

# Interspecific Differences in the Reaction to Atropine and in the Histology of the Esophagi of the Common California Sea Hares of the Genus *Aplysia*

LINDSAY R. WINKLER and BERNARD E. TILTON<sup>1</sup>

DURING A STUDY of the effects of certain cholinergic agents on the tissues of *Aplysia*, it was noted that the esophagi of the two California species (*A. californica* and *A. vaccaria*) reacted divergently to atropine. This is of interest to both the taxonomy and physiology of the genus as well as potentially to a better understanding of the mode of action of atropine. Other drugs commonly known to show activity on muscle tissue were also tested on the two species to determine if any other interspecifically divergent reactions existed. These pharmacological reactions will be reported later.

Botazzi (1898) observed the periodic contractions of the esophagus of the European *Aplysia* and made a thorough study of its physiology. The physiology of the crop was studied extensively by von Brücke (1905). Straub (1907) reported the reaction of the heart of an unspecified species of *Aplysia* to muscarine and noted the lack of atropine antagonism in the heart ventricle. Hogben (1924) studied the reaction of the crop of an European *Aplysia* to epinephrine.

## MATERIALS AND METHODS

Small- to medium-size specimens of *Aplysia* (*Neaplysia*) *californica* Cooper and *Aplysia* (*Aplysia*) *vaccaria* Winkler were collected during the summer months at Lunada Bay, Palos Verdes, Los Angeles Co., California. The animals were packed in wet *Pelvetia fastigata* and transported to the laboratory where they were maintained in a 10-gal. salt water aquarium until needed. A Cole-Parmer model all-plastic impeller-type pump drew the water from the aquarium through a filter containing glass wool and activated charcoal and pumped it serially

through two 5 gal. carboys maintained in a refrigerator. It was thus possible to keep the water clean and cooled to approximately the temperature of the intertidal environment of these animals. Parsley obtained in the local market was eaten in quantity by *A. californica* but was refused by *A. vaccaria*. Consequently all specimens of the latter were used as soon as practicable.

Animals were sacrificed by incising the entire length of the foot, turning the animal inside out and removing the esophagus after tying it at both ends. The esophagi were suspended from a plastic holder in conventional baths using 30 ml. of sea water. The movable end of the esophagus was ligated to a Grass force-displacement transducer, which was connected to a Grass amplifier-recorder.

To identify the two types of excursions noted in the tracings, cross sections of esophagus tissue were tied on opposite sides of the ring thus formed so as to obtain tracings of the contractions of the circular muscle with little or no effect from the longitudinally oriented tissue. Atropine was used in 1:1000 solution (1 mg/ml) in all cases.

For histological examination both longitudinal and cross sections were made using standard techniques. These were stained with eosin and hematoxylin. Sections were also made of all the noticeably differentiated areas of the digestive tract.

## EXPERIMENTAL RESULTS

The normal pattern of contraction as recorded from the isolated esophagus consists primarily of comparatively rapid short excursions which vary in frequency and amplitude. These may be interspersed with occasional contractions of greater amplitude, especially in *A. californica* (Fig. 1a). Since the latter contractions were ab-

<sup>1</sup> Department of Pharmacology, Loma Linda University School of Medicine, Loma Linda, California. Manuscript received January 13, 1961.

