Isolation and Characterization of Acetylcholinesterase and Other Particulate Proteins in the Hemolymph of Aplysia californica*

Frederick A. Bevelaqua, Kwang Shin Kim, M. H. Kumarasiri, and James H. Schwartz†

From the Department of Microbiology, New York University Medical Center, and the Department of Neurobiology and Behavior, The Public Health Research Institute of the City of New York, Inc., New York, New York 10010

SUMMARY

Hemolymph of the marine mollusk, Aplysia californica, contains four large particles: acetylcholinesterase, hemocyanin, a hemagglutinin, and a structure tentatively identified as erythrocurorin. We purified the acetylcholinesterase 20-fold by differential centrifugation and filtration through a column of 4% agarose. The freshly isolated esterase complex was found to have a sedimentation coefficient of 69, but the negatively stained enzyme lacked a definite structure in the electron microscope, and appeared as irregular aggregates of a 60 A subunit. The complex was unstable below pH 5 or during storage at 7°C. Under these conditions, enzymatic activity remained essentially unchanged. Treatment of the purified enzyme with trichloroacetic acid, organic solvents, and sodium dodecyl sulfate broke the complex down into two major subunits with molecular weights of about 70,000. Exposure of the enzyme to [3H]diisopropylfluorophosphate resulted in the labeling of one of these subunits. Although similar in specificity, the cholinesterase of the blood differed from the enzyme in Aplysia nervous tissue, which is associated with membrane. Treatment with sodium deoxycholate activated the membrane-associated enzyme but inhibited slightly that of the hemolymph; tyrocidine inhibited the hemolymph enzyme but not the enzyme of nervous tissue; and mild digestion with trypsin released the membrane-bound enzyme in an active, soluble form, but inactivated the enzyme of hemolymph.

The other particulates of Aplysia hemolymph were partially characterized. Aplysia hemocyanin was similar in structure to other molluscan hemocyanins. When negatively stained, the unit particle appeared to be a disc with a diameter of 280 A and a width of 45 A. These discs were stacked to form long cylindrical arrays. The purified hemocyanin was found to contain 0.26% copper (dry weight). Using differential centrifugation and gel filtration we also obtained a 9-fold purification of Aplysia hemagglutinin. This particle was 120 A in diameter with a dark staining central core of 40 A consisting of 6 subunits. The particle tentatively identified as erythrocurorin appeared as a structure 200 A in diameter consisting of 5 V-shaped subunits.

Acetylcholinesterase of nervous tissue is bound to membranes in Aplysia (1, 2) as it is in other animals (see Refs. 3 and 4 for review). Giller and Schwartz (2) described an esterase free in the hemolymph of Aplysia with substrate specificities similar to the enzyme from nervous tissue. The specific activity of the acetylcholinesterase in the blood was about an order of magnitude greater than that in ganglia; an equivalent volume of hemolymph contained about 6 times more activity than did the cell body of R2, the giant cholinergic neuron of the abdominal ganglion.

Esterase of electroplax can be released from membranes by treatment with toluene (5) to yield a soluble oligomeric enzyme (6-8). The acetylcholinesterase of Aplysia hemolymph is unusual since it is a large enzyme complex which is not associated with membranes under physiological conditions. Most of the protein in Aplysia blood is particulate. Examination of negatively stained preparations by electron microscopy revealed the presence of four large complexes. In order to identify the acetylcholinesterase, we separated these complexes by differential centrifugation and gel filtration. The major particulate component was the respiratory protein, hemocyanin. This copper-containing protein is present in the blood of many invertebrates (9). In addition to the acetylcholinesterase, we also isolated a hemagglutinin. Pauley et al. (10) have previously reported hemagglutinating activity in Aplysia blood. We have tentatively identified another particle as erythrocurorin, an iron-containing protein, which has been described in the hemolymph of other invertebrates (11).

MATERIALS AND METHODS

Aplysia californica (Pacific Bio-Marine Supply Co., Venice, Calif.) were maintained in well aerated aquaria containing artificial sea water (Instant Ocean, Aquarium Systems, Eastlake, Ohio) at 15°C. An amount of hemolymph approximately one-quarter to one-half the total body weight of the animal was separated through the foot and kept on ice for about 10 min. We were careful not to contaminate the blood with ink or any other secretions. After a week at 7°C unfractionated blood thickened and became slightly turbid. This phenomenon, which reminded us of clotting.
of vertebrate blood, was not studied further, but did not result from bacterial growth. No bacteria were seen upon microscopic examination; addition of toluene or 0.25% sodium azide was without effect. Hemolymph from two animals was never combined.

