DEVELOPMENT OF FEATHER KERATIN DURING EMBRYOGENESIS OF THE CHICK

EUGENE BELL, Ph.D., and Y. T. THATHACHARI

From the Department of Biology, Massachusetts Institute of Technology, Cambridge, and the Biophysics Laboratory, Stanford University, California. Dr. Thathachari's present address is Kodambakkam, Madras, India

ABSTRACT

The development of keratin in 9 to 17 day embryonic chick feathers has been studied by x-ray diffraction and cytochemical methods. The x-ray diffraction pattern given by the 9-day feathers contains none of the features seen in the adult pattern. In the 10 to 11 day patterns, besides two diffuse rings centered at 4.7 A and 10 A, two sharp, rather weak rings are seen at 35 A and 4.2 A with slight preferred orientations about the equator and the meridian, respectively. At 12 days, in addition to the foregoing, a sharp intense equatorial reflection at ~56 A is observed. On treatment with lipid solvents, the 35 A ring is removed; prolonged extraction removes the 4.2 A ring, while blurring the 56 A reflection and enhancing the central low angle scatter. The 14-day pattern shows, besides all the features seen in the earlier patterns, a 23 A meridional reflection and other meridional and near meridional reflections. All the basic features of the adult pattern are seen at this stage and remain essentially intact on lipid extraction. Beyond 14 days, the pattern remains essentially the same, only improving in clarity and detail. The 4.2 A ring seen in the 10 to 15 day pattern is scarcely detectable in the 16-day pattern. Cytochemical evidence indicates that extensive —S—S bond formation occurs between the 13th and 14th days. It is suggested that lipids serve as a framework for the developing keratin structure which acquires permanent stability through hydrogen bonds and disulfide cross-links. The relation between keratin synthesis and tissue architecture as well as cytodifferentiation is discussed.

INTRODUCTION

The emergence of morphological characters in cells, intercellular matrices, and tissues during embryonic development may in some instances be expected to bear a close relation to, if not depend upon, the synthesis of new structural proteins.

The problem of whether there exists a correlation between protein synthesis and differentiation at cell and tissue levels in the developing feather is one of the subjects of the present study. In dealing with this problem it is of particular interest to determine at what point, in the emergence of the feather, keratin synthesis begins, and to what extent the development of macromolecular organization may be related to cytological differentiation and tissue architecture. The gap between macromolecular organization and histological appearance will, of course, not be bridged by demonstration of temporal correlations of events at different levels of organization.

Such correlations may, however, be suggestive for further work.

The x-ray diffraction pattern of adult feather keratin has been described in detail among others by Bear (1944, 1951) and Schor and Krimm (1961 *a*). Excellent reproductions of the patterns given by sea gull feather rachis and turkey feather calamus are found in their papers. Krimm (1960) has also shown that 14-day embryonic feathers give an x-ray diffraction pattern that contains all the basic features of the adult pattern. At low resolution the pattern consists of a strong equatorial reflection (at about 35 A), a strong meridional reflection (at about 23 A), and two diffuse rings centered at about 4.7 A and 10 A with intensification about the equator. There are other reflections seen along the meridian—some truly meridional and related to the 23 A reflection. With better resolution the 10 A ring splits into two well ordered rows of spots. Equatorial reflections of larger spacings noticed by Corey and Wyckoff (1936) and Astbury and Bell (1939) have been considered by Bear (1944) as radiation artifacts associated with the strong ~35 A reflection. However, Schor and Krimm (1961) have shown that at least the ~56 A reflection is definitely real and is the most intense reflection in the pattern given by heterozygous frizzle feather, the ~35 A reflection being practically absent.

Several models of feather keratin structure have been proposed, on the basis of these data, by Pauling and Corey (1951 *b*), Ramachandran and Dweltz (1960), Astbury and Beighton (1961), and Schor and Krimm (1961 *b*).

A number of attempts have been made to gain insight into the structure of feather keratin by studying changes in the x-ray patterns due to various chemical treatments of the feather (Bear and Rugo (1951); Fraser and MacRae (1959)). The present study of the step-by-step emergence of the adult diffraction pattern during embryogenesis was undertaken in the hope that it would contribute to an understanding of the relationship of the synthesis and macromolecular organization of feather keratin to cell differentiation.

