Tract Specificity in the Structure of Down Feathers of the Newly-Hatched Chick
(plumage/pterylae/papillae/skin grafts/microscopy/mesoderm/embryogenesis)

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ABSTRACT Comparative study of completed down feathers from seven of the major tracts of newly-hatched Silver Campine chicks reveals tract-specific differences in the total length of the primary group of barbs and in the length of their barbule-free tips. Two classes of barbs can be distinguished on the basis of overall length of barbs of any one of the tracts: (a) those barbs that form from the first group (complement) of 9-11 barb ridges that arise in strict dorsoventral order around the circumference of the epidermal cylinder of the feather germ, and (b) those barbs that are added secondarily, later, either by the formation of a newly organized ridge between the bases of two of the original group of barb ridges, or by the splitting of one or more of the original complement of barb ridges. The primary (majority) group of ridges, only, are of use in gauging tract specificity. The statistical significance of measurements of total barb-length and the length of the barbule-free tips have been evaluated; differences between the feathers of each of the seven tracts examined can be distinguished statistically. The fact that the first feathers to form from the papillae are tract-specific shows, unquestionably, that specificity is present in the papillae from the very beginning of their organization, as might be inferred from their orderly pattern of origin in time and space within each of the various tracts.

The present study is one of several concerning the origin of regional specificity in the ectodermal derivatives of the domestic fowl (1, 2). Initially (1), it was shown that each of the various types of derivative (feather, scale, beak, and spur) acquires its distinctive characteristics during embryogenesis in response to a specific stimulus from the underlying mesoderm (prospective dermis). The question as to whether this mesodermal component also determines the tract specificity—so apparent in the succeeding plumages of the juvenile and adult birds—has, more recently, been under experimental investigation. The experiments were designed to permit combinations of embryonic ectoderm and mesoderm of different tract origins to be carried (as skin grafts) through the normal sequence of plumage changes from down juvenile to adult (Rawles, unpublished data). During the course of this experimentation, requiring long periods of time for completion, microscopic examination of the down feathers of certain of the heterologous grafts suggested strongly that the down feather itself possesses tract-specific properties. Because the down feathers for nearly a century have been considered "structurally uniform," this observation led immediately to the present report, which is based on a morphological study of the completed down feathers located within the major tracts of the normal newly hatched chick. It establishes that morphological differences exist in these first feathers to arise from the papillae of the tracts (pterylae).

MATERIALS AND METHODS

The majority of the down feathers studied were taken from seven principal tracts of newly hatched chicks of the Silver Campine, a pigmented variety of domestic fowl, the plumage of which shows varying shades of gray coloration. For comparison, the down plumage from chicks of two all-white varieties of domestic fowl, a White Leghorn and a hybrid from a cross of White Leghorn—New Hampshire Red, was also examined. In all cases, chicks were removed from the incubator as soon as their feathers had become dry and fluffy, i.e., after the normal shedding of the embryonic, external feather-sheaths. The specimens were killed by extended etherization, fixed in toto in 10% formalin, rinsed thoroughly, and kept immersed in frequent changes of 70% alcohol until ready for study. Since the barbs of the individual feather spread apart quickly as the feather dries, but immediately cling together closely again when wet, the feathers were handled as far as possible while moist with alcohol.

The feathers were removed, as needed for study, from the more central portions of each of the following tracts, head (posterior), neck (hackle), saddle, breast, humeral, thigh, and wing, as described and figured by Holmes (3). One at a time, the moistened feathers were placed, ventral side downward (i.e., the side next to the body of the bird) on a glass slide for examination with a binocular dissecting microscope at a usual magnification of ×15.