The small number of cells (121) and other debris was removed by centrifugation at 11,500 x g for 15 min. All purification procedures were carried out at 2°C. Samples were diluted in saline (0.15 M NaCl in 10 mM Tris-HCl, pH 7.6). Acetylcholinesterase was assayed spectrophotometrically using acetylthiocholine (Sigma Chemical Co., St. Louis, Mo.) (13) at 22°C. A unit of acetylcholinesterase hydrolyzed 1 μmol of acetylcholine per min at 22°C. Protein was estimated spectrophotometrically (14). Hemocyanin was detected by its A436.

Purification of acetylcholinesterase was carried out with fresh blood and was completed within 72 hours.

**Initial Discontinuous Gradient**—Eight milliliters of the freshly isolated and centrifuged blood were layered above two 1-ml density steps: 0.6 M sucrose in saline and 0.9 M sucrose in saline. Ten of these gradients were centrifuged at 105,000 X g for 105 min in the fixed angle type 40 rotor (Beckman Spinco, Palo Alto, Calif.). The top 8 ml of each of these ten gradients contained most of the hemaggulitin. The 0.6 M sucrose layers were combined for further purification and characterization of the acetylcholinesterase and of the particle which we have tentatively identified as erythrocurorin. These layers contained hemocyanin.

**Second Discontinuous Gradient**—The combined 0.6 M sucrose layers from the initial gradients were diluted to 25 ml and placed over three 5-ml saline layers: the bottom, 0.9 M sucrose; the middle, 0.6 M sucrose; and the upper, 0.45 M sucrose. The gradient was centrifuged for 4 hours at 95,000 x g in the SW 27 rotor. The 0.6 M layer was collected by hand, diluted to 8 ml, and concentrated to a volume of 1 ml by repeating the initial discontinuous gradient centrifugation at 105,000 x g for 90 min.

**Gel Filtration**—The concentrated enzyme was applied to a 4% agarose (Bio-Gel A-15m, 100 to 200 mesh, Bio-Rad, Richmond, Calif.) column (1 x 10 cm), which was eluted with saline.

**Isoelectric Focusing**—A gradient was prepared from a less dense solution consisting of 61 ml of water, 1 ml of 15% Ampholine, pH 3 to 10 (LKB Instruments, Inc., Rockville, Md.) and 5 ml of enzyme solution (0.6 M sucrose layer of second gradient centrifugation), and a more dense solution consisting of 57 ml of water, 8.5 ml of 8% Ampholine (pH 3 to 10), and 25 g of sucrose. Focusing was done at 4°C for 30 hours with a final potential of 600 volts (15) in a 110-M electrolysis column (LKB) (16).

**Purification and Assay of Hemaggulitin**—A 1-ml sample of the top layer from the initial discontinuous gradient (see above) was used as starting material for the further purification of the enzyme. Hemaggulitin was assayed using a modification of the procedure of Paulay et al. (10). Fresh (7 to 10 days after collection) chick red blood cells in Alsevere's solution (Glory Laboratories, Inc., Rockville, Md.) were washed three times at 10°C in saline, and adjusted to a final concentration of 2%, 0.05 ml was added to duplicate serial 2-fold dilutions of samples in 2 ml of 0.15 M NaCl in 10-ml round-bottom glass test tubes. After 1 hour at 22°C, the tubes were kept for 18 hours at 14°C. When unfractionated hemolymph or undialyzed gradient fractions were assayed, appropriate amounts of artificial sea water or sucrose saline were added to controls. The titer end point was the highest dilution which produced agglutination visible to the unaided eye. We arbitrarily defined a unit of agglutinating activity as that amount producing detectable agglutination under these standard assay conditions.

**Purification of Hemocyanin**—The 0.9 M sucrose layer of the initial discontinuous gradient contained highly purified hemocyanin. Greater purification was achieved, however, on a discontinuous gradient with several more density layers. After brief centrifugation at 11,500 x g, 20 ml of hemolymph were layered on four sucrose steps each in 5 ml of saline: 0.2, 0.6, 0.9, and 1.2 M. The gradient was centrifuged at 95,000 X g in the SW 27 rotor for 4 hours. The 0.9 M sucrose layer contained the purified hemocyanin used for the microscopic studies and for the determination of copper (17).