MATERIALS AND METHODS

X-ray diffraction patterns were taken of feathers cut from the mid-dorsal region of white Leghorn embryos of 9 to 17 days of incubation. Eggs were incubated at 99.75°F. A sequence of patterns spaced a day apart was so obtained. Since 9-day feathers were too small to examine apart from the skin, the epidermis was separated from the mesoderm by means of 0.28 per cent trypsin (Difco) and 0.11 per cent pancreatin (Nutritional Biochemical Corporation) in Ca^{++} and Mg^{++}-free Tyrode's solution. The sheet of epidermis rich in feather germs was then washed in Tyrode's solution and in distilled water. The 9-day epidermis and 10-day feathers were encased in thin collodion sandwiches as they were too small to be handled otherwise.

The 11 to 17 day feathers which ranged in length from less than 2 mm to over 2 cm were washed in distilled water and, while still wet, were picked one by one and laid on a standard microscope slide in close packed parallel arrays several layers thick. The feathers stuck together nicely and, when dry, could be lifted off the slide as a rigid bundle which could be handled with ease. Typically, about thirty 12-day feathers formed a bundle 2 mm long and 0.1 mm thick. The bundles remained intact after immersion in benzene, ether, or alcohol for any length of time.

A Norelco copper anode x-ray tube operated at 40 kv and 20 ma served as the source of x-rays (CuK, alpha) and a flat plate camera with pin-hole collimation was used to record the diffraction patterns from the central portion of each bundle. The air in the camera was replaced by a continuous stream of helium to reduce the parasitic background scattering. A specimen-to-film distance of about 7 cm was normally employed. A distance of about 10 cm was employed to study the low angle region and a distance of about 3.5 cm was used in the preliminary surveys to secure greater speed. At a distance of about 10 cm from the specimen, the diameter of the incident x-ray beam was about 1 mm and, with a direct beam trap of about 3.5 mm diameter, spacings less than approximately 90 A could be recorded. The spacings were measured to an accuracy better than ± 1 A at 35 A and ± 4 A at 56 A. The 10 to 13 day specimens were less than 0.1 mm thick and an exposure of over 80 hours was required for the details to be made barely visible. A Joyce-Loebl Mark III microdensitometer was used to determine spacings but was in general less satisfactory than visual estimates. Background on the original negatives was in no instance heavy enough to reduce the reliability below the limits indicated.

Diffraction patterns were taken from at least three specimens of feathers of each day of age from 9 to 17 days. A larger number of specimens was examined in the interesting range of 11 to 14 days.

Feathers and skin were also prepared for histological examination. One series was stained for —S—S— groups by the Barrnett-Seligman method (1954) and another by Mallory's aniline blue col-

lagen stain which stains keratin a distinctive red. A third series was dried down on slides and examined for birefringence.

RESULTS

1. *Histological Observations*

Since the staging of feather development depends upon variables such as breed, incubation temperature, and location of feather on the embryo as well as incubation time, a brief description of 9 to 17 day feathers used in this study follows. Also, cytochemical observations on the time and place of appearance of keratin or —S—S— groups are included.

9 DAYS: Clearly defined feather germs were observed on the dorsum of the chick embryo. The epidermis appeared pseudostratified in the basal layer overlying the mesodermal condensation. Earlier, the basal layer consisted of a single layer of cuboidal cells. The cuboidal layer was covered by a single layer of flat epitrichial cells. The epidermis was elevated in the region of the germ which had grown out to a length of about 0.5 mm.

10 DAYS: At 10 days the cells of the basal layer of the epidermis were heavily stratified and the feather was about 1 to 1.5 mm long. Rugae or barb ridges were observed at the distal end of the feather in the intermediate cell layer. The epitrichial layer was still single.