With the aid of a fine camel's hair brush (of the quality used by artists) and a steel beading needle, the barbs of each individual feather were carefully spread outward and upward obliquely from the calamus and in one plane, a dorsal barb usually alternating with a ventral barb, resulting in a fan-like configuration. The dried remnant of pulp was broken off and discarded. To hold the almost weightless feather in place on a glass slide during the manipulation of its barbs, the apex of a triangular piece of glass (cut from the corner of an ordinary slide) was placed temporarily over the calamus. A glass coverslip thereafter served to hold the barbs in a flattened position for study. While the mounting of the feather in this manner obscures the normal circular arrangement of the barbs around the calamus, it permits morphological comparisons under manifaction of feathers of different tract origin. Comparisons were based on measurements of the length of the barbs and the ratio of the length of the barbs

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These data, of shorter bimodal fashion, studied demonstrates measured lengths are, actually, regard total seem to from left to right, as indicated by sample numerals. See text for explanation. (X3).

**OBSERVATIONS**

An obvious first difference between the down feathers of the major tracts is their overall size (Figs. 1–3), which is clearly dependent on the length of their longest barbs. Close examination under low magnification of a single, typical, completed down feather from any one of the principal tracts (e.g., Fig. 1, from the dorsal neck tract) showed that while the majority of the barbs appear to be of the same length, there are also a few barbs that are noticeably shorter. When barbs are counted from left to right in Fig. 1, barbs 3, 5–11, and 14 seem to be of similar lengths, whereas barbs 1, 2, 4, 12, and 13 are definitely of shorter, variable lengths. Measurements of the total length of the individual barbs confirm that there are, actually, two separate and distinct classes of barbs in regard to overall length. A frequency distribution of barb-lengths measured in feathers of each of the seven tracts studied demonstrates that barb-length not only distributes in bimodal fashion, but that the spread of values of the group of shorter barbs is twice that of the groups of longest barbs. These data, shown graphically in Fig. 4 from the saddle tract, are representative also of the other six tracts examined. Graphs of this kind illustrate, furthermore, that the majority of the barbs, the longest and most numerous, come very near to being of equal length. Within any one tract, the majority or primary group of barbs falls within a very small range of values.

The significance of the existence of two separate and distinct groups of barbs is suggested by the observation of Watterson (4). In his study of the development of the down feathers of the wing tract, Watterson reports that barb ridges, in addition to those of the first complement, are frequently added secondarily after the development of the first complement has begun. These additional barb ridges are formed by either of two developmental processes; (a) by the formation of a new barb ridge in a triangular space that may occur occasionally between a pair of ventral barb ridges that fail to increase correspondingly with the increase in diameter of the feather germ (epidermal cylinder), or (b) by the splitting of one or more of the original complement of barb ridges.

Since barbs of the second order begin to develop later, and by processes that may be considered irregular, it might be expected that these barbs would be shorter and show greater variation in length than those of the original (primary) group that arise in strict dorsoventral order around the circumference of the feather germ.
Further evidence that barbs of the shorter and variable type most probably represent the group that Watterson found were added later is the fact that they can be distinguished from the primary group of longer barbs by the morphology of their distal ends. The primary group of barbs, the longest and most numerous, begin to develop with a barbule-free tip. Proximal to the tip, barbules begin to form and increase progressively in length towards the basal portion of the barb. Some of the barbs of the shorter group, however, bear no tip at all, but begin development with the formation of long barbules (e.g., barbs 1, 2, and 13, Fig. 1). Barbs of this type would correspond to those described by Watterson as forming secondarily by the splitting of one of the original group of barb ridges. Watterson states that when splitting occurs, the split is always below (proximal to) the barbule-free, apical portion of a barb ridge, and may occur at almost any level between this point and the base of the barb ridge. Indeed, a ridge derived from splitting may, in turn, split into two ridges. Although the number of barbs derived from splitting is extremely variable, the average number of splits per feather appears to be three. Others of the group of shorter barbs begin development with barbule-free tips, resembling somewhat those of the primary series of barbs except that the barbule-free portion is always very much shorter (e.g., barbs 4 and 12 in Fig. 1). This group of shorter barbs would correspond to those that arise from a newly organized barb-ridge interposed in the gap that occurs occasionally between two of the original ridges of the first complement (4). Because the development of the shorter group of barbs is distinctly different from that of the longer, primary group, this study of differences in barb morphology between feathers of various tracts has been restricted to the primary group of 9–11 barbs that are more constant in number and morphology from feather to feather.