**Preparation of Complex Tentatively Identified as Erythrocurorin**—In order to separate the erythrocurorin from acetylcholinesterase in the 0.6 M sucrose layer from the initial discontinuous gradient, we diluted a 1-ml sample to 4 ml with saline and layered it on 14 ml of a continuous linear density gradient (0.4 M to 0.7 M in saline) over a cushion of 2 ml of 0.9 M sucrose. This gradient was centrifuged at 95,000 x g in the SW 27 rotor for 6 hours. Fractions were collected dropwise by puncturing the bottom of the tube.

**Electron Microscopy**—Negatively stained specimens were prepared either with 2% ammonium molybdate (pH 5.2) or with 1% phosphotungstic acid neutralized with NaOH on Formvar-supported carbon-coated 200-mesh copper grids (18).

The negative grids were examined with a Siemens Elmiskop 1A electron microscope using a short focal length objective equipped with a decontamination device at an accelerating voltage of 80 kV.

**Special Materials**—Purified bacteriophage ϕ1, labeled with [3H]thymidine, and unlabeled ϕ2 were obtained from Dr. Peter Modell, The Rockefeller University. Tyrocidine was the gift of Dr. Fritz Lipmann, The Rockefeller University. Diisopropyl-[1,3-3H]-phosphorofluoridate (3.3 Ci per mmol) was purchased from Amersham-Searle.

**RESULTS**

**Distribution of Four Protein Complexes of the Hemolymph**

Most protein in *Aplysia* hemolymph is particulate. Using a two-step density gradient, we found that the major constituent was hemocyanin. Three other particle types were also partially separated from each other (Table I). All four particles were present in the 0.6 M sucrose layer of the gradient. The distribution of the particle we have tentatively identified as erythrocurorin was determined only by electron microscopy, since we had no other method for assaying the presence of this component. This initial centrifugation resulted in fractions enriched in the three particles which we could assay. Hemocyanin, the largest component, was the predominant material in the 0.9 M sucrose layer. The hemaggulitin remained primarily in the supernatant. Although both the 0.6 and 0.9 M sucrose layers contained similar proportions of the acetylcholinesterase, the enzyme in the 0.6 M layer had the highest specific activity, and was used as starting material for the further purification of the enzyme.

**Purification of Acetylcholinesterase**

Using differential centrifugation and gel filtration, we obtained a 20-fold purification of the esterase with a 10% recovery of initial activity (Table II). Loss of enzyme resulted primarily from discarding cruder fractions. Enzyme activity was relatively stable. Within a week of collecting the blood, less than 10% of the total activity was lost. We nevertheless carried out the purification within 72 hours of collection, since we were concerned that the polymeric structure of the esterase complex might not be stable. Each step of the purification procedure yielded enzymatic activity of which 90 to 100% was sedimented at 105,000 X g for 90 min. In some experiments we included 0.2% glutaraldehyde in the gradient for a total exposure time of 8 hours. Under these conditions, 80% of the enzymatic activity was lost with no change in the sedimentation of the surviving enzyme activity.

**Table I**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Acetylcholinesterase</th>
<th>Hemocyanin</th>
<th>Hemaggulitin</th>
<th>Erythrocurorin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant...</td>
<td>35</td>
<td>26</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>0.6 M layer...</td>
<td>4</td>
<td>37</td>
<td>10</td>
<td>6.5</td>
</tr>
<tr>
<td>0.9 M layer...</td>
<td>63</td>
<td>37</td>
<td>90</td>
<td>13.5</td>
</tr>
</tbody>
</table>

*Detected by electron microscopy.*
TABLE II
Purification of hemolymph acetylcholinesterase

| Fraction                                | Volume (ml) | Units | Protein (mg) | Specific activity (unit/mg protein) | Purity
|-----------------------------------------|-------------|-------|--------------|------------------------------------|--------
| Hemolymph                               | 8           | 0.58  | 56           | 0.01                               | 1      
| Initial gradient centrifugation (0.6 M layer) | 1           | 0.20  | 1.5          | 0.15                               | 15     
| Second gradient centrifugation (0.6 M layer) | 5           | 0.13  | 0.75         | 0.17                               | 17     
| Concentration step (repeat of initial gradient centrifugation—0.6 M layer) | 1           | 0.11  | 0.63         | 0.17                               | 17     
| Agarose gel filtration (peak fraction)  | 0.5         | 0.06  | 0.30         | 0.20                               | 20     

FIG. 1. Gel filtration of hemolymph acetylcholinesterase and hemagglutinin on 4% Agarose. The column was equilibrated with saline at 7°C and a hydrostatic pressure of 20 cm. The flow rate was 15 ml per hour. A, protein; O, acetylcholinesterase. Arrows indicate the excluded volume. A, 0.11 unit of partially purified hemolymph acetylcholinesterase applied to column. B, 1300 units of partially purified hemagglutinin (O) applied to column.

