A STUDY OF FEATHER CHARACTER IN LIMBS TRANSPLANTED BETWEEN EMBRYOS OF DIFFERENT BIRD SPECIES

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This paper reports some observations made upon pigment and feathers in transplanted limbs of avian embryos. The transplants include all possible reciprocal combinations between guinea fowl, duck, turkey and chick embryos and, in addition, grafts of chukar partridge and quail limb buds to embryonic chick hosts. For a preliminary report of this work see Eastlick.2

Three- to four-day embryos were used as donors and hosts. An opening 5–7 mm. square was cut in the shell directly over the host embryo, the vitelline membrane slit and the amnion torn open whenever necessary. A limb bud was severed from a donor embryo, transferred to the lateral body wall of the host and either placed into the intra-embryonic coelom (Hamburger3) or attached to the right lateral body wall of the host (Eastlick4).

Transplants placed into the intra-embryonic coelom usually interfere with the retraction of the yolk sac so that the host dies before hatching. However, in one case a host to which a duck leg had been transplanted was autopsied 26 days after hatching and a graft 5 cm. in length was found within the coelom. Transplants to the lateral body wall interfere much less frequently with the development of the host so that many are able to hatch and survive. The oldest host of this type, a chicken bearing an extra duck leg, was 15 weeks old on October 12. So far, 48 chick hosts bearing extra limbs from various avian embryos have hatched; this represents approximately 17% of the total number of embryos with flank grafts allowed to reach the age of hatching.

Chick to Chick Transplants.—In these experiments White Silkie Bantam embryos were used as donors while the hosts were White Leghorn, New Hampshire Red and Barred Plymouth Rock embryos. Silkie feathers, with the possible exception of the proximal portions of the major wing and tail feathers, are distinctive since they have a plume-like or “hairy” appearance due to the absence of hooklets (barbicels) on the barbules. Of the 115
transplants made, 62 were successful and 8 hatched. Two of the transplanted legs possess Silkie type feathers since barbicels are lacking at 8 weeks of age, while the other leg has feathers which are intermediate between donor and host types in that some of the barbules have barbicels while others do not. All of the transplanted wings possess feathers of the intermediate type with the distal ends of the feathers usually presenting a frayed appearance while the proximal ends are of host type. The frayed condition is brought about by the barbules tending to separate because of the absence of barbicels or the lack of a number sufficient to hold them together. Frequently two or more adjacent barbules have numerous hooklets and resemble the host feather type while other barbules in the immediate vicinity are bare. It is suggested, tentatively, that this result is brought about by the mesoderm of the host invading the graft and progressively altering the development of the donor follicles in some unknown manner. More experiments will be made in an attempt to gain further information on this point.

It is well known that Leghorns are fast feathering, that Barred Rocks feather slowly while Silkies seem to be intermediate. The data secured in this study suggest that the feathers of a transplanted limb maintain their inherent (donor) growth rate rather than assuming that of the host. For example, a Brown Leghorn wing bud transplanted to a Barred Plymouth Rock produced feathers which developed more rapidly than the plumage of the host (Fig. 1). Moreover, the feathers of Silkie wings developed on White Leghorn hosts did not grow as fast as the wing feathers of the host but a Silkie leg grafted to a Barred Rock produced feathers which grew more rapidly than those on the host's own legs.

These results differ from those obtained by Willier and Rawles who made skin grafts of White Silkie Bantams to other breeds and found that the transplanted Silkie skin developed feathers which possessed the form, arrangement and growth rate of the host. Willier and Rawles transplanted a restricted piece of ectoderm plus small amounts of adhering mesoderm, while in the present study the entire limb bud with its ectoderm and mesoderm was used. It is suggested that the amount of mesoderm which is included with the donor tissue may account for the divergent results which have been secured in the two laboratories.

When a comparison is made between grafts of White Leghorn and Silkie limb buds, the latter may be distinguished by their smaller size at comparable stages and the presence of feathers along the outer portions of the shank and foot in more advanced stages.

Two chickens have hatched in which Silkie limbs were transplanted to New Hampshire Red hosts. In both of these the melanophores of the host invaded the transplant and pigmented the feathers.

When a posterior limb bud of a 60-hour Silkie Bantam was removed by
making the cuts at the level of the posterior cardinal vein and transplanted to a White Leghorn host, the resulting graft lacked dermal pigment on the shank and possessed colorless skin due to the absence of subcutaneous chromatophores. It is suggested that these two types of pigment cells are derived from the neural crest since all grafts which included any of the crest were pigmented.