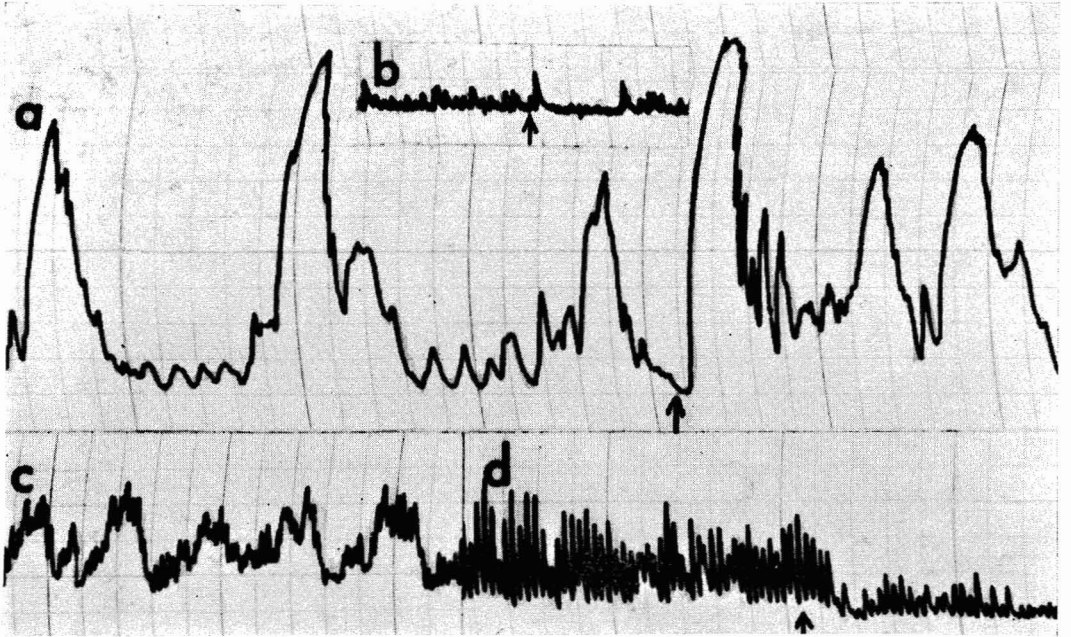


FIG. 1. The normal tracing of the esophagus of *A. californica* is indicated in *a* and the effect produced by 0.2 mg. of atropine solution added to the 30 ml. bath is shown at the arrow. The depressing of the circular contractions of a ring of esophagus by 0.2 mg. atropine is shown in *b*. Tracings *c* and *d* show two types of normal tracings obtained from *A. vaccaria* esophagus and the effect of 0.4 mg. atropine added at arrow.

sent when the rings of esophagus were used (Fig. 1*b*), they are attributed to longitudinally oriented muscle in contrast to a primary circular muscle response. For some unknown reason the rapidity of contraction was increased when the esophagus was arranged in rings. The deep, intermittent excursions are not as pronounced in *A. vaccaria* (Fig. 1*c*) and may be absent (Fig. 1*d*). The response of circular muscle, however, is consistently more rapid and more pronounced than is the case in *A. californica*.

In *A. californica* even very small doses (0.05 mg. in some cases) of atropine produce an immediate and unailing but transitory contraction of the longitudinal muscle (Fig. 1*a*). The esophagus of *Aplysia vaccaria*, however, does not react to atropine except in comparatively large doses of 0.4 ml. or more (in a 30 ml. bath), in which case the excursions of the circular muscle are depressed (Fig. 1*d*). The circular muscle of *A. californica*, as demonstrated by the muscular ring preparations, is noticeably depressed by atropine in even smaller doses than that required to depress the circular type muscle in *A. vaccaria*

(Fig. 1*b*). This depression was not noticed in the usual tracings of *A. californica* muscle since it was obscured by the longitudinal contractions. Other muscle-active drugs with specific reactions of interest in themselves (which are to be reported later) did not produce divergent results between the two species.

In an attempt to gain an insight into possible reasons for the divergence of reaction between the two species, histological sections were made and stained with eosin and hematoxylin. Strongly eosinophilic bundles of coarse, cylindrical muscle strands were observed in the esophagus of *A. californica* (Fig. 2*a, b*). These strands appear coarsely striated in some preparations (Fig. 2*b*) and coarsely granular in others, the differences possibly representing problems in killing and fixing. The nuclei are larger and more sparse than those of *A. vaccaria* (Fig. 2*c, d*) and of the surrounding more conventional muscle of the present species. Not only are the nuclei three times as large but they tend to be arranged across the muscle strands, thus appearing rectangular in section. This muscle is

contrasted with the less eosinophilic, more undulatory muscle of the circular (Fig. 2a) and *A. vaccaria* type muscle (Fig. 2c, d), which is very heavily nucleated. While strands of a type of muscle which seems morphologically to be somewhat similar to the former type of muscle appear sparsely in the crop, and to even a lesser extent in the area between the gizzard and "stomach" of the digestive tract in *A. californica*, none of the eosinophilic muscles observed in other parts of the digestive tract of *A. vaccaria* possessed the distinctive tubular shape with the vacuolation, striation or granulation, and lack of undulatory characteristics.

#### DISCUSSION AND CONCLUSIONS

While it might be tempting to assume that the histological differences represent the im-

mediate cause of the divergent pharmacological reactions observed, they are better interpreted as visible, easily demonstrable, morphological differences which parallel and are closely associated with neurohumoral and even biochemical differences which are themselves the underlying mechanistic causes. This is especially true since the effect of atropine on conventional tissue systems is thought to be on the neuromuscular receptors. The contractatory response observed in *A. californica* appears to be the transitory result of stimulation, since the mechanical stimulation of washing will produce similar contractions. However, the sensitivity to this chemical stimulation is very great, being as low as 0.05 mg. atropine placed in the 30 ml. bath—a concentration of  $1:6 \times 10^5$ . Such a sensitivity represents a very delicately balanced chemical system.

