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Germinal Basis of Thyroxin and Female Hormone Effect on Barb Origin in Saddle Feathers.

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The subcutaneous injection of thyroxin or female hormone into the Brown Leghorn male or capon brings about modifications in saddle feathers which, on the basis of c-isochrone relations, signify the origin and growth of definitive barb primordia in a previously undifferentiated ventral field of the germ (Fraps and Juhn,[1] Fraps[2]). With sufficient concentration of thyroxin, a greater or lesser number of barb apices appear to arise practically simultaneously. Female hormone brings about only slight changes of a similar nature in breast feathers, suggesting that the ventral regions of these germs normally carry a full complement of barb primordia. The only direct observation bearing on this indicated difference in germinal organization was made by Lillie and Juhn,[3] who noted that the unoccupied ventral region of saddle feather germs appeared to be considerably wider than the comparable region in breast germs; Lillie and Juhn, however, attributed this difference largely to the lowness of ridges in saddle germs. A comparison of breast and saddle feather germs prepared before and following injection of thyroxin has therefore been made with a view toward establishing the embryonic basis of the ventral differentiations indicated by c-isochrone analyses of regenerated feathers.

Figs. 1-5 represent, rather schematically, the ventral regions of regenerating germs split along their dorsal faces and spread into the plane of the paper. Apical levels are cut away (excepting in Fig. 1), as are also the undifferentiated germ bases. The sloping lines indicate barbs and barb primordia visible in the several preparations. Increasing diameters of barb bases with increasing distances of bases from sites of origin are not shown in the figures, nor are differences in slopes of barb primordia in different germs or regions. The essential relations illustrated by the several figures follow.

Fig. 1. Germ of breast feather, male or capon, showing open

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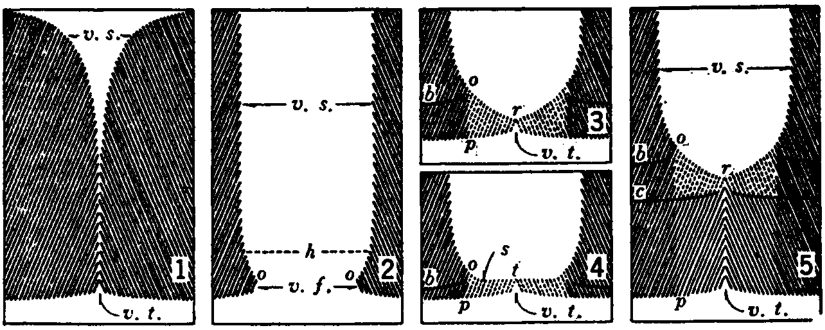
[1] Fraps, Richard M., and Juhn, Mary, *Phys. Zool.*, 1936, **9**, 319.

[2] Fraps, Richard M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 201.

[3] Lillie, Frank R., and Juhn, Mary, *Phys. Zool.*, 1932, **5**, 124.

ventral area or strip, *v. s.*, extending a short distance below feather apex. This open area decreases rapidly in width with axial growth of the germ, and at an early stage barb primordia come to arise from opposite faces of a typical ventral triangle, *v. t.* Injection of female hormone modifies only slightly breast feathers in the stage of development here represented. There is some evidence that supranormal concentrations of thyroxin cause differentiation in the open apical levels of the breast germ (*v. s.* of Fig. 1 when this region lies at the base of the germ), but comparison of mounted feathers will be required to clear up this point.

The serial origin of barb primordia from a ventral triangle is characteristic of most feather germs from completion of apical levels to formation of the after-feather (Lillie and Juhn[3, 4]).



FIGS. 1-5.

Sections from ventral regions of regenerating feather germs, each prepared by splitting the germ along its dorsal axis and spreading into the plane of the paper.

FIG. 1. Breast feather germ, near apex of feather: *v.s.*, ventral strip (compare Fig. 2); *v.t.*, ventral triangle.

FIG. 2. Saddle feather germ, uninjected male or capon, at main vane levels: *v.s.*, ventral strip; *v.f.*, ventral field; *o*, sites of origin of definitive barb primordia; *h*, distal collar level.

FIG. 3. Germ of Fig. 2 following action of thyroxin and growth of base of Fig. 2 to level *b*, Fig. 3. The locus of definitive barb apices forming in the ventral field is indicated by *or; v.t.*, ventral triangle; *op*, projected face of ventral field of Fig. 2.

FIG. 4. Same as Fig. 3, but assuming more rapid thyroxin effect on ventral field: *os*, first phase of thyroxin action; *st*, barb apices theoretically forming simultaneously in left collar half.

FIG. 5. Germ represented in Fig. 3 following growth raising base line of Fig. 3 to level *c* of Fig. 5, and assuming maintenance of thyroxin concentration.

Fig. 2. Saddle feather germ from an uninjected male or capon. A longitudinal mid-ventral strip, *v. s.*, separates visible (definitive) barb apices of opposite vane-halves. Basal levels of this open area, approximately levels below *h*, lie in the collar of Lillie and Juhn (1932) and may be denoted the ventral field, *v. f.* Definitive barb

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[4] Lillie, Frank R., and Juhn, Mary, *Science*, 1937, 86, 38.

apices become visible (or differentiated) at opposite faces, *o*, of the ventral field. At proximal levels of the main vane structure the ventral field becomes narrower and is finally closed; barb primordia then arise from opposite faces of a ventral triangle until formation of the after-feather (Lillie and Juhn[4]).