No antagonistic reactions have been observed between donor and host.
in these transplantations which is in marked contrast to the intense inflammation and destruction of the epidermis of duck, guinea hen, chukar partridge and quail limb buds on chick hosts.

**Reciprocal Transplants between Chick and Duck Embryos.—**Of the 132 operations made by grafting Mallard and White Pekin duck limb buds to White Leghorn and Barred Plymouth Rock hosts, 62 were successful and 15 hatched. Typical web-footed duck legs developed upon the chick hosts. The extra duck legs persist after the hatching of the host and, for a time, grow more rapidly that the host's own legs.

Transplants of White Pekin limb buds to the flanks of Barred Rock hosts invariably develop pigmented down. The melanophores of the chick migrate into the extra leg in large numbers as is shown by the fact that the skin of the shank, toes and web of the graft as well as the feathers became pigmented (Fig. 2). White Pekin transplants made to White Leghorn hosts always develop non-pigmented feathers. Mallard limb buds transplanted to White Leghorns develop pigmented down when neural crest cells are included with the donor tissue but non-colored down when the crest cells are excluded. Similar results have been obtained by Eastlick in transplantations of limb buds between pigmented and non-pigmented chick embryos.

Mallard melanophores are able to migrate extensively in White Leghorn hosts for in a number of cases the pigment cells wandered out from the transplant and pigmented the feathers on the right wing and the major portion of the down on the right lateral body wall of the host. In such cases the feathers beyond the limit of the transplant are unquestionably of host origin and are merely pigmented by the donor melanophores. The down was replaced by feathers having the same form, distribution and rate of growth as host feathers on the unoperated side.

The down on the extra limb, which probably is of donor origin, is lost following an intense inflammation in the epidermis of the graft. This reaction does not proceed beyond the limits of the duck skin as may be noted by the difference in color of the skins of the donor and host. Later feathers which appear to be of host origin develop and cover the graft. A more extensive account of the tissue reaction and replacement phenomena will be reported later.

Of the 60 operations in which chick limb buds were transplanted to duck hosts, 20 cases were successful but none survived beyond the time of hatching. White Leghorn grafts attached to the mesenteries of Mallard hosts remain non-colored while transplants made to the lateral body wall always possess pigmented down. In the former, melanophores of the host were unable to migrate into the graft while they succeeded in doing so in the latter cases. At present it is not clear why melanophores are unable to migrate across the mesenteries. Transplants of Barred Rock limb buds to White Pekin ducks were colored or non-colored depending upon the pres-
ence or absence of neural crest cells. Barred Rock melanophores are able to migrate extensively since the wing and body wall of a number of hosts became pigmented when the transplant included neural crest cells.

Sixteen reciprocal transplants have been made between Mallard and White Pekin embryos in which 11 cases were positive but none hatched. The White Pekin transplants remained non-colored when attached to the mesenteries but became pigmented when attached to the lateral body wall of the hosts. Mallard transplants which contained neural crest cells sometimes pigmented large areas of the Pekin hosts but when neural crest cells were absent from the donor tissue, non-colored grafts resulted.

Reciprocal Transplants between Guinea Fowl and Chick Embryos.—Of the 96 guinea fowl to chick transplants which were made, 49 were successful and 9 hatched. Pearl guinea fowl embryos were used as donors in all cases and most of the hosts were White Leghorns although a few were Barred Rocks. It was noted that the majority of grafts possessed non-colored feathers even when the protocols stated that neural crest cells should have been included with the donor limb buds. In an effort to insure that crest cells were included with the transplant, the donor buds were severed by making the cuts adjacent to the neural tube. Even in such instances the grafts were covered with only partially pigmented down and the melanophores failed to migrate into the adjacent tissues of the host. It seemed that this reaction might be due to (1) the failure of neural crest cells of the guinea to migrate laterally from the cord as rapidly as is the case in chick or duck embryos or (2) an antagonism between chick and guinea tissues. In an attempt to gain evidence on this point a half-portion of the neural tube and the neural crest between two or more somites were transplanted to the flank of White Leghorn hosts. Rather small pigmented areas resulted although similar transplants of Barred Rock tissues produced extensive areas of pigmented down. Moreover, an extra guinea fowl limb on a chick host fails to survive more than a few days after hatching, while an extra duck limb persists and grows. These results suggest that chick hosts afford an unfavorable environment to the cells and tissues of the guinea fowl. An explanation of the factors underlying the inability of the guinea tissue to survive awaits further study.

