

*ON A BIOASSAY FOR ACETYLCHOLINE AND ON SOME
PROPERTIES OF THE LONGITUDINAL MUSCLES OF
HOLOTHURIA GRISEA (ECHINODERMATA)*

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(4 figs.)

Smooth muscles of some Invertebrate animals are recommended for determination of the amount of Ach in extract of tissues. The body wall muscles of Sea Cucumber (*Holothuria grisea*) one of the most common Echinoderm found along the Brazilian coast, have been preferred for a bioassay and used in a very successful way.

The longitudinal muscles of that Echinoderm contract in the presence of as little as 1×10^{-14} g Ach/ml. The technique is very simple and inexpensive. As perfusion fluid filtered sea water is very convenient. The preparation of fresh animals does not have spontaneous movements, gives regular responses and has the great advantage of rapid relaxation. In average the muscles have a latent period of contraction of 10-15 seconds and relax 2-3 minutes after washing. These conditions permit a great number of assays in a short time. Eserinization is not necessary. Several experiments indicate regular proportion between the amount of Ach and the contraction (fig. 1).

Some precaution must be observed during the bioassay. Fresh recently captured animals give better results. The longitudinal muscles can be dissected easily and free from connective tissues. Ordinary atmospheric air or pure oxygen is used to oxygenate and stirr the bath. The bubbles must be fine and not much rapid in order not to disturb the muscle mechanically. The sensitivity to Ach is so high that small drops of the esther attached to the suspension thread must be avoided.

This method is largely used not only in the marine laboratories (Marine Biological Laboratory of São Sebastião and Aquario of Santos) but also in São Paulo where the Holothurians were kept in the laboratory in running filtered sea water.



Fig. 1 — Contractions of the longitudinal muscle of the body wall of *Holothuria grisea* after excitation by Acetylcholine. 1 = 10^{-10} ; 2 = 10^{-8} ; 3 = 10^{-6} ; 4 = 10^{-4} .

The uneserized longitudinal body wall muscles of *H. grisea* was adopted as a bioassay for Ach by Ambache & Sawaya (1953) and the method is one of the most sensitive (Welsh & Tawarog 1960, p. 195). These authors refer that “the isolated longitudinal wall muscle of *Holothuria* is a method of Ach assay of great potential value in laboratories located near a source of marine animals”. And add: “it would appear to be a specific assay, but this aspect requires further study”.

In order to verify the specificity of the method for Ach, several experiments were performed in different conditions.

The body wall muscles of *H. grisea* is insensitive to many drugs as histamine, curare, adrenaline, nor-adrenaline, as Sawaya & Ancona Lopez (1950) have pointed out, and to the salts barium chloride and potassium chloride. Those authors have also verified that atropine does not antagonize the effect of Ach.

All those drugs were brought into action at physiological concentration, about 10^{-6} (1 μg). Some of them (histamine, adrenaline and nor-adrenaline) at higher doses (1×10^{-3} or 1×10^{-4}) provoke relaxation of the muscles. von Euler, Chaves & Theodosio (1952, p. 104) refers to the effect of histamine on the relaxation of the muscles and mention that adrenaline and dl-noradrenaline has no influence on those structures. In several experiments with histamine the relaxation effect was observed as concentration of 20 μg . At this doses adrenaline and nor-adrenaline have no effect at all.

Former studies of Sawaya & Ancona Lopez (1959) show that only nicotine induces strong contraction in doses of 1 μg . The influence of that alcaloid differs from that of Ach chiefly because the relaxation of the muscle after nicotine does not take place before 20-30 minutes and with Ach this period is regularly 20 sec. and never more than 3 minutes. In the case of nicotine there is an actual contracture from which the muscle recovers very slowly.

Another point to be mentioned is the behaviour of the muscle under eserine, physostigmine and other allied drugs. For the bioassay the uneserinized muscle is used. Eserine has no effect on the contraction but potencializes Ach. However, the after effect of eserine is a contracture of the muscle in the same way as the mentioned nicotine effect. The relaxation time is here about 20-30 minutes. This delay in relaxation and the strong sensitivity of the muscle to Ach indicate the use of uneserinized muscle in the bioassay.

The reactions of the longitudinal muscle to different concentrations of Ach are very constant. Fig. 2 and 3 shows the effect of small doses of Ach. These records could be obtained several times with the same muscle during more than 12 hours of working. In some cases, the same muscle after that period of work was kept in an ice-box at 2° or 3°C and on the next day experimented again with good results.