Y-shaped barbs have been observed in feathers of all seven of the tracts studied, but more frequently in the saddle tract (see Fig. 2, barbs 9 and 10). Such barbs undoubtedly represent a fusion of the formative zones of two of the primary barb ridges. Thus, it would appear that fusion, as well as splitting, of organizing centers may occur.

Measurements were made of the total barb-length and of the length of the barbule-free tip of each of the primary group of long barbs of 11–13 feathers from seven of the major tracts; (this is only a fraction of the total number examined under magnification). These measurements are presented graphically in Fig. 5. The averages are derived by pooling the measurements of the longest barb in each feather to give a value for each of 11 to 13 barbs for each tract (see statistical analysis below). A comparison of these averages shows that the size of the down feather, which is dependent upon the longest barb, decreases in the following order in the seven tracts examined: neck > breast > wing > thigh > humeral > saddle > head. Feathers of three of the tracts (wing, thigh, and humeral) show smaller differences between average lengths of the longest barbs. Feathers of the other four tracts differ sub-

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**Fig. 4.** The distribution of barb length in down feathers from the central region of the saddle tract. Measurements of 127 individual barbs were made, from 11 different feathers. Each class size spans 0.4 mm in length.

**Fig. 5.** Graph showing average length of the barbs and the length of their barbule-free tips in each of the seven tracts (pterylae) studied. Each mean and standard error is based on the measurement of the longest barb from each of 11–13 feathers.
stantially in the length of the longest barbs. Even when differences in overall length are not so dramatic, or in the extreme case (thigh and wing) where the overall length is about the same, the lengths of the barbule-free tips are sufficiently different to permit one to distinguish between the tract origin of the feathers.

The larger feathers with longest barbs (e.g., neck) have, by actual count, more barbules per barb than the smaller feathers (e.g., head). Irregular numbers of barbules occur on the two sides of any one barb. Their spacing is about the same regardless of tract. Barbules increase in length more gradually from tip to base of the barb in feathers of some tracts than in others (see Figs. 1 and 2). As a diagnostic feature of tract origin, the barbules are of lesser importance.

STATISTICS

The significance of the quantitative differences between feathers from the seven major tracts measured was evaluated by an analysis of variance. To rule out any significant contribution due to variation from feather to feather within a tract, only the longest barb from each feather was included in each tract class. Selection, in this manner, gave class sizes of from 11 to 13 measurements per tract. Group analysis of these classes yields an $F$ ratio of 361.32. Since $F_{0.95, 11}$ for the number of samples and class size is 4.75, these mean differences are significant at the 0.05% level. Next, comparisons of mean differences between tracts taken two at a time were analyzed. All comparisons of paired means proved significant at the 1% level, except for comparisons within the thigh–wing–humeral group. Paired comparisons of means within this group were not statistically meaningful. However, when the individual measurements of the thigh, wing, and humeral groups are pooled, and the mean of this “new” class is compared with each of the means of the remaining separate classes (namely, neck, saddle, breast, and head), again each difference was significant at the 1% level. Thus, on the basis of barb length alone, thigh, wing, and humeral tracts are statistically indistinguishable. This result might have been predicted by inspection of the means for barb length plotted in the graph in Fig. 5. This same graph, however, indicates that although overall barb length is quite similar in feathers of the thigh, wing, and humeral tracts, the lengths of the barbule-free tips in feathers of these three tracts show marked differences. To test whether these three groups could be distinguished on the basis of the length of the barbule-free tip, a comparison of mean differences was performed on these three groups; again, the tip lengths of only the longest barb in each feather were used, since an analysis of variance indicated significance at the 0.05% level. The results of these comparisons show significance at the 1% level, indicating that, indeed, these three tracts differ from one another on the basis of the lengths of the barbule-free tips, although the barbs in these three tracts are similar in overall length.