11 DAYS: Rugae were well defined along the length of the feather by the 11th day of incubation. The intermediate layer (which arises from the basal layer) was six to seven cells deep and the epitrichium now consisted of two to three ply of cells. Many mitoses were observed in the intermediate layer. The entire feather had begun to sink into the dermis, an event which marked the beginning of follicle formation.

12 DAYS: Differentation of future barb cells, recognized by their large size and nearly central position, was seen on the 12th day near the tip of the feather. The follicle of the feather was better developed than before. No indication of keratinization was observed in Mallory-stained sections or in sections stained for —S—S groups.

13 DAYS: By the 13th day the first histochemical sign of keratinization was noted and then only in the feather sheath near the tip of the feathers. There was further differentiation of barb cells and clusters of differentiating barbule cells were seen peripheral to the barb cells.

14 DAYS: By 14 days the feather sheath appeared heavily keratinized. Similarly, within the intermediate layer of the feather, the barbules which made up about two-thirds of the feather substance were well developed and strongly keratinized as indicated by the —S—S stain and by the Mallory stain. The barbs, on the other hand, which by now were morphologically well separated from the barbules (except at points of attachment), had not yet begun to keratinize, by cytochemical criteria.

15 TO 17 DAYS: Between 15 and 17 days the process of keratinization was intensified and the elements of the protoptile or the down feather, that is, barbs and barbules, had begun to separate from each other and from the sheath.

2. *Birefringence of the Developing Feather*

Feathers from embryos of 9 to 10 days of incubation showed no birefringence when examined longitudinally under the polarizing microscope. Birefringence of the 11-day feather was confined to the sheath layer near the tip, but was too weak to permit measurement of the sign. From 12 days on the sign of the birefringence was positive. The magnitude of the birefringence and changes of magnitude between 12 and 14 days could not be measured accurately with the equipment available. At 12 days the sheath was birefringent down the length of the feather, and the peripheral barbule zone near the feather tips showed slight birefringence. By 13 days this zone as well as the sheath became strongly birefringent, and at 14 days the entire feather well down the shaft was birefringent.

3. *Lipid-Protein Ratio of the 13-Day Feather*

Total protein (milligrams per milliliter of homogenate) determined by the method of Salton (1953) and total lipid (milligrams per milliliter of homogenate) by the method of Mokrasch and McGillevry (1956) were obtained for 13-day feathers pooled from three embryos. The protein-lipid ratio was found to be 5:1.

4. *Sequential Development of the X-Ray Diffraction Pattern of Feather Keratin*

The x-ray diffraction pattern given by the 9-day feathers (more correctly, dissociated 9-day

epidermis rich in feather germs) showed none of the features of the adult pattern. Besides a central scattering there were two halos, one centered at about 7.2 A and the other, a very diffuse one, centered at about 4.5 A. The 10-day pattern showed two diffuse rings centered at 4.7 A and 10 A similar to the rings seen in the diffraction patterns of denatured proteins and resembling the rings seen in the adult feather keratin pattern at low resolution. Besides these, a rather sharp ring was observed at about 35 A and another sharp ring at 4.2 A. The 11-day pattern was essentially the same, only the 35 A ring showed a slight preferential orientation about the equator, and the 4.2 A ring, a slight preferred orientation about the meridian, revealed by the slight weakening of the rings about the meridian and the equator, respectively. All these features were present in the 12-day pattern and, in addition, a sharp, intense reflection appeared at 56 ± 4 A, very well oriented about the equator. This reflection was so strong, compared to the ring at 35 A, that the central area of the film was completely blackened when the 35 A ring was just visible. The two reflections could not be seen clearly in the same film.

