erties of an enterococcus. Such an organism would apparently be inhibited by low concentrations of bacitracin or aureomycin, however. The results of our *in vitro* experiments suggest that resistance developed in *mitis* streptococci by contact with other agents does not necessarily bring about the overall increase in resistance shown to accompany sulfonamide resistance.

*Summary.* 1. At a certain level of sulfonamide resistance properties characteristic of enterococci were acquired by 4 strains of *S. mitis*. 2. Increased resistance to sulfonamides was accompanied by increased resistance to penicillin, streptomycin, bacitracin, and aureomycin in all 4 strains. The resistance of these trained strains to the antibiotics and sulfathiazole was similar to that of a strain of *S. fecalis*. 3. Acquisition of ability to grow on bile or in high concentrations of streptomycin did not generally produce the biochemical properties of enterococci, nor resistance to sulfathiazole or to the antibiotics.

Received July 28, 1949. P.S.E.B.M., 1950, v73.

## Influence of Certain Neurotropic Substances on Central and Synaptic Transmission in Callianassa. (17610)

R. S. TURNER,* W. A. HAGINS, AND A. R. MOORE.†

*From the Department of Anatomy, School of Medicine, Stanford University, and Hopkins Marine Station, Pacific Grove, Calif.*

Although acetylcholine and cholinesterase have been shown to be present in the nervous system of crustaceans, evidence for the functional significance of these substances is generally lacking(1,2,3,4). A possible exception may occur in the anomuran *Petrolisthes armatus*, in which Welsh and Haskin(5) have found that injection of acetylcholine increases the tendency to autotomy of the walking legs. In this animal eserine in doses of 1.5 γ per gram elicited extreme activity and convulsions, while atropine exerted a depressive action. No attempt was made by Welsh and Haskin to localise the site of action of these substances. In an effort to obtain further information on the action of these and other neurotropic agents, we have carried out experiments on the macrurous anomuran *Callianassa californiensis* in which application of reagents was made at 3 different loci: 1. On the abdominal nerve cord and ganglia. 2. At the point of synapse between giant fibers of the nerve cord and the nerves to the flexors of the telson. 3. On the flexor muscles of the abdomen after severance of their connections with the central nervous system.

*Methods.* The abdominal nerve cord of *Callianassa* usually was exposed from the ventral side, freed entirely from adjoining tissues and left continuous with the thoracic cord. The abdomen of the animal was then cut away and the head and thorax, with the attached nerve cord, placed in a bath of sea water. Preparations kept well in running sea water for periods up to 24 hours. The drugs were made up in sea water and added in the proper concentrations directly to the (40 cc) bath. Electrical stimulation and recording were carried out by means of micromanipulated platinum electrodes. Responses were amplified through a Grass P3 amplifier and visualised on the screen of a DuMont 208 oscilloscope, the sweep of which was triggered from the stimulating circuit. The flexor muscles of the abdomen were denervated by ex-

* Special Fellow, National Institute of Health, 1948-49.

† Department of Biology, University of Portland, Ore.

1. Prosser, C. L., *Physiol. Rev.*, 1946, v26, 337.

2. Welsh, J. H., and Schallek, W., *Physiol. Rev.*, 1946, v26, 447.

3. Bacq, Z. M., *Biol. Rev.*, 1947, v22, 73.

4. Katz, B., *Biol. Rev.*, 1949, v24, 1.

posing and removing the entire abdominal nerve cord from the dorsal side, leaving the muscles and their attachments intact. The synapses between the nerves to the flexors of the telson and the giant fibers of the cord (there are two giant fibers in this form, one on each side of the abdominal cord) made a favorable preparation for the study of possible synaptic effects. Unless otherwise stated, stimuli were delivered at approximately 30 per minute.

*Observations. Eserine.* Intact animals in which eserine (physostigmine salicylate) was allowed access to the ventral nerve cord displayed vigorous and sustained muscular responses. On the isolated nerve cord eserine ($2.5 \times 10^{-3}$ M) after a latent period of 15 to 30 seconds caused marked bursts of activity (Fig. 1). Activity of the ventral nerve cord could also be brought about by weaker solutions ($2.5 \times 10^{-5}$ M) after a latent period of 3 to 3.5 minutes. In concentrations of $2.5 \times 10^{-4}$ M the reaction was prompt, 15 to 30 seconds, and the amplitude of the response was maximal, since increase of the concentration to $2.5 \times 10^{-3}$ M produced no further increase in amplitude. This "spontaneous" activity continued undiminished for an hour. At concentrations as high as $2.5 \times 10^{-3}$ M eserine had no effect on the conduction characteristics of the giant fibers, nor on transmission through the giant fiber-telson nerve synapse. Denervated muscle likewise showed no response to eserine in these concentrations.

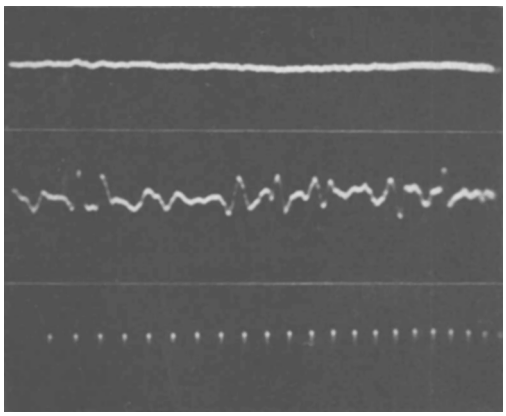

FIG. 1.
Top: resting baseline. Middle: activity of ventral nerve cord produced by $2.5 \times 10^{-3}$ M eserine. Bottom: Time line, 1,000 cycles per sec.

