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TABLE OF CONTENTS

The Role of the Ectodermal Crest In the Developing Wing Of the Chick Embryo

by

Yvonne T. Grantling

I. Introduction

The embryo of the chick has been repeatedly employed in extensive studies on morphogenesis and differentiation. Recently, however, the development of the appendage buds of the chick embryo have been studied with the purpose of following the pattern of differentiation in a more or less independent system. These studies involve the development of the young appendage buds on the chorio-allantoic membrane. Much fruitful information has been obtained from this type of investigation. Most recently Saunders (1948) and Zwilling (1952) have emphasized an interaction between the ectodermal crest of the wing bud and the underlying subadjacent mesoderm in the proximal distal growth of the appendage components.

Warren (1934) reported that definite bones would be missing in the definitive wing upon removal of the anterior or posterior halves of the wing bud. He indicated that a mosaic pattern of wing parts is resident in the mesoderm of the three-day old wing bud.

Hansborough (1954) removed plugs of mesoderm varying in sizes from 5% to 50% without disturbing the distally located ectodermal crest of the four-day old embryo wing bud. He observed that since complete regulation can take place after the removal of one-third to one-half of the tissue from the bud, the mosaic arrangement of materials for the future wing parts if present is not a rigid one.

Wolff and Hampe (1956) excised an intermediate segment of the three day old limb bud and transplanted it on the chorio-allantoic membrane. The terminal cap was replaced on the base of the limb bud. The operated bud forms a perfect leg and the transplanted fragment gives rise to several bone elements. Their evidence showed that regulation is sub-total in that only the fibula and first digit is absent. They termed this "regulation intermediaire."

Hunt (1932) grafted wing buds of embryos ranging in age from forty-eight hours to seven days old to embryos eight to ten days old for the purpose of analyzing the dif-

ferentiation of the limb buds and the extent to which the chief components or limb, bone and muscle are self differentiating. He observed that the muscle of the chick limbs are capable of initial independent differentiation, but they do not maintain this independency.

Zwilling (1952) transferred the ectoderm from three day chick embryo wing buds to the mesoderm of the leg bud denuded of its ectoderm. He concluded that although the presence of the apical ectoderm is a specific requisite for limb out growth, it has no influence on the determination of the limb type (i.e. leg or wing).

II. Materials and Methods

The eggs used in this experiment were obtained from New Hampshire Red Hens from the Poultry Department of the University of Maryland. They were incubated at 38 degrees Centigrade for a period of 3½ days (stages 21-24 of the Hamburger-Hamilton series). Before operating each egg was candled and the site of the embryo marked. The small end of the egg was sterilized with 70% alcohol and a small hole was made from which ½ cc. of albumin was removed with a 5 cc. syringe. With a rotary drill, a window 1.5 cm. square was drilled in the egg through which the operations were made.

After exposing the embryo, the amnion and chorion were deflected to expose further wing bud. An incision was made into the ectodermal crest with number 12 sewing needles which were sharpened to a fine point. Portions of the crest were stripped off with micro-forceps. After the operations were completed camera lucida sketches were made of the bud. The amniotic folds were then pressed together with the micro-forceps to insure their union and to prevent the deformation of the bud during future development. The window was firmly sealed with cellophane tape and the eggs were replaced in the incubator. Development was allowed to continue until the tenth day at which time the embryos were recovered and fixed in 10% formalin. Each operated wing was observed for morphological changes and camera lucida sketches were made for comparison with the controls. The embryos were not stained for the study of their skeleton.

III. Observations

TABLE I

Crest Removed		*No. Operations*	*No. Survivals*	*Results*	
				Normal	*Abnormal*
Anterior	⅓	121	68	13	55
Middle	⅓	47	11	0	11
Posterior	⅓	87	58	0	58
	Total	255	137	13	124

Table I shows that a total of 255 embryos were operated upon of which 137 survived. The embryos operated upon were divided into three categories. In the first group in which the anterior third of the crest area was removed, there were 121 such operations of which 68 embryos survived. In the second group in which the middle third of the crest was excised, there were 47 operations of which 11 embryos survived. In the third group where the posterior third of the crest was removed, there were 87 operations of which 58 embryos survived. In the first group (ant. ⅓ removed) 13 of the 68 surviving embryos were normal. In the second group (middle ⅓ removed) there were no normal embryos of a total of 11 survivals. In the third group (posterior ⅓ removed) all of the 58 surviving embryos were abnormal.

Results for the different excisions:

A. **Anterior one-third excised**

Upon removing the anterior one-third of the ectodermal crest of the wing bud, there were 13 normal definitive wings and 55 abnormal wings. The abnormal wings in this group were expressed in the complete absence of digit two.

B. **Middle one-third excised**

Removal of the middle-third of the ectodermal crest of the bud produces drastic abnormalities in the definitive wing. There is a characteristic absence of all the wing parts distal to the humerus. In other cases, the entire wing was absent.

C. **Posterior one-third excised**

When the posterior third of the ectodermal crest is removed, the abnormalities are more pronounced than those produced when the anterior one-third of the crest is excised, but less so than when the middle third is removed. The abnormalities range from the complete absence of digits 2, 3, and 4, to the absence of the radius and ulna.

IV. Interpretations

In the light of the data reported here and those reported by Warren (1934), Hansborough (1954), Wolff and Hampe (1956), it can be concluded that the factor which influences the outgrowth of wing components resides in the ectodermal crest of the early bud. Its absence in the wing bud of the wingless embryo as Zwilling (1949) has demonstrated, is responsible for the lack of a definitive wing in the older chick. Its injury or removal, as Saunders has demonstrated, causes a deficiency in wing parts of the definitive wing or their complete absence. The work reported here is the initial step in plotting the areas of the

crest ectoderm which seem to be specific in their influence on the elaboration of distal wing parts. Further studies may result in a complete fate map of inductive regions and their specific influences on the differentiation and growth of wing components.

V. Summary and Conclusions

1. Portions of the ectodermal crest of the wing bud have been excised for the purpose of studying the factors operating in the growth and the elaborations of the parts of the definitive wing.
2. When the apical ectodermal crest of the wing bud is damaged or injured an abnormal wing develops.
3. Removal of the anterior third of the crest results in the growth of a normal wing or the absence of digit 2.
4. When the middle third of the crest is removed, there is a complete absence of the distal wing parts or the presence only of the humerus.
5. Removal of the posterior third of the apical crest results either in the absence of all the digits or the complete absence of the digits, radius and ulna.
6. The ectodermal crest of the wing bud determines the growth and the differentiation of the future wing parts of the definitive wing.

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