Fig. 1 shows the sharp, equatorial reflection at 56 A in the 12-day pattern. Many of the 13-day patterns were exactly the same as the 12-day pattern. Fig. 5 shows a 13-day pattern in which the two diffuse rings at 10 A and 4.7 A can be seen, along with the ring at 4.2 A which is sharp and has intensification about the meridian. The ~35 A ring can be distinguished in the overexposed central region. Some of the 13-day patterns (probably given by slightly more mature feathers) showed, in addition to the features seen in the 12-day pattern, a sharp reflection at 23 ± 1 A, very well oriented about the meridian. In Figs. 2 and 3, the 23 A meridional reflection is just visible. In Fig. 2 the 56 A equatorial reflection is clear and sharp while the exposure is not enough to register the ring at 35 A. In Fig. 3 the ring at 35 A is seen clearly and the ~56 A reflection can be just distinguished. The 14-day pattern contained all the basic features of the

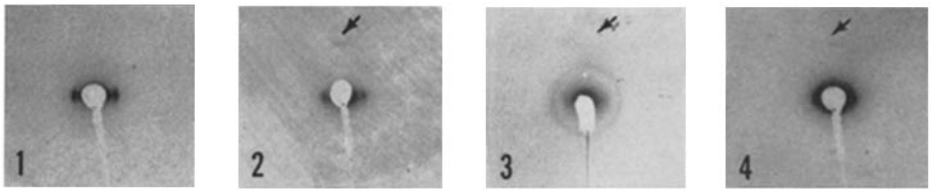


FIGURE 1

Low angle x-ray diffraction pattern of 12-day embryonic feathers. The sharp equatorial reflection at about 56 A is seen clearly. The specimen-to-film distance is 10.3 cm.

FIGURE 2

Low angle x-ray diffraction pattern of 13-day embryonic feathers. The arrow points to the weak 23 A meridional reflection. The stronger equatorial reflection occurs at about 56 A. The specimen-to-film distance is 10.3 cm.

FIGURE 3

Low angle x-ray diffraction pattern of another bundle of 13-day embryonic feathers. The disoriented ring at about 35 A is seen clearly. The arrow points to the extremely weak meridional reflection at 23 A. The 56 A equatorial reflection is blurred. The specimen-to-film distance is 10.3 cm.

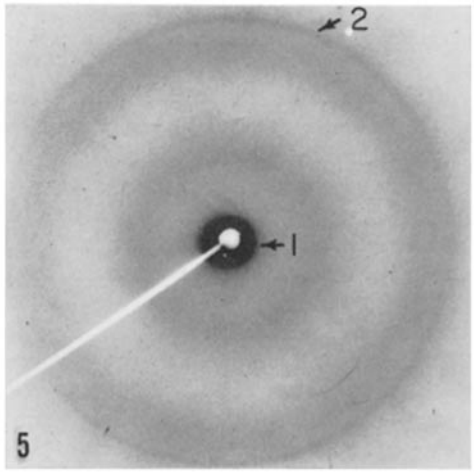
FIGURE 4

Low angle x-ray diffraction pattern of a bundle of 12-day embryonic feathers (the same as that used for Fig. 1), after extraction with alcohol for 40 hours. The 56 A equatorial reflection could still be distinguished from the central, continuous scatter. The arrow points to the weak 23 A meridional reflection. The specimen-to-film distance is 10.3 cm.

adult pattern. The two diffuse rings at 4.7 A and 10 A were intensified about the equator; beside the meridional 23 A reflection, the other meridional and near meridional reflections were observed (Fig. 6). The sharp ~56 A equatorial reflection appeared quite as intense as in the other photographs. There was a well oriented equatorial reflection at 34 ± 1 A, very nearly the same spacing as the ring seen in the earlier patterns. In some patterns, a weaker, disoriented ring passed right through the center of the equatorial spot at 34 A. This can be seen in Fig. 6 in which the 56 A spot is lost in the beam trap. In a few 14-day patterns, besides the above features, a fairly strong, disoriented ring at ~44 A could be seen. The diffraction pattern given by the 15-day and older feathers was essentially similar to the 14-day pattern: only the patterns improved in clarity and detail. The 34 A equatorial spot increased in sharpness and intensity. The 4.2 A ring could scarcely be seen in the 16-day patterns and later. The 56 A equatorial reflection was rarely observed as a strong reflection in mature feathers.