Transplants of guinea fowl limb buds to the flank of Barred Rock hosts invariably bear pigmented down even when donor neural crest cells are excluded. This is due to the migration of chick melanophores into the graft. The down is not replaced by juvenile feathers since the graft undergoes necrosis soon after the time of hatching.

Forty chick to guinea fowl transplants have been made of which 20 were positive but none hatched. White Leghorn buds grafted to the flank of guinea fowl hosts became fully pigmented when a broad attachment existed between the host and transplant but in case of a narrow bridge, the feathers
on the graft usually became colored only in part. All grafts connected with
the mesenteries were non-pigmented.

Reciprocal Transplants between Chick and Turkey Embryos.—Of the 122
chick to turkey grafts which have been made, 60 were positive and one em-
broyo hatched but lived only a day. White Leghorn limb buds transplanted
to the coelom remained non-pigmented when attached to the mesenteries.
Grafts within the coelom, however, developed colored down if attached to
the muscles of the inner body wall. Melanophores are unable to migrate
across intact parietal peritoneum or mesenteries but can cross a muscular
bridge between donor and host so that donor limbs within the coelom or at-
tached to the lateral body wall of the host become darkly pigmented. The
melanophores not only pigment the down of the graft but the skin of
the shank and foot as well.

Eighty-four turkey to chick transplants were made, 51 being successful.
Two of the latter hatched but lived only a day. Usually turkey limbs do
not develop a normal amount of muscle when transplanted to chick hosts
but the skeletal elements grow quite rapidly. Some of the grafts are larger
than any of the chick to chick transplants. The turkey transplants
possess pigmented down only when neural crest cells are included with the
donor tissue. Turkey melanophores were found to migrate rapidly into
chick tissues.

Fifty-four turkey to turkey transplants have been made of which 15
were positive but none hatched. Both donors and hosts were of the Bronze
variety. Transplants which became attached to the mesenteries developed
colored plumage only when neural crest cells were included while all grafts
on the outer body wall of the hosts were colored.

Reciprocal Transplants between Turkey, Duck and Guinea Fowl Embryos.
—Successful grafts have been made in all possible combinations but none
of the embryos hatched and no facts at variance with the preceding have
been noted.

Transplants of Quail and Chukar Partridge Limb Buds to Chick Embryos.
—Fifty-three transplants of quail limb buds to chick hosts have been made;
26 were successful and 7 hatched. Of the total of 63 chukar to chick grafts
made primarily to White Leghorn hosts 27 were successful and of these 6
hatched. The quail and chukar partridge limb buds developed normally
but the grafts are much smaller than chick to chick transplants.

Non-pigmented grafts were secured in both types by excluding the crest
cells from the donor tissue. The feathers on the transplants grew for a few
days but degenerated and gradually sloughed off during the period of in-
flammation and destruction of the epidermis which occurred usually be-
tween 8 and 14 days after hatching. After varying periods of time, the
grafts ceased growing and were resorbed gradually. In two cases a few
feathers, seemingly of host origin, appeared upon the graft during the
period of resorption. While the original feathers on these transplants possessed typical chukar partridge pigmentation and markings, the regenerated feathers were non-colored.

In several instances melanophores spread from the transplant pigmentation a considerable area of the host down which later was replaced by feathers possessing, entirely or in part, the markings of the donor but which grew at the host rate. In many cases the tips of the definitive feathers were more heavily pigmented than the bases; frequently the terminal ends were darkly colored, the mid-portions were mosaics and the bases non-colored. When such a feather was pulled the one replacing it was non-colored and regenerated at the same rate as a similar feather on the unoperated side. These feathers which seemed to be of host origin, but possessing donor type of pigmentation, were not influenced during the period when the epidermis of the transplant was destroyed.

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**THE BREEDING OF SOME RABBITS PRODUCED BY RECIPIENTS OF ARTIFICIALLY ACTIVATED OVA**

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In a previous paper1 I reported that three litters of young were obtained from rabbit does which had received transplantations of eggs previously subjected to artificially activating treatments. One was a litter of fifteen, of which three were males and twelve females. Two were litters of one, each female. Since the latter were produced by does made pseudopregnant by pituitary extract there could be no doubt that the young arose by parthenogenetic development. This was confirmed by the fact that the coat color of the offspring was that to be expected from the transplanted ova. In the case of the first litter, however, a probability exists that some of the offspring might have been developed from fertilized eggs since the recipient female was made pseudopregnant by mating with a vasectomized male. If this male were imperfectly vasectomized, or retained sperm for some time after vasectomy, he might have been the parent of the young.

The ova transplanted into the first female were superovulated by pitui-