# PROTEOLYTIC ENZYMES OF THE LOBSTER

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Few investigations have been made of the proteolytic enzymes of invertibrates. Originally these enzymes were distinguished mainly by their different pH ranges; but now synthetic substrates are available which are often specific for single enzymes. By this method J. Mansour-Bek[1], who gives a review of the earlier literature, studied the proteolytic enzymes of *maja squinado* and found a carboxypolypeptidase by its action on chloracetyltyrosine, an aminopolypeptidase by action on leucylglycylglycine, a dipeptidase capable of splitting glycylglycine and trypsin, characterised by splitting proteins at pH 7.4. He obtained similar results[2] with *murex anguliferus*. Synthetic substrates are now also available for trypsin[3] and chymotrypsin[4], and a fairly comprehensive survey of the enzymes of digestive juices is possible without effecting any separations.

In the present experiments extracts from the large digestive organ of the lobster were examined. The organ was removed and crushed in a mortar and strained through gauze. The solid material left in the extract and the oily layer which came to the surface, were removed as completely as possible by centrifuging. The extract had a pH about 7.5. The following table shows the results obtained when the

TABLE 1

| Temperature C | Substrates | Increase in formol titration for 1 cc. of mixture (*h*) | Increase in formol titration no substrate | Notes |
|---|---|---|---|---|
| 15 | Casein | 4.35 | — | (*a*) |
| 15 | Edestin (formolized) | 0.20 | — | (*b*) |
| 35.5 | Glycylglycine | 0.23 | 0.0 | (*c*) |
| 35.5 | *l*-leucylglycylglycine | 1.36 | 0.13 | (*d*) |
| 35.5 | *d*-leucylgylcylglycine | 0.08 | — | (*d*) |
| 35.5 | Chloracetyltyrosine | 0.46 | 0.04 | (*e*) |
| 35.5 | Benzoyl-*l*-arginine amide | 2.92 | 0.15 | (*f*) |
| 35.5 | Benzoyl-*l*-tyrosylglycine amide | 1.07 | 0.25 | (*g*) |

NOTES:

(*a*) 5 cc. of 5% casein in M/10 phosphate buffer, $p_h$ 7.5, as prepared by Northrop and Kunitz (5), +1 cc. extract.

(*b*) 5 cc. of formolized edestin, as prepared by Anson (6), +1 cc. extract. Titrated directly.

(*c*) 5 cc. 0.01M glycylglycine+1 cc. 7.5 buffer+1 cc. extract.

(*d*) 5 cc. 0.01M leucylglycylglycine+1 cc. buffer+1 cc. extract.

(*e*) 5 cc. 0.01M chloracetyltyrosine+1 cc. buffer+1 cc. extract.

(*f*) 5 cc. 0.05M benzoyl-*l*-arginine amide+1 cc. buffer+1 cc. extract.

(*g*) 5 cc. substrate (3 mgm./cc.)+1 cc. buffer+1 cc. extract.

(*h*) Formol titration. To 0.5 cc. sample is added 0.5 cc. of 40% formaldehyde and 5 drops of 0.1% phenolphthalein, and titrated to first definite pink color with 0.1N sodium hydroxide.

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test mixtures were left for 23 hours at the designated temperature. A formal titration was carried out on samples at the beginning and end of this period and the figure given is the increase of titre of 0.01 N alkali observed. Blank experiments were performed with similar mixtures containing no substrate. The substrates themselves undergo no significant hydrolysis in this period under the circumstances and in the absence of extract.

It can be seen that the extracts have an appreciable action on casein, *l*-leucylglycylglycine (aminopolypeptidase) but not *d*-leucylglycylglycine. benzoyl-*l*-arginine amide(trypsin), benzoyl-*l*-tyrosyl glycine amide (chymotrypsin). The presence of the latter was also confirmed by the powerful milk clotting action of the extract. The comparatively slight action on chloracetyltyrosine and formolized edestin cannot be regarded as conclusive evidence for a carboxypolypeptidase. The general and rather surprising conclusion is that the extracts are very similar in enzymic constituents to those of the mammalian pancreas.

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## EFFECT OF HEAD ECTODERM ON DEVELOPMENT OF LIMB MESODERM IN AMBLYSTOMA PUNCTATUM

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The present investigation is concerned with the substitution of head ectoderm for normal limb ectoderm in Amblystoma punctatum embryos. The mesoderm of the fore limb was shown by Harrison (1918) to comprise a harmonic, equipotential system. That final expression of the limb-forming potencies, resident in this mesoderm, may be influenced by its surrounding tissue has been demonstrated by Harrison (1921) in studying the relation of limb development and the orientation of limb mesoderm with reference to the dorsoventral axis of the embryo and by Nicholas (1924) in studying posture regulation of the limb in response to changes in the mesoderm immediately surrounding the limb area. That realization of limb-forming potencies on the part of the mesoderm may be hindered by replacing normal ectoderm with head ectoderm has been suggested by Harrison. Similar suppression but of the balancer rather than the limb, by covering the region with other head ectoderm, has been reported by Carpenter (1937).

In a series of 140 cases the result of replacing the normal limb ectoderm with ectoderm from the head region of Amblystoma punctatum was studied. The age of the donors ranged from yolk-plug