5. *The Effect of Lipid Extraction on the Developing Feather Keratin Pattern*

The 11 to 13 day feather specimens were soaked in alcohol, ether, and benzene for various lengths of time from 30 minutes to over 40 hours, and the changes in the diffraction patterns were studied. In each case, the diffraction data were recorded before and after the treatment, employing the same exposure conditions and using the same region of the specimen. It was found that immersion of the specimen in any of these solvents for over 30 minutes resulted in the disappearance of the ~35 A ring seen in the 11 to 13 day patterns. The oriented 34 A equatorial reflection seen in the 14-day pattern remained intact while the



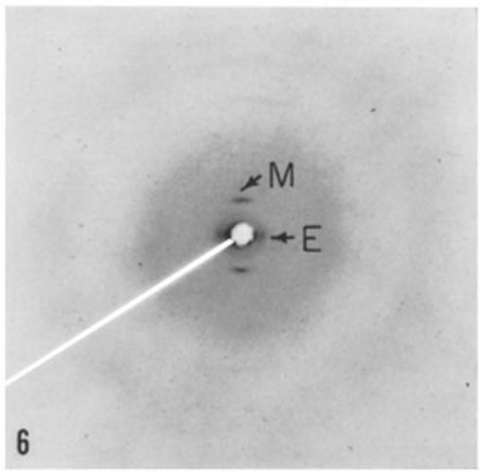


FIGURE 5

X-ray diffraction pattern of 13-day embryonic feathers. Diffuse rings occur at 4.7 A and 10 A. Arrow 1 points to the ring at 35 A which can be seen in spite of the overexposed central region. Arrow 2 points to the ring at 4.2 A. The ring has definite intensification about the meridian. The specimen-to-film distance is 7.9 cm.

FIGURE 6

X-ray diffraction pattern of 14-day embryonic feathers. Two diffuse rings are centered at about 4.7 A and 10 A. The intensification of the rings about the equator is evident—particularly in the inner ring at spacing 10 A. Arrow *M* points to the strong 23 A meridional reflection. At least four other reflections can be seen along the meridian. Arrow *E* points to the equatorial reflection at about 34 A. A weaker disoriented ring passes through the middle of the fairly sharp equatorial spot. The specimen-to-film distance is 7 cm.

disoriented ring passing through this spot (whenever present) disappeared. On the other hand, the sharp ~56 A reflection and the 4.2 A ring were less affected when feathers were subjected to lipid extraction. Treatment with alcohol for about 20 hours resulted in the nearly complete absence of the 4.2 A ring; this ring still could be seen after the specimen had been treated for over 40 hours with ether or benzene. After extraction with benzene, ether, or alcohol for over 40 hours the sharp ~56 A reflection still could be distinguished, though it appeared to be much less sharp and the central scattering had increased (Fig. 4). It also seemed that the spot had moved slightly inward, suggesting a slightly larger spacing. It appeared that alcohol extraction of the feathers caused a greater amount of blurring of the 56 A reflection and a greater central scatter than benzene or ether extraction. The disoriented ring at 44 A seen in some 14-day patterns was completely removed on soaking the specimen in alcohol for about 20 hours, while the other features of the pattern remained intact. When 14-day feathers were treated with 1 per cent urea for 2 hours, the diffraction pattern showed only the two diffuse rings centered at 4.7 A and 10 A besides some low angle central scatter. The 4.2 A ring was still to be seen, though weak. When the original specimen showed the disoriented ring at 44 A, treatment with urea left the ring intact.

DISCUSSION

It is clear that the macromolecular organization of the feather undergoes a sequence of developmental changes between 9 and 14 days of incubation.

The x-ray diffraction pattern given by the 9-day feather has none of the features of the adult feather pattern. At 10 to 11 days, besides two diffuse rings centered at 4.7 and 10 A, two rings at ~35 A and 4.2 A with slight preferred orientation about the equator and meridian, respectively, were observed. In addition to the foregoing, at 12 days, an intense equatorial reflection at ~56 A appeared; at 14 days a 23 A meridional and other meridional and near meridional reflections were observed.

It is important to ask whether this series of patterns actually reflects sequential steps in the final organization of keratin. What indeed do the reflections at ~56 A, ~35 A, and 4.2 A seen in the 10 to 13 day feathers signify, and how, if at all, are they related to the development of keratin?

