junction blocking doses are compared.

Conclusions. 1. The curariform activity of a new series of bis-quaternary ammonium compounds is reported. 2. Moderate increase of activity is obtained by shortening the distance between nitrogen atoms from approximately 15 Å to 9 Å. 3. Replacement of N-ethyl groups with N-methyl in the 6-carbon chain analog decreases both the activity and the subcutaneous absorption in mice, but has no effect on the activity as measured in dogs and rabbits.

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Peptidases in Isolated Blastomeres of *Mytilus edulis.** (20967)

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Investigations of mosaic eggs of annelids and mollusks in attempts to characterize the various cytoplasmic regions in chemical terms have demonstrated regional differences in pH, rH, enzymes and oxidative metabolism, Spek(1), Ries(2), Berg and Kutsky(3). The following investigation was undertaken to see if regional differences of peptidases might occur in a mosaic egg.

Peptidases have been found in eggs and embryos of invertebrate animals, Holter(4), Linderstrøm-Lang and Holter(5), Holter, *et al.*(6). No correlation of enzymatic activity with cell determination has been shown in the regulative egg of the sea urchin, Holter and Lindahl(7). Alanylglycine peptidase has been found in the mosaic eggs of *Tubifex,* Holter *et al.*(6), and of *Chaetopterus,* Holter(4); however, the localization of the enzyme and correlation with morphological differentiation was not investigated. The eggs of *Mytilus edulis,* used in this study, undergo a mosaic development as shown by differentiation of isolated blastomeres, Rattenbury and Berg (8). The most obvious segregation of factors important to subsequent differentiation occurs at the first cleavage, and methods have now been developed for removal of the egg membranes and separation of the first cleavage blastomeres, Berg(9). Measurements of peptidases in first cleavage blastomeres, reported here, are in part the results of an investigation of the physiological and chemical properties of the isolated early cleavage blastomeres.

Methods. The gametes of *M. edulis* were obtained by allowing individual animals to spawn in dishes of sea water. Vitelline membranes were removed with a membrane lysin and the first cleavage blastomeres separated in calcium free sea water. Peptidases were determined by a modification of the titrimetric method of Linderstrøm-Lang and Holter(10). The segregated blastomeres were cytolyzed in 7.4 μl glycerol-phosphate solution. 7.4 μl of substrate (alanylglycine or leucylglycine) were added and the mixture incubated

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TABLE I. Alanylglycine Peptidase Activity in Early Developmental Stages of *Mytilus edulis*.

Stage of development		Peptidase activity, μl 1/20 N HCl/embryo
Unfertilized egg		.34 ± .013
Fertilized "		.36 ± .013
Blastula	6 hr	.36 ± .007
Gastrula	12 "	.34 ± .017
Trochophore larva	24 "	.30 ± .015
Veliger "	48 "	.12 ± .012

at 40°C for 18-20 hours. After stopping the reaction with 30 μl of 0.05 N alcoholic acid, 150 μl of acetone were added and the mixture was stirred. 150 μl of this were transferred to a cup shaped micro glass electrode and, using a Beckman pH meter, titrated to an end point with 0.05 N alcoholic acid from a micro burette. Electrometric titration was found to be more convenient and reliable than the colorimetric titration with naphthyl red as originally used by Linderstrøm-Lang and Holter. Since extremely small amounts of peptidase may be quantitatively measured by this method, relatively few blastomeres were needed for each determination. The majority of tests were carried out on 9 CD and 18 AB blastomeres which represent equivalent amounts of cytoplasm. To obtain accurate measurements of amounts of cytoplasm, the diameters of the two types of blastomeres were measured with an ocular micrometer. Peptidase activities were expressed as μl of acid used for titration. The experimental error in this technic is considerable; accordingly, sufficient determinations were made to permit a statistical evaluation of the results.

Results. Alanylglycine peptidase activity was found to be present in unfertilized eggs and all stages of early development of *Mytilus* (Table I). Ten or more measurements of peptidase were made on homogenates of the developmental stages. There is no significant variation of the total amount of the enzyme activity during cleavage and gastrulation; however a significant decrease occurs at the trochophore stage and the veliger contains only one-third the activity of early stages.

