

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*-tyrosylglycine 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

(Harrison stage 12) to tail-bud stage (Harrison stage 29). Ectodermal grafts of stages 25-29 were taken from across the front of the animal so as to avoid inclusion of cells over the optic cup and olfactory placode. Care was exercised in placing the graft to disturb the limb mesoderm as little as possible. Camera lucida sketches were made of the graft after it had healed in place. The experimental animals were observed for at least 15 days following the operation, the maximum period being 30 days. Of 131 cases which lived, 85 showed complete suppression of the limb on the operated side, 41 showed partial suppression, in 5 cases a normal limb developed. In the cases classified as partial suppressions an incomplete appendage developed. This consisted in each case of a shaft-like structure supported by a single rod of cartilage and lacking in digits. The occurrence of these cases as well as of those in which a complete limb developed was correlated with the eccentric position of the grafted head ectoderm in relation to the limb-forming area. In 10 cases in which limb ectoderm (stage 29) was replaced by belly ectoderm (both stage 13 and stage 29 were tested) a normal limb developed. It is concluded therefore that head ectoderm as opposed to flank or belly ectoderm will, when grafted over developing limb mesoderm, suppress the development of the mesoderm and prevent limb formation.

Serial sections of animals (10) fixed at intervals (1-5) days after grafting of the head ectoderm over the limb were examined. Significant differences in the number of mitoses in the mesoderm of the normal and the operated sides were not found. Whereas the basement membrane of the graft was not abnormally thick the layer of epithelial cells was conspicuous because of its thickness, varying from two to three times that of the normal.

Five representative cases in which limb suppression was obtained by grafting head ectoderm (stage 19-22) over limb mesoderm were selected for study in serial sections. In one of these cases a short, rudimentary limb was present, in the others the appendage was completely lacking. Examination of the sections revealed the presence of a pectoral girdle in every case. Coracoids, constantly present, showed slight irregularities in contour and interestingly enough had acquired a glenoid cavity in only one case, that being the one in which a rudimentary appendage was present. The scapula-suprascapula cartilage was present in each case but tended to be reduced in size and less platelike than the normal. A procoracoid was identified in two cases. Myotomes had developed normally and the usual sheets of segmental muscle were present in each specimen.

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