It has been pointed out that feather contains lipid impurities that show up in the diffraction patterns (Rudall, 1947). In our work the estimated ratio protein:lipid in the 13-day embryonic feathers is 5:1. Hence reflections due to the presence of lipids cannot be neglected. A spacing of 4.2 A can be associated with the cross-section of lipid chains; and spacings of 56 A and 35 A, with spacings along the lipid chains. It is possible for the lipid chains to be oriented with their lengths perpendicular to the fiber axis so that the 4.2 A reflection has intensification about the meridian and the larger spacings have preferred orientations about the equator. In order to explain the different degrees of preferred orientation of the ~35 A and ~56 A reflections and the effects of lipid extraction of the feathers on these reflections, we may have to assume the presence of more than one lipid phase.

If these reflections are due in part at least to lipids present in the developing feather, we would like to know whether the lipids are associated with a prekeratin—possibly in the form of a lipoprotein complex—or whether they occur just as impurities that have nothing to do with the development of keratin. We are inclined to take the former view for the following reasons. The spacing of the ring at ~35 A is very close to the value 34 A, the equatorial spacing found in the adult feather rachis. The equatorial spacing is slightly variable, depending on the degree of hydration, and we can expect it to be slightly smaller in the fully developed structure than in the developing structure where the cross-links are fewer and the fibrillar units are likely to be rather loosely held. In some of the 14-day patterns a disoriented ring passes right through the center of the spot which is known to be the equatorial reflection associated with keratin structure. The disappearance of this ring with lipid extraction of the specimen suggests that it has the same origin as the corresponding ring in the earlier patterns. Again it was found that in some of our x-ray diffraction pictures of sea gull feather rachis and also in the x-ray diffraction pattern of turkey feather calamus (Schor and Krimm, 1961 *a*) disoriented rings passed right through the known equatorial reflections of feather keratin at about 34 A and 55 A. These

rings certainly are not due to a disoriented phase of feather keratin itself since in that case we could have expected a similar ring through the strong 23 A meridional spot; such a ring was not present, however. In all these cases it appears that the disoriented ring that is absent after lipid extraction seems to take exactly the same value for its spacing as the corresponding equatorial spot associated with keratin structure, and this equatorial spacing has varied detectably in the examples cited. Hence, it appears reasonable to suppose that the ~35 A ring is related to feather keratin structure.

The ~56 A reflection is relatively more resistant to lipid extraction. We can, however, expect that when more than one phase of a lipid is present the degree of attachment of the lipids to the protein fibrils may vary, causing variation in the ease of extraction of the lipids. Swanbeck (1959) has observed a similar effect in his studies on epidermal keratin. The ~56 A reflection is very sharp and intense and indeed very well oriented about the equator; it is difficult to consider that it is due to a lipid impurity and has nothing to do with keratin structure. One is tempted to assume that it is due to keratin and reflects the formation of some ordered micellar structure around the fiber axis, the structure being completed when groups are brought into register along the vertical axis, perhaps with the development of cross-links like —S—S bonds. It is also possible at the same time that the structure of the "micelles" themselves may be better organized through the formation of hydrogen bonds. It is most unlikely that this reflection is a radiation artifact since at 12 days it is the strongest reflection in the pattern.

It may be remarked that heterozygous frizzle feather is also found to give a very strong ~56 A reflection, the ~34 A reflection being practically absent (Schor and Krimm, 1961). Krimm (personal communication) found that the x-ray diffraction pattern of the residue left after the feather is solubilized contained a reflection of about this spacing. As yet, however, we cannot make a definite statement about the precise origin and significance of this reflection. It is interesting to note also that in the studies on the sequential development of the x-ray diffraction pattern of developing feather the completion of the pattern occurs at about the time when extensive —S—S bonds are first observed in cytochemical studies—between about the 13th and the 14th days of incubation.

The disoriented ring seen at about 44 A in some of the 14- and 15-day feathers appears to be due to some lipids; the ring is absent after the specimen is extracted with alcohol. It is not known, however, whether this reflection has any significance in relation to keratin structure.