TABLE II. Alanylglycine Peptidase Activity in Isolated Blastomeres of *Mytilus edulis*

Blastomere	Titration value, μl 1/20 N HCl/cell	Avg vol of cells, 10^{-5}mm^3	Peptidase activity, 10^3 μl 1/20 N HCl/ mm^3 cytoplasm
AB	.13 ± .01	4.1 ± .08	3.2 ± .31
CD	.24 ± .02	8.4 ± .13	2.9 ± .32

Localization of peptidases was investigated by measuring both alanylglycine and leucylglycine peptidases in isolated cleavage blastomeres. Table II summarizes the results of determinations of alanylglycine peptidase in AB and CD blastomeres. The results are averages of 9 experiments in each of which 4 to 6 separate determinations of peptidase were made on the isolated blastomeres. Analyzed statistically as a paired series, the activities of this peptidase are not significantly different in the 2 types of blastomeres.

Table III summarizes the measurements of leucylglycine peptidase in isolated blastomeres. Six hundred AB and 300 CD blastomeres were homogenized in 150 μl of extraction medium and 12 determinations of leucylglycine peptidase were carried out on the extract. The values for cell volumes from Table II were used for calculating relative enzyme activities. The activity of this enzyme was less than of alanylglycine which probably accounts for the greater variation of the measurements. Analyzed statistically as an unpaired series, there is no significant difference in activity of the enzyme in the blastomeres.

TABLE III. Leucylglycine Peptidase Activity in Isolated Blastomeres of *Mytilus edulis*.

Blastomere	Titration value, μl 1/20 N HCl/cell	Avg vol of cells, 10^{-5}mm^3	Peptidase activity, 10^2 μl 1/20 N HCl/ mm^3 cytoplasm
AB	.021 ± .004	4.1 ± .08	5.1 ± .95
CD	.043 ± .005	8.4 ± .13	5.1 ± .66

It should be emphasized that these results are based on measurements of enzyme activity under optimal *in vitro* conditions and not the *in vivo* activity. As pointed out by previous investigators, enzymes in the living cell may be influenced by activators, inhibitors, conditions of pH, etc., and thus measurements of activity of an enzyme in homogenates may or may not reflect the actual activity in the cell.

Expressed as relative *in vitro* activities, the results may be compared with those obtained on other forms. The data of Table I indicate

that during early development through gastrulation of *Mytilus*, the activity of alanylglycine peptidase remains constant. The uncleaved egg thus seems to be provided with sufficient amounts of this enzyme for subsequent development. These results are similar to those obtained by Holter and Lindahl(7) where the amount of alanylglycine peptidase remained constant to the pluteus stage of *Paracentrotus*. Linderstrøm-Lang and Holter(5) found a decrease in peptidase in later stages of development of *Urechis* which is similar to that which occurs in the veliger stage of *Mytilus*. This may be due to a decrease in growth rate with a lowering of synthetic metabolism in which peptidases are thought to play a role.

Holter and Lindahl(7) and Holter, *et al.* (11) found no correlation of peptidase activity with cell determination of the regulative egg of the sea urchin. Animal and vegetal regions of the embryo as well as isolated germ layers contained equal activities of peptidase. Likewise, according to the data in Tables I and II, in the mosaic egg of *Mytilus*, there is no correlation of peptidase activity with cell determination. Development of isolated blastomeres of *Mytilus* demonstrates a definite segregation of developmental potentialities at the first cleavage, however, these cannot be related to differences in peptidase activity. Ries(2) has shown by cytochemical technics a localization of the enzymes peroxidase and indophenol blue oxidase (presumably cytochrome oxidase) in some mosaic eggs; however, it would be desirable to measure these enzymes quantitatively. Cytochrome oxidase, as measured by a micro-spectrophotometric method has been found in both types of isolated blastomeres of *Mytilus*; however, the variability of the results prevented comparison of concentrations. As more micro methods are developed for measuring enzymatic activity it may be possible to demonstrate a localization of other enzymes in mosaic eggs. Also, the large scale segregation of isolated polar lobes from the eggs of *Mytilus*, Berg(12), brings the amount of specific cytoplasm available for quantitative biochemical studies into a range where ultra micro-chemical methods will not be needed.

Summary. Alanylglycine peptidase occurs in unfertilized eggs, cleavage and later developmental stages of *Mytilus edulis*. Peptidases, with alanylglycine and leucylglycine as substrates, were determined quantitatively in isolated first cleavage blastomeres. No significant differences in activities of these enzymes were found and it is concluded that the segregation of developmental factors at the first cleavage is not related to activities of these enzymes.

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