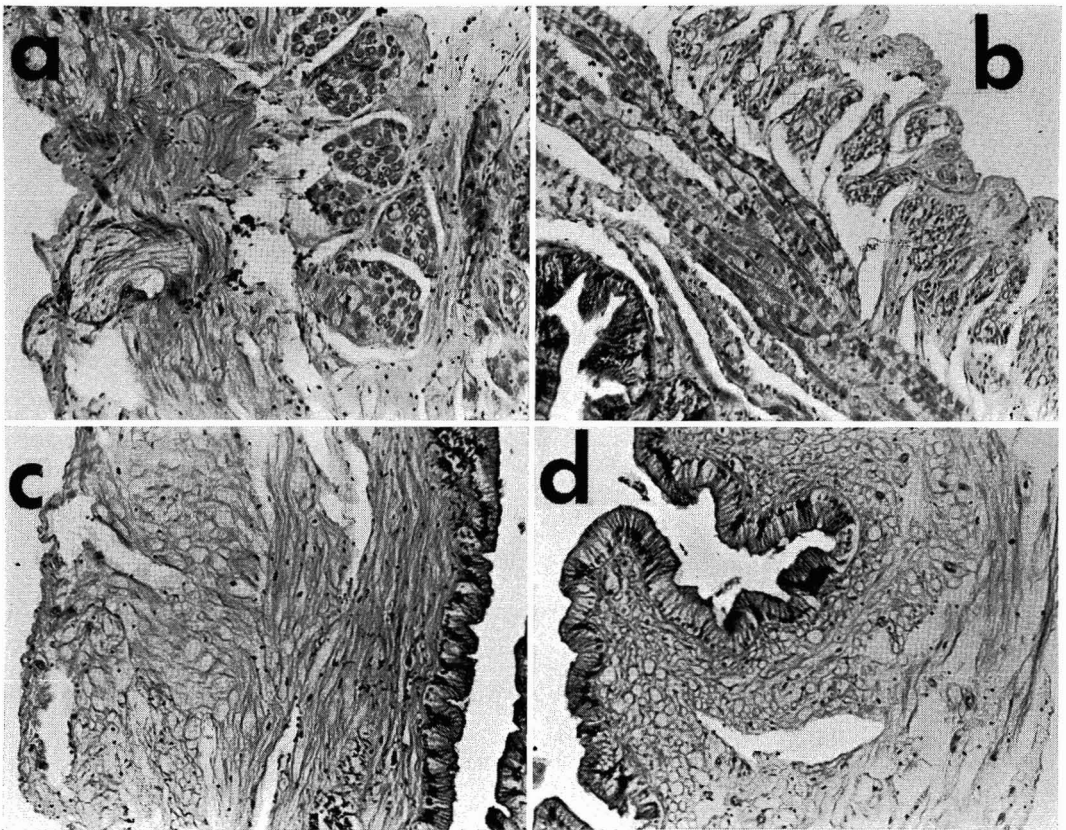


FIG. 2. *A. californica*: a, cross section; b, longitudinal section. *A. vaccaria*: c, longitudinal section; d, cross section. The midsection of the esophagi were used for cross sections.

Moreover, the mechanism is of special interest since it is unique among known muscle preparations in that it is stimulated rather than depressed by atropine.

The drug reaction and histological difference accentuates the divergence present within the genus. *A. vaccaria* belongs to a large and widespread subgenus (*Aplysia*), while *A. californica* (subgenus *Neaplysia*) is unique to the Californians. When it becomes possible to study comparatively the members of the two other subgenera<sup>2</sup> (*Varria* and *Pruvataplysia*), a taxonomic generalization may be possible. However, Bottazzi (1898) in his physiological study of the esophagus indicated that *A. limacina* (almost certainly *A. fasciata* of subgenus *Varria*) was much more atonic than the close relative of *A. vaccaria* (subgenus *Aplysia*) with which he also worked. Since it is thought that *A. californica* is an offshoot of this subgenus *Varria*, and since its esophagus is far from atonic by any interpretation, it may be that the musculature of the former has diverged considerably from its fore-runners.

It would also seem quite probable that more comparative studies of the tissues of the species contained in other genera of animals would reveal occasional divergences of at least equal magnitude. Such a possibility cannot safely be overlooked in experimental biology in any of its phases, and accentuates the need for accurate taxonomy before proceeding with studies which may be of a nontaxonomic nature.

<sup>2</sup> Specimens of esophagus tissue from these subgenera were kindly supplied from existing museum material on hand by Dr. N. B. Eales but, unfortunately, the conventional preservation method employed by the collectors was not adequate for any type of conclusions.

#### SUMMARY

1. The normal pattern of esophageal contractions in *Aplysia californica* consists of short rapid contractions attributed to circular muscle, interspersed at intervals by deep excursions attributed to the longitudinal musculature. These secondary contractions are less pronounced and less regular in *A. vaccaria*.

2. Atropine causes marked contraction of the esophagus in *A. californica*, even in low concentration, but in *A. vaccaria* it depresses circular activity in higher concentrations.

3. The esophagus of *A. californica* has longitudinal bundles of cylindrical muscle appearing coarsely striated or granular. These have not been found in *A. vaccaria*, and are suggested as being associated with the mechanistic cause for the differences in reaction noted.

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