*Acetylcholine* ($1 \times 10^{-2}$ M) alone did not affect the reactivity of the cord, the synapses or denervated muscle. When applied within a few seconds after eserine there was no clear augmentation of response, such as is characteristic in indisputably cholinergic forms.

*Atropine* ($2.5 \times 10^{-3}$ M) after a latent period of approximately 10 minutes brought about a significant increase in "spontaneous" activity of the abdominal cord. This activity often reached an intensity comparable to that seen with eserine, and was maintained for 30 minutes or longer. No effects were observed on synaptic transmission nor on conduction in the giant fibers, nor did any depression phase appear over a period of 90 minutes.

*Nicotine* was found to block synaptic transmission reversibly in concentrations in the neighborhood of $1 \times 10^{-5}$ M to $4 \times 10^{-5}$ M. Conduction in the giant fibers was unaffected by nicotine in these concentrations.

*Methylamine hydrochloride* blocked synaptic transmission in concentrations of $1 \times 10^{-3}$ M. Washing with sea water effected complete recovery of synaptic function.

*Triethylamine hydrochloride* in concentrations of $1 \times 10^{-3}$ M produced no observable effects, but $4 \times 10^{-3}$ M completely blocked synaptic transmission. Function could be effectively restored by simple washing with sea water.

*Di-isopropyl fluorophosphate* (DFP) at concentrations of $1.3 \times 10^{-2}$ M immediately blocked both conduction and synaptic transmission for a few seconds, after which these functions spontaneously recovered and remained normal for an experimental period of 60 minutes. Concentrations of $2.8 \times 10^{-2}$ M blocked synaptic transmission for 20 minutes and conduction in the giant fibers for approximately 10 minutes. At concentrations above $3.0 \times 10^{-3}$ M spontaneous activity was noticeable and persisted even after 10-12 hours washing in sea water.

*Discussion.* While in general the reactions of *Callianassa* to the reagents tested followed the pattern of other crustacea (Table I), there are certain points of difference, especially in the responses to eserine and atropine. Eserine in concentrations of $1 \times 10^{-3}$ pro-

TABLE I.

| Drug | No. Exp. | Block conduction in giant fibers | Block synapses of giant fibers | Increase "spontaneous" activity in cord |
|---|---|---|---|---|
| Eserine | 15 | — | — | ++++ |
| Acetylcholine | 12 | — | — | — |
| Atropine | 5 | — | — | +++ |
| Nicotine | 5 | — | + | — |
| DFP | 3 | + (transient) | + (transient) | + |
| Triethylamine | 3 | — | + | — |
| Methylamine | 3 | — | + | — |

duces increased activity in the nerve cord of the crayfish (Prosser(6)); whereas solutions only 1% as strong are effective in exciting the nerve cord of *Callianassa*. Thus the neurons of *Callianassa* are responsive to eserine in amounts similar to those to be expected in a normal physiological system. This central excitatory action of eserine most probably underlies the responses observed in *Petrolisthes* by Welsh and Haskin(5). Prosser(6) is inclined to attribute the effects of eserine to a direct toxic action on the nerve cells. It would appear that atropine also exerts such an action, at least in the concentrations ($2.5 \times 10^{-3}$ M) employed by us. (Prosser(6) noted no effect of $10^{-4}$ atropine in the crayfish.) The depressive action of atropine observed in *Petrolisthes* by Welsh and Haskin(5) may have been peripheral in origin, since an effect of atropine similar to that of novocaine and procaine has been observed in nerve-muscle preparations of the crayfish by Ellis, Thienes and Wiersma(7).

The actions of nicotine, methylamine, triethylamine and DFP on *Callianassa* are in essential accord with the data obtained on the crayfish by Wiersma and Schallek(8).

It is to be noted that, although eserine exerts an excitatory action on the neurons of the abdominal cord of *Callianassa* in concentrations low enough to be physiologically significant, the addition of acetylcholine to an eserinised preparation has no observable further effect. This is true even when the eserine is causing "submaximal" excitation, or if acetylcholine is added during the latent period preceding marked activation of the neurons. Thus, even though it might be possible to attribute the excitatory effects of eserine and DFP to their anticholinesterasic activity(9), the failure of acetylcholine itself to produce any characteristic effect leads us to conclude that a cholinergic mechanism does not play a predominant role in the excitation and conduction phenomena of *Callianassa*. The action of eserine is probably therefore referable to its properties as a central excitant.

*Summary.* Eserine ($2.5 \times 10^{-5}$ M to $2.5 \times 10^{-3}$ M) and atropine ($2.5 \times 10^{-3}$ M) produce a marked increase in the "spontaneous" activity of the ganglion cells of the ventral nerve cord of *Callianassa californiensis*. These substances have no appreciable effect on conduction characteristics of the giant fibers in the nerve cord, on the transmission across the synapse between the giant fibers and the nerves to the flexors of the telson, nor on denervated muscle. Nicotine ($4 \times 10^{-5}$ M), methylamine ($1 \times 10^{-3}$ M), triethylamine ($4 \times 10^{-3}$ M) and di-isopropyl fluorophosphate (DFP) ($1.3 \times 10^{-2}$ M) reversibly block synaptic transmission for varying periods of time.

5. Welsh, J. H., and Haskin, H. H., *Biol. Bull.*, 1939, v76, 405.

6. Prosser, C. L., *J. Cell. Comp. Physiol.*, 1940, v16, 25.

7. Ellis, C. H., Thienes, C. H., and Wiersma, C. A. G., *Biol. Bull.*, 1942, v83, 334.

8. Wiersma, C. A. G., and Schallek, W., *J. Neurophysiol.*, 1948, v11, 491.

9. Nachmansohn, D., *Ann. N. Y. Acad. Sci.*, 1946, v47, 395.

Received November 1, 1949. P.S.E.B.M., 1950, v73.