected during (0.05 and 0.02 ml, *b*) but not before (0.05 ml, *a*) or after (0.05 ml, *c*) stimulation of the phrenic nerve. The active material released on stimulation of the nerve sensitized the tissue to subsequent doses of bradykinin. The diaphragm bath fluid (1 ml) was acidified (*p*H 2) and extracted three times with ether (1:1 by volume); the extract was dried under reduced pressure at 30°C and reconstituted for testing in 0.5 ml of De Jalon solution.

second, 0.03-msec pulse width. The samples were stored at 4°C before bioassay on the isolated non-oestrus uterus of the rat that was suspended in De Jalon solution containing atropine (0.15 μg/ml) and 2-bromo-lysergic acid diethylamide (0.25 μg/ml) in a 0.5-ml bath at 22°C.

No spontaneous release of any pharmacologically active substance into the bath fluid was detected when this bioassay was used. However, in eight out of nine experiments during the 15-minute period of nerve stimulation, release of an oxytocic substance occurred. The activity of this substance was not antagonized by acetylcholine or serotonin antagonists, nor was it destroyed by incubation with chymotrypsin (1 mg/ml) at 37°C for 30 minutes. Furthermore, the substance was soluble in diethyl ether (Fig. 1) but insoluble in petroleum ether (boiling point, 30° to 60°C) on partition at *p*H 2. The only substances that are known to stimulate smooth muscle and to possess these properties are the prostaglandins (*4*).

When the samples obtained during nerve stimulation were pooled, acidified to *p*H 2, and extracted three times with an equal volume of ether, 95 percent of the pharmacological activity was always detected in the dried, ether-soluble residue. When such an ether extract was subjected to thin-layer chromatography on silica gel G (Merck) and developed in a mixture of benzene, dioxane, and acetic acid (20:20:1) (*9*), 95 percent of the pharmacological activity applied to the plate was detected by elution and subsequent bioassay on the isolated uterus at an R_F of 0.45; this R_F coincided with that obtained for prostaglandin E_1, E_2, and E_3. When this active material was redeveloped in a mixture of ethyl acetate, acetic acid, methanol, 2,2,4-trimethylpentane, and water (110:30:35:10:100) (*9*), the major fraction of the activity coincided with the R_F obtained for prostaglandin E_1; some activity was detected at the R_F corresponding to that of prostaglandin E_2.

On stimulation of the phrenic nerve, approximately 1 ng of prostaglandin-E_1 equivalent per minute was liberated into the bath fluid. The question then arose of whether the prostaglandin was released from the nerve or nerve endings on stimulation or from the diaphragm muscle on contraction. On three occasions the phrenic nerve was isolated and suspended over a pair of platinum electrodes in a bath containing Tyrode solution at 37°C and aerated with O_2 containing 5 percent CO_2; stimulation of the nerve did not result in release of prostaglandins.

However, when the whole diaphragm was homogenized in water at *p*H 4, boiled for 10 minutes, acidified at *p*H 2, and extracted three times with petroleum ether (1:1 by volume) and then three times with ether (1:1 by volume), then the dried ether residue contained approximately 125 ng (S.E., ± 33, $n = 7$) of prostaglandin-E_1 equivalents per gram (wet weight) of diaphragm. Furthermore, when the diaphragm was directly stimulated a prostaglandin was detected in the bath fluid in four out of ten experiments.

Acetylcholine is released from the phrenic nerve–diaphragm of the rat on stimulation (*7, 8, 10*); however, it seems unlikely that the release of prostaglandins from this preparation is associated with muscular contraction, since addition of eserine (5 μg/ml) to the bath fluid, in the presence or absence of acetylcholine (50 ng/ml), neither potentiated nor inhibited the release of prostaglandin obtained by stimulating nerve or muscle. Furthermore, the presence of *d*-tubocurarine (300 μg/ml) in the bath fluid sufficed to block the response of the muscle to nerve stimulation, but did not affect the concomitant release of prostaglandin.

We have recently demonstrated that the addition of epinephrine or norepinephrine (0.5 to 5.0 μg/ml) to the bath fluid in the presence of ascorbic acid (0.6 μg/ml) elicits release of prostaglandin from the phrenic nerve–diaphragm. The release of prostaglandins when the nerve is stimulated may be due to the prior release of catecholamines.

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