The final purification step was filtration through a column of 4% Agarose, with a nominal exclusion limit of 15,000,000 daltons. All of the enzyme activity was completely excluded (Fig. 1A). The A_260: A_280 ratio of the purest fraction was 1.15.

Characterization of Purified Acetylcholinesterase

Stability of Esterase Complex—Although cholinesterase activity was almost unchanged after several weeks, the enzyme complex was apparently unstable. When freshly isolated, the blood's cholinesterase activity was 90% sedimented at 105,000 X g for 90 min; about 5 to 7% of the activity could not be sedimented after repeated centrifugation of the supernatants. We have not determined whether this small fraction of the total esterase activity is another enzyme or whether it represents smaller (but active) subunits of the enzyme complex. After storing unfractionated hemolymph or purified fractions at 7°C, we found that about 30% of the esterase activity per week remained in the 105,000 X g supernatant.

Treatment with detergents and low pH also altered the sedimentation behavior of the enzyme (see below). Lyophilization (The VirTis Co., Gardner, N. Y.), both in the presence and absence of phosphotungstate and ammonium molybdate, did not affect either the enzymatic activity or the proportion of the enzyme sedimenting at 105,000 X g.

Sedimentation Constant—Purified esterase sedimented as a single component of 69 S in a continuous sucrose gradient (Fig. 2). Bacteriophage f1 (40 S) and f2 (80 S) were included as standards.

Isoelectric Focusing and Effect of Low pH—Most of the enzymatic activity banded at pH 4.8 (Fig. 3), a small proportion had an isoelectric point of 3.6. We are uncertain about the molecular size of the enzyme in either of these bands, however. Treatment of the enzyme for short periods of time at low pH resulted in loss of structure, but not of enzymatic activity. We incubated the esterase at room temperature in unbuffered saline made pH 3 or 5 with dilute HCl for 1 hour. After neutralization with 1 M phosphate buffer, all of the enzymatic activity was recovered, but only 20 to 30% could be sedimented at 105,000 X g.

Effect of Detergents—The acetylcholinesterase of hemolymph did not appear to be associated with membrane. Treatment of the enzyme with Triton X-100 or sodium deoxycholate at a detergent to protein ratio of 3.3:1 did not decrease substantially the amount of esterase which was sedimented at 105,000 X g (Table III). Enhancement of membrane-bound enzymes is frequently observed after treatment with detergents (see for example Ref. 20). Kumarasiri and Schwartz have found that acetylcholinesterase of Aplysia nervous tissue was activated by deoxycholate. Treatment with detergents did not activate the enzyme.

1 M. H. Kumarasiri and J. H. Schwartz, unpublished experiments.
hemolymph enzyme (Table III). On the contrary, deoxycholate brought about a loss of 24% in its total activity.

Changeaux et al. (21) have shown that the acetylcholinesterase of the electric organ of Electrophorus electricus, when in the membrane, was insensitive to tyrocidine, but that the enzyme in solution after extraction from nervous tissue was inhibited slightly by tyrocidine. We obtained similar results. The solution after extraction from nervous tissue was inhibited of the esterase. The pattern obtained on polyacrylamide gel electrophoresis in the detergent (22) is shown in Fig. 5A. The specific activity 0.17 unit per mg. The pH (X) of each 4-ml fraction was determined. After neutralization, the fractions were assayed for acetylcholinesterase (O).

**TABLE III**

<table>
<thead>
<tr>
<th>Addition</th>
<th>Sedimented</th>
<th>Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>83</td>
<td>96</td>
</tr>
<tr>
<td>Sodium deoxycholate</td>
<td>85</td>
<td>66</td>
</tr>
</tbody>
</table>

Effect of detergents on structure and enzymatic activity of partially purified hemolymph acetylcholinesterase

Partially purified hemolymph acetylcholinesterase, 0.10 unit, was incubated for 1 hour at room temperature with 0.4% of the detergents. Samples were centrifuged at 105,000 × g for 90 min at 22°C.