In summary, the present data suggest that lipids may provide a framework around which the more highly ordered forms of keratin are assembled. By the 10th day, micelles may be present in at least random distribution, while by 12 days there may be ordering of the micelles about the fiber axis. It is not certain of what the micelles consist. The possibility that they may be entirely lipid in make-up and unrelated to the emergence of adult keratin has been alluded to, but considered less likely than the possibility that they are a lipid-prekeratin complex or indeed some form of prekeratin itself. If the latter suggestion is correct the scaffold may no longer be required after the preliminary feather structure is stabilized (between 13 and 14 days) by formation of —S—S bonds and hydrogen bonds. It will be recalled that after this time lipid extraction no longer affects the principal features of the adult-like pattern. Further support of the suggestion that lipid plays a transitory role in the development of feather keratin is the observation that the 4.2 A ring, attributable to lipid, had nearly disappeared by the 15th to 16th day.

The morphogenic events which lead to the formation of the down feather appear to precede the emergence of definitive keratin. Barb ridges, barbs, and barbule cell differentiation, development of the follicle, and sheath formation are observed before "mature" keratin can be demonstrated by x-ray diffraction methods or by cytochemical techniques.

The problem of when embryonic keratin or prekeratin first appears has not yet been dealt with, nor have criteria for its diagnosis been established. Until such criteria are known and the timetable of synthesis elaborated, the possibility that prekeratin influences or even directs cell morphology cannot be dismissed. It has been suggested (Picken, 1960) that elongation of cells in the hair cortex or flattening of cells in the hair cuticle precedes keratinization. The time of appearance of keratin as defined by the adult x-ray diffraction pattern, the appearance of fibrils

in the electron micrographs, or by histochemical stains does not, of course, rule out the possibility that monomer keratin may be important in determining cell morphology. The present study indicates that precursor protein is probably present by the 10th day and possibly earlier. Hence, its role cannot be overlooked even though mature keratin appears late in relation to the time sequence of feather morphogenesis.

It is to be pointed out that feather keratin probably has a polyphase structure—consisting of a system of well organized fibrils embedded in a less well organized matrix. It has been shown by Schroeder and Kay (1955) that at least three kinds of keratin are found in the mature feather, as determined by amino acid analysis (barb, calamus, and rachis). It is not unlikely that barb and barbule keratin are also different in amino acid composition and this may be important for the differentiation of these cell types. Rudall (1947) and Earland *et al.* (1962) have remarked on the presence of α-keratin in the feather cap. The latter authors have also pointed out that the orientation of the fibrils varies markedly in different regions of the feather. For instance, they find that the fibrils in an outer part of the calamus of about one-third of the total thickness are oriented at right angles to the fibrils in the inner part of the calamus. The study of the morphogenesis of such polyphase structure would require x-ray microdiffraction from small selected regions in different parts of the developing feather.

Whether the "induction" of feathers triggers the synthesis of keratin is as yet undetermined and will remain so until an assay for prekeratin is worked out. Since keratinization occurs in other parts of the skin as well as the feather, it is quite likely that the feather "inducer" itself is something other than keratin. In fact the initiation of feather germ formation appears to depend upon a humoral factor to which the skin responds at about 6 days of incubation. After this initial stimulus, early development of the germ involves an elaborate interaction between mesoderm and epidermis (Sengel, 1957) which results in the elevation and thickening of the epidermis. There is also evidence that barb ridge formation, a process which occurs over an extended period (10 to 13 days of incubation), depends upon mesodermal participation. If 8-day dissociated epidermis without mesoderm is returned as a centrifuged aggregate to an embryonic environment, it will develop barbs and barbules but no barb ridges, while dissociated whole skin forms perfect feathers (Bell and Schuler, 1962). Perhaps, after an initial mesodermal stimulus, keratin formation commences in the epidermis and then plays a directing role in the formation of feather parts.

The authors wish to express their thanks to Dr. A. Rich and Dr. H. H. Pattee for providing the x-ray diffraction facilities and for helpful discussions. Thanks are also due to Dr. S. Krimm for discussion of some of the results.

The work reported in this study was supported by grants from the National Institutes of Health, the National Science Foundation, and the United States Office of Naval Research, Contract Nonr 225 (51).