Fig. 3. Saddle feather germ comparable to that of Fig. 2 following reaction to thyroxin in sufficient concentration to close the ventral field. The locus of barb bases shown in Fig. 2 becomes the transverse locus, *b*, and indicates approximately the axial growth occurring during differentiation of the ventral field. Definitive barb apices arise successively from *o* to *r* (in each collar limb), with formation of the ventral triangle, *v. t.*, when the ventral field is completely occupied. The first stages of this process are demonstrated by comparison of the distance between faces of the ventral field (*o* to *o*, Fig. 2) in normal and thyroxin treated germs.

Fig. 4. (Theoretical case.) The same relations described for Fig. 3, but assuming more rapid action of thyroxin in differentiation of the ventral field. Apices between *o* and *s* arise successively and with increasing rapidity as thyroxin concentration increases; apices between *s* and *t* are presumably of simultaneous origin. It has not been possible to identify positively this limiting effect in the germ. Possibly barb primordia of simultaneous origin form vertically in the ventral field, as do apical-most complements (*cf.* Lillie and Juhn[3]), but such a formation has not been observed. The lower level of the base line, *b*, compared with the same locus of Fig. 3 indicates the lesser growth required (in Fig. 4) during complete differentiation of the ventral field in this case.

Fig. 5. A germ such as represented in Fig. 3 after growth has raised the locus of barb bases (of Fig. 3) to the level *c*. The concentration of thyroxin is assumed to remain at a sufficiently high level to maintain the ventral triangle shown in Fig. 3. The approximate increase in length of barbs laid down following thyroxin over barb lengths of the normal male or capon feather is represented by segments lying between *op* and *or*.

In saddle feather germs from Brown Leghorn, Black Minorca, or Barred Rock males it has proven impossible to identify in the split preparation barb primordia or apices between such a contour as is represented in Fig. 2. In cross-sections through the collars of comparable germs, however, the entire circumference is seen to be occupied by barb primordia or very similar structures.† This observa-

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† The author wishes to express his appreciation of Professor Frank Lillie's generosity in placing at his disposal the series of cross-sections of saddle germs on which the observations reported here are based.

tion may be taken to mean that the ventral field of the split preparation (Fig. 2) carries normally differentiated primordia which in these preparations cannot be made visible, but a number of considerations make this conclusion highly improbable. Firstly, the apex of a barb primordium rises practically vertically above its site of origin in the collar; if *definitive* barb apices were originating from a ventral triangle in the saddle germ they would become visible, after differentiation, in approximately the center of the ventral strip instead of at its faces. Secondly, cross-sections show a considerably greater number of barb primordia (or apparent primordia) than are indicated by c-isochrone counts in regenerated feathers at comparable levels. Thirdly, the number of barb primordia (or apparent primordia) visible in cross sections of normal saddle germs is of the same order as is the number of barbs lying on c-isochrones through levels of maximum thyroxin or female hormone effect,[2] indicating perhaps (but not conclusively) that the hormones bring about definitive differentiation of rudimentary structures. Fourthly, the apparent primordia of the ventral collar levels become highly attenuated above levels of growth in the germ. Finally, the order of events characterizing the origin and development of definitive barb primordia in the ventral field following thyroxin injection (Figs. 3 and 5) makes it practically certain that definitive barb apices and corresponding segments do not occupy this region in the normal saddle feather germ.

The width of the ventral field of Fig. 2 is somewhat narrower than the distal ventral strip. This seems to be a common condition, even at those levels of the germ producing practically uniform regions of the feather vane. There is a suggestion in this out-curving of barb apices that secondary processes of dedifferentiation may involve not alone rudimentary structures invisible in split preparations, but also limited lengths of primordia identifiable in these preparations. As against this, it must be recognized that a part or all of the structures appearing in cross-sections of the ventral field may conceivably be independent of the visible differentiations of split preparations. In either event, however, the ventral field seems beyond reasonable doubt to constitute the embryonic basis for the rapid or simultaneous formation of definitive barb apices indicated by c-isochrone analysis of feathers grown under supra-normal concentrations of thyroxin.

Hypothyroidism in the Brown Leghorn male (as well as in the male of other breeds) is associated with increased length of feather in proportion to width, and restriction of barbulation in saddle and other feathers (Greenwood and Blyth,[5] Parkes and Selye[6]). These changes, taken in connection with the effects of hyperthyroidism reported here, suggest a relative increase in extent of the ventral field of saddle feathers grown under conditions of hypothyroidism. The appearance in the breast tracts of longer, saddle-like feathers with restricted barbulation following thyroidectomy is in all probability likewise associated (among other germinal changes) with a ventral field of greater or lesser extent. It should be a matter of considerable interest to alter experimentally the balance of other hormones in thyroidectomized birds in order to test the specificity of thyroxin in determining and maintaining the limited ventral triangle of feather germs of different tracts.

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Evaluation of Germicides by a Manometric Method.*

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The need for a method capable of evaluating germicides for chemotherapeutic use is universally recognized. Obviously, the first requirement is that the method should simulate as nearly as possible the conditions met in the animal body. In addition it should be relatively simple and should yield information regarding the action of the test substance on bacteria, and on animal tissues, in terms which would permit a direct comparison of the respective effects. The method of Lambert,[1] employed more recently by Salle,[2] does not seem to fulfill these requirements, since the parasitotropic and organotropic properties of the disinfectant are tested under profoundly different conditions. Moreover, it is based on a tissue-culture technic which is time consuming and laborious.

The manometric method proposed here has proved simple, rapid, and remarkably flexible. It permits the conditions of testing to be modified at will, and at the same time allows wide latitude in the

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[5] Greenwood, A. W., and Blyth, J. S. S., *Proc. Roy. Soc. Edinb.*, 1929, **49**, 313.

[6] Parkes, A. S., and Selye, H., *J. Genetics*, 1937, **34**, 297.

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[1] Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 683.

[2] Salle, A. J., McOmie, W. A., and Schechmeister, I. L., *J. Bact.*, 1937, **34**, 267.