Fig. 4. Effect of tyrocidine on Aplysia acetylcholinesterases. Esterase 0.20 unit, from nervous tissue (A) or hemolymph (B) were assayed in a volume of 0.2 ml containing amounts of tyrocidine dissolved in 1 ~1 of ethanol to give the indicated final concentrations in the reaction mixtures. In order to prepare the enzyme from nervous tissue, frozen Aplysia ganglia were homogenized at 0°C in about 5 volumes of 0.2 m sucrose containing 0.3 m NaCl and Tris-HCl (pH 7.6) in ground glass tissue grinders (Micrometric Instrument Corp., Cleveland, Ohio). The homogenate was centrifuged at 20,000 × g for 10 min. The resulting pellet was washed three times with buffered sucrose in saline.

Fig. 3. Isoelectric focusing of partially purified hemolymph acetylcholinesterase (specific activity 0.17 unit per mg). The pH (X) of each 4-ml fraction was determined. After neutralization, the fractions were assayed for acetylcholinesterase (O).
Hemocyanin—The purified material contained 0.26% copper (dry weight), an amount expected for a molluscan hemocyanin (23). The presence of hemocyanin in the blood of the Californian species is remarkable, however, since analysis of hemolymph from the otherwise closely related Mediterranean species (A. depilans and A. lamacina) has failed to show any hemocyanin (24).

Hemagglutinin—Using differential centrifugation and Agarose gel filtration, we obtained partial purification of a hemagglutinin (Table IV). Although purified 9-fold, the material was contaminated with other protein (Fig. 1B) and electron microscopy revealed the presence of the other particles of hemolymph (Fig. 7B). The hemagglutinin of Aplysia was similar in size and structure to those of other invertebrates (25). The complex is 120 A in diameter containing a dense, circular core of 40 A in diameter surrounded by a transparent ring 40 A thick. This ring appears to be composed of six subunits.

The purified hemagglutinin was contaminated with a small amount of acetylcholinesterase (Fig. 1B). This enzymatic activity was apparently associated with particles smaller than those in the purified enzyme, since it was not entirely excluded during filtration.

Purification of Erythrocurorin—The 0.6 M layer from the initial density gradient was examined by electron microscopy and found to contain a particle which we have tentatively identified as erythrocurorin, as well as acetylcholinesterase, some hemagglutinin, and fragments of the hemocyanin. This particle was purified by centrifugation in a continuous sucrose density gradient in which the esterase was found in the center of the gradient, and the hemocyanin at the bottom of the gradient. A band of protein toward the top contained flower-like particles, which appeared to be uncontaminated (Fig. 7C). The complex is 200 A in diameter, and consists of 10 petals. In some particles it can be seen clearly that each pair of petals is joined toward the center. Thus the particles consist of 5 V-shaped subunits. This configuration is similar to that of erythrocurorin isolated from hemolymph of other invertebrates (11).

DISCUSSION

More than 90% of the acetylcholinesterase activity present in the hemolymph of Aplysia was associated with a 69 S particle which appeared to be a distinct enzyme complex unassociated with membrane. Two other particles, hemocyanin (23) and erythrocurorin (11) were also isolated, and these are known to have respiratory function in other invertebrates. Since these particles are abundant in A. californica, it is surprising that Giurietti et al. (24) failed to find any proteins capable of carrying oxygen in the hemolymph of two other species of Aplysia. We found the structures of the hemocyanin, erythrocurorin, and hemagglutinin of A. californica to be similar to those of other animals.
The acetylcholinesterase complex, when isolated, was unstable and dissociated into smaller active particles. Exposure to low pH decreased the sedimentability of the enzyme without destroying enzymatic activity. Isoelectric focusing of a partially purified enzyme preparation yielded a distribution of activity with two isoelectric points, showing that there are at least two possible active forms of the enzyme. Although we have not studied the breakdown of the hemolymph acetylcholinesterase complex in detail, we have also found that it tended to dissociate into smaller units during storage for several weeks at 7°C. The decrease in sedimentability under these conditions was considerably greater than the total loss of enzymatic activity, and this is another indication that the complex is capable of dissociation into smaller active particles. In addition to the complex, about 7% of the enzymatic activity exists normally in the hemolymph as smaller particles which were not sedimented at 105,000 x g. This activity may be a different enzyme, but we have not studied its properties further.