DISCUSSION AND CONCLUSION

The down feather is the first in a succession of plume types to arise from the same organ primordium, a dermal papilla with its thin ectodermal covering, and melanocytes that originate in the neural crest. These highly complex organs, the papillae, arise as dome-like elevations on the surface of the body between the sixth and tenth days of embryogenesis, in a definite number within each tract and in a specific sequence in time and space (3). The feather itself arises from the epidermal component of the papilla, and is a keratinized dead structure when completed. The dermal component is richly supplied with blood vessels that nourish the epithelial feather while developing, and dwindle to a vestige when the papilla is dormant between generations of feathers. Thus, the papilla is the seat of the specificity that manifests itself in differences in the structure of feathers throughout the life of the bird.

As the first and simplest structure to arise from the cutaneous papilla, the down feather is small and consists of a circle of slender, flexible barbs springing from a short cylindrical calamus and bearing two rows of barbules along a major portion of their length. There is no shaft or rachis, hence no obvious bilateral arrangement of the barbs, or no dorsal ventral surface, although bilateralcy is apparent in the order of origin of the barb ridges and in the alignment of the melanocytes (4) and dorsoventrality is evident in the conical shape of the growing organ and its enzymatic patterns (5). Without a shaft, the barbs are attached radially to a common, short, cylindrical base, the calamus or quill, within which they merge. Since there are no barbicels (microscopic hooklets) to hold the barbs together in a coherent vane, the feathers are loose, soft, and fluffy. Thus, the structural features of the succeeding juvenile and adult feathers that arise later from the same papilla, and which make them so distinctive in the different tracts, are not present, or are incipient, or are less fully developed in the down. The obvious differences between the contour feathers of the various tracts are principally in the size and shape of the coherent vane and in the length of the basal, downy portion of the shaft that lacks barbicels. Knowing that the down, juvenile, and adult feathers all develop from the same papillae and follicle established during embryogenesis, and that the later generations of feathers are highly specific within each tract, one might expect from a posteriori reasoning that specificity would be present in the first series of feathers to arise from the papillae of the tract.

The present study was initiated during an investigation of the origin of tract specificity in juvenile and adult feathers, in which embryonic ectoderm and mesoderm from different prospective tracts were recombined and grown on the chorioallantoic membrane. The resulting grafts bearing structurally normal down feathers were then grafted to the skin of a newly-hatched host chick, and carried through to the adult stage. In the nine chicks that reached adulthood, the dermis determined the specificity of the resulting juvenile and adult feathers (Rawles, unpublished data). This procedure, however, is arduous and complex, and much time and effort went into getting results from grafts that had to survive for months. The results of the present study make such procedures unnecessary, and provide a more practical criterion for determining tract specificity.

Differences in the morphology of completed down feathers become apparent when feathers from different tracts are compared microscopically. Since previous investigations of the down plumage have usually been restricted to feathers of one tract, specificity has been overlooked until now.

The tract specificity of down feathers was detected by measured differences in the length of the barbs and in the lengths of their barbule-free tips, both of which are dependent on rates of growth. It is known, for example, that barbicels
(microscopic hooklets) fail to develop on the barbules of juvenile and adult feathers when the rate of growth diminishes to a certain threshold, hence the variations between tracts in the length of the downy base in such feathers (6). Undoubtedly there are other specificities within each tract, so apparent in juvenile and adult feathers, that could be detected morphologically or physiologically in the down feather. In the later generations of feathers, where the inherent bilaterality of the feather is clearly expressed, there are differences in the lengths of the barbs on the two sides of the shaft that lead to asymmetries in the width of the vane, and often to differences in color patterns, because of disparities in growth rates (7). Patterns of coloration, e.g., stripes on the head and back, appear in the down of the Campine (8), Brown Leghorn (9), and many other varieties of domestic fowl. Such manifestations of physiological differences in down feathers within a tract suggest that further study by new approaches will give insight into such intriguing problems as the orderly appearance of the feather at a specific time, and the precise location of each feather germ at a particular point within the tract, the mirror image of one bilateral tract to another (e.g., the right- and left-breast tracts), and the gradients within each tract that control symmetries of form and color.

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