Received for publication, May 10, 1962.

REFERENCES

ASTBURY, W. T., and BEIGHTON, E., Structure of feather keratin, *Nature*, 1961, **191**, 171.

ASTBURY, W. T., and BELL, F. O., X-ray data on the structure of natural fibres and other bodies of high molecular weight, *Tabulae Biologica*, 1939, **17**, 90.

BARRNETT, R. J., and SELIGMAN, A. M., Histochemical demonstration of sulfhydryl and disulfide groups of proteins, *J. Nat. Cancer Inst.*, 1954, **14**, 769.

BEAR, RICHARD S., X-ray diffraction studies on protein fibers. II. Feather rachis, porcupine quill tip and clam muscles, *J. Am. Chem. Soc.*, 1944, **66**, 2043.

BEAR, RICHARD S., and RUGO, H. J., The results of x-ray diffraction studies on keratin fibers, *Tr. New York Acad. Sc.*, 1951, **53**, 627.

BELL, E., and SCHULER, M., Studies on the development of chick skin (Paper delivered at the meeting of the Tissue Culture Association, May 31, 1962).

COREY, R. B., and WYCKOFF, R. W. G., Long spacings in macromolecular solids, *J. Biol. Chem.*, 1936, **14**, 407.

EARLAND, C., BLAKEY, P. R., and STELL, J. G. P., Studies on the structure of keratins, *Biochim. et Biophysica Acta*, 1962, **56**, 268.

FRASER, R. D. B., and MACRAE, T. P., Molecular organization in feather keratin, *J. Molecular Biol.*, 1959, **1**, 387.

KRIMM, S., Structure of frizzle mutant feather keratin, *J. Molecular Biol.*, 1960, **2**, 274.

MOKRASCH, L. C., and MCGILVERY, R. W., Purification and properties of fructose-1,6-diphosphatase, *J. Biol. Chem.*, 1956, **221**, 909.

PAULING, L., and COREY, R. B., The pleated sheet, a new layer configuration of polypeptide chains, *Proc. Nat. Acad. Sc.*, 1951 *a*, **37**, 251

PAULING, L., and COREY, R. B., The structure of feather rachis keratin, *Proc. Nat. Acad. Sc.*, 1951 *b*, **37**, 261.

PAULING, L., and COREY, R. B., Two rippled sheet configurations of polypeptide chains, and a note about the pleated sheets, *Proc. Nat. Acad. Sc.*, 1953, **39**, 253.

PICKEN, L. E. R., The Organization of Cells and Other Organisms, Oxford, University Press, 1960, 286.

RAMACHANDRAN, G. N., and DWELTZ, N. E., Structure of feather keratin, Seminar on Collagen, Central Leather Research Institute, Madras (November 29–30, 1960).

RUDALL, K. M., Protein chain types in the vertebrate epidermis, *Biochim. et Biophysica Acta*, 1947, **1**, 549.

SALTON, M. R. J., Studies of the bacterial cell wall. IV. The composition of the cell walls of some Gram positive and Gram negative bacteria, *Biochim. et Biophysica Acta*, 1953, **10**, 512.

SCHOR, R., and KRIMM, S., Studies on the structure of feather keratin. I. X-ray diffraction studies and other experimental data, *Biophysic. J.*, 1961 *a*, **1**, 467.

SCHOR, R., and KRIMM, S., Studies on the structure of feather keratin. II. A β-helix model for the structure of feather keratin, *Biophysic. J.*, 1961 *b*, **1**, 489.

SCHROEDER, W. A. and KAY, L. M., The amino acid composition of certain morphologically distinct parts of white turkey feathers; and of goose feathers barbs and goose down, *J. Am. Chem. Soc;* 1955, **77**, 3901.

SENGEL, P., Analyse experimentale du development in vitro des germes plumaires de l'embryon de poulet, *Experientia*, 1957, **13**, 177.

SWANBECK, G., Macromolecular organization of epidermal keratin, *Acta Dermato-Venereol.*, 1959, **39**, suppl. 43, 1.