Acetylcholinesterases exist in two forms. One form is soluble and is similar to that found in the blood of many mollusks (26) and other invertebrates (3). The other form is associated with membranes, as is the enzyme in all vertebrate tissues (3, 4). Although similar in substrate specificity (2), the hemolymph acetylcholinesterase differs in several important respects from the esterase bound to membrane of Aplysia nervous tissue. Thus, treatment with detergents activated the membrane-bound enzyme, but was ineffective on the hemolymph enzyme. Tyrocidine, which inhibited the hemolymph enzyme, did not have any affect on the enzyme associated with membranes of nervous tissue. Also, digestion with trypsin rapidly inactivated the hemolymph enzyme, but released the nervous tissue enzyme in an active, soluble form with a molecular weight of about 250,000. Despite these dissimilarities, it is nevertheless possible that the various properties observed only reflect differences in the physical state of the enzyme in the two tissues and that the two enzymes are related. The source of Aplysia hemolymph acetylcholinesterase is presently unknown, but it does not appear to be associated with cellular elements of the blood. Perhaps it is secreted by nervous tissue into the hemolymph.

There is some evidence that acetylcholinesterase of electric tissue might exist in the membrane as a large complex, since Lawler (27) extracted an enzyme particle with a molecular weight of 13.4 x 10^6. Most of the esterase extracted with toluene from the electroplax membrane is an 11 S particle with a molecular weight of approximately 250,000 (6-8). This particle is built up of four subunits, each with an average molecular weight of
FIG. 7. Electron micrographs of purified particles of *Aplysia* hemolymph. A, hemocyanin stained with ammonium molybdate. Magnification: $\times 250,000$. B, hemagglutinin (arrows) stained with ammonium molybdate. This fraction also contains some fragments of hemocyanin as well as the other particles of the hemolymph. Magnification: $\times 250,000$. C, the purified particle tentatively identified as erythroceurin stained with ammonium molybdate. Magnification: $\times 300,000$. 
about 64,000 (4, 8, 21, 29). Toluene extraction possibly involves a group of three (21, 29); in the larger complexes, presumably clusters in groups of four, and occasionally in the electron microscope. Electron micrographs of the enzyme isolated from electroplax membrane showed 50 to 60 A single particles which correspond to the 60 A particle which we have seen with the electron microscope. Electron micrographs of the enzyme isolated from electroplax membrane showed 50 to 60 A single particles, revealing groups of the small particles correspond to the 17 to 18 S particles, Rieger et al. (29) observed as many as 10 subunits clustered en grappe. Changeaux et al. (21) found that the electroplax enzyme appeared to be more ordered when treated with tyrocidine. Electron micrographs of the Aplysia esterase were similar to those of the tyrocidine-treated electroplax enzyme, revealing groups of the small particles in ordered clusters. These clusters were not uniform in size, however, and probably do not reflect accurately the large, complex structure of the enzyme as it most likely exists in solution in the hemolymph. We do not know the reason why the material on examination by electron microscopy appeared to be smaller and less well ordered than might have been expected from the behavior of the enzyme during centrifugation and gel filtration. We have noted however that cecine appears to stabilize the complex to some degree. In the presence of this inhibitor, which interacts with the active site of the esterase, the enzyme complexes appeared in micrographs to be more ordered and extensive.

Its great abundance in molluscan hemolymph suggests that the acetylcholinesterase might have a function similar to that of soluble pseudocholinesterases and of the acetylcholinesterase of erythrocyte stroma found in vertebrate blood. Loewi's experiment (30) on the frog heart showed that nerve stimulation can release acetylcholine into the blood. The cholinesterases found in both vertebrate and invertebrate blood might function to protect distant nervous and other responsive tissues from the transmitter substance which has escaped into the circulation.

Acknowledgments—We thank Dr. A. W. Bernheimer for his help with electrofocusing, and Dr. L. Day for discussions on the physical properties of the esterase complex. We are grateful to both Dr. Day and Dr. R. T. Ambron for their critical reading of the manuscript.

REFERENCES
30. LOEWI, O. (1921) Arch. Ges. Physiol. 189, 239-242
Isolation and characterization of acetylcholinesterase and other particulate proteins in the hemolymph of Aplysia californica.
F A Bevelaqua, K S Kim, M H Kumarasiri and J H Schwartz


Access the most updated version of this article at http://www.jbc.org/content/250/2/731

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/2/731.full.html#ref-list-1