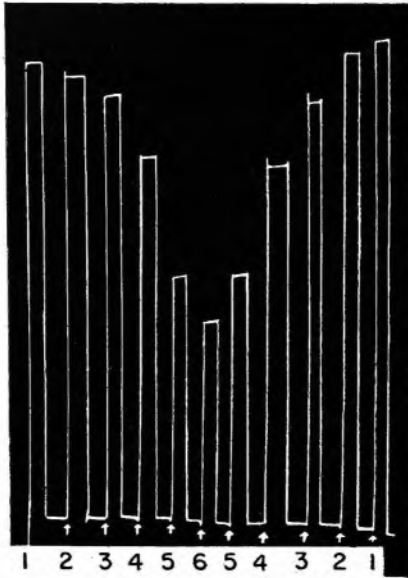


Fig. 2. — Contractions of the longitudinal muscle of the body wall of *Holothuria grisea* after excitation by Acetylcholine. 1 = 10^{-4} ; 2 = 10^{-5} ; 3 = 10^{-6} ; 4 = 10^{-7} ; 5 = 10^{-8} ; 6 = 10^{-10} .

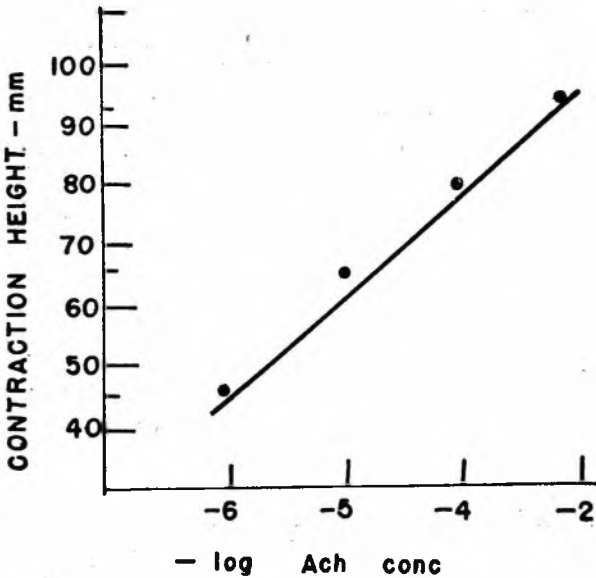


Fig. 3 — Contractions of the longitudinal muscle of the body wall of *Holothuria grisea*.

The perfusion fluid used was always filtered sea water. von Euler, Chaves & Teodosio (1952, p. 101) have employed sea water diluted with distilled water to 80% Such a dilution has no effect in our preparations.

In order to see if the dilution of sea water with distilled water has some influence on the contraction, the longitudinal muscles have been submitted to different concentration of sea water and after 5 to 15 minutes of contact of the perfusion diluted fluids the same doses of Ach were injected into the bath.

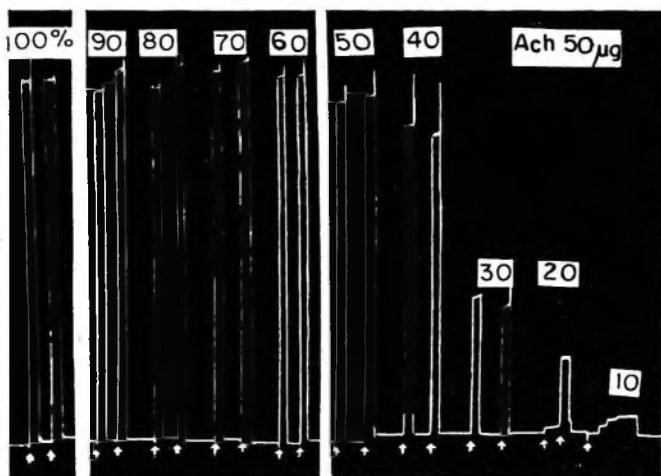


Fig. 4 — Contractions of the longitudinal muscle of the body wall of *Holothuria grisea* after excitation by 50 μ g of Acetylcholine diluted in different concentration of filtered sea-water.

Fig. 4 shows that the dilution of sea water with distilled water can be made to 60% without influence on the reaction of the muscle to Ach. After 60% the contraction is less and decreases when the perfusion fluid is diluted down to 20%. Dilution of sea water below 10% causes inhibition of the influence of Ach.

DISCUSSION

According to the results of several experiments the body wall muscles of *Holothuria grisea* can be used for Ach bioassay. Prati-

cally the contraction of those muscles are specific for Ach and nicotine. The contracture determined by the latter indicate the difference of those muscles' reactions.

As have been said the muscles are insensitive to several drugs of common use in pharmacology.

The physiology of the muscles of Echinoderms is very little known. Hanson & Lowy (1960, p. 265) in their study on muscles of Invertebrate animals do not give information on the muscles of Echinoderms.

SUMMARY

1. The body wall muscles of Sea Cucumber (*Holothuria grisea*) are very sensitive to acetylcholine and nicotine.
2. Reaction of the muscles under Ach is very characteristic, that is, the muscle reacts to as little as 1×10^{-14} , the latent periods is of 10-20 sec. and the relaxation occurs on 2-3 minutes after working.
3. Nicotine at physiological doses ($1 \mu\text{g}$) provokes contracture of the muscles.
4. The body wall muscles of *Holothuria grisea* can be recommended as very useful material for Ach bioassay.

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