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Differentiation of Avian Keratinocytes. Characterization and Relationships of the Keratin Proteins of Adult and Embryonic Feathers and Scales†

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ABSTRACT: The keratin proteins of embryonic and adult feathers and scales of the chicken were investigated, with the aim of providing a basis for the study of cellular differentiation in these tissues at a molecular level. Results from gel electrophoresis, isoelectric focusing, immunodiffusion, and amino acid analysis have demonstrated that the proteins of feathers are different from those of scales. Differences also occur between adult and embryonic tissues. The results suggest that in each cell line which originates from embryonic epidermis, a different and restricted set of structural genes for keratin proteins is selected from a larger set.

In developing embryonic chick skin, inductive events originating from the dermis result in the transformation of epidermal cell groups into feather and scale primordia (Wessells, 1962–1965; Rawles, 1963; Bell, 1963, 1964; Sengel, 1971). These primordia develop until they reach a form approximating the definitive organ. Tissue specific proteins (keratins) then become detectable by histological criteria and their synthesis continues rapidly until the cells fill with keratin (Bell and Thathachari, 1963). In suitable culture systems the developmental events can be experimentally investigated by recombining isolated epidermis and dermis from various regions (see Rawles, 1963). Such studies have shown that the developmental fate of the epidermis is determined by the nature of the underlying dermis. For example, epidermis from a prospective scale region (anterior tarsometatarsus) can be experimentally induced to form feathers in the presence of dermis from a prospective feather region (Rawles, 1963).

Studies on the molecular events occurring in response to these inductive influences have been hampered by lack of a suitable assay system for the keratin proteins which the tissues synthesize during their subsequent development. The component proteins of embryonic feathers and scales have not previously been identified. It was consequently not known whether the inductive events described above, control only tissue morphology or result as well in the synthesis of different tissue-specific proteins. Identification and characterization of the component keratin proteins of embryonic feathers and scales is a prerequisite in order to answer this question and to provide a quantitative and qualitative basis for the study of specific gene action during the differentiation of these tissues.

Malt and Bell (1965) and Ben-Or and Bell (1965) studied the proteins of embryonic feathers by chemical and immunological methods, but did not identify any individual keratin proteins. Harrap and Woods (1964a,b, 1967) and Woods (1971) extended the earlier studies of Ward *et al.* (1946), Woodin (1954, 1956), and Schroeder and Kay (1955) on the proteins of adult feathers. They prepared soluble proteins from adult feathers by reduction and S-carboxymethylation and showed that each of the morphological parts of the adult feather (Schroeder and Kay, 1955) contains several proteins. These were all of the same molecular weight (10,500) but were electrophoretically and chromatographically distinguishable.

In the present paper, soluble SCM[1]-proteins of adult and embryonic feathers and scales from the chicken have been investigated by gel electrophoresis, isoelectric focusing, peptide mapping, and immunological methods. The proteins of feathers are different from those of scales. Differences occur between the proteins of each tissue in their adult and embryonic states.

Materials and Methods

Preparation of Reduced S-Carboxymethylkeratins. Adult feathers and leg scales (from anterior tarsometatarsus) were obtained from approximately 1-year-old White Leghorn chickens, strain Para 3, a pure-bred strain obtained from Parafield Poultry Station, Parafield, South Australia. The so-called "embryonic" (down) feathers and scales were from newly hatched (21 day) chickens of the same strain. Adult feathers were separated into their four morphological parts, namely, rachis, calamus, barbs, and medulla (Schroeder and Kay, 1955). All tissues were washed, reduced and carboxymethylated essentially as described by Harrap and Woods (1964a) except that the conditions for reduction were altered to 3 hr at 37° in a solution containing 8 M urea, 0.5 M ethanolamine, and 0.1 M mercaptoethanol (pH 10.5).

The extracted SCM-proteins were dialyzed exhaustively against glass-distilled water and freeze-dried. Amino acid analysis demonstrated that the reduction and carboxymethylation reactions had gone to completion. Yields of freeze-dried material were greater than 70% of the dry weight of the start-

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[1] Abbreviation used is: SCM, *S*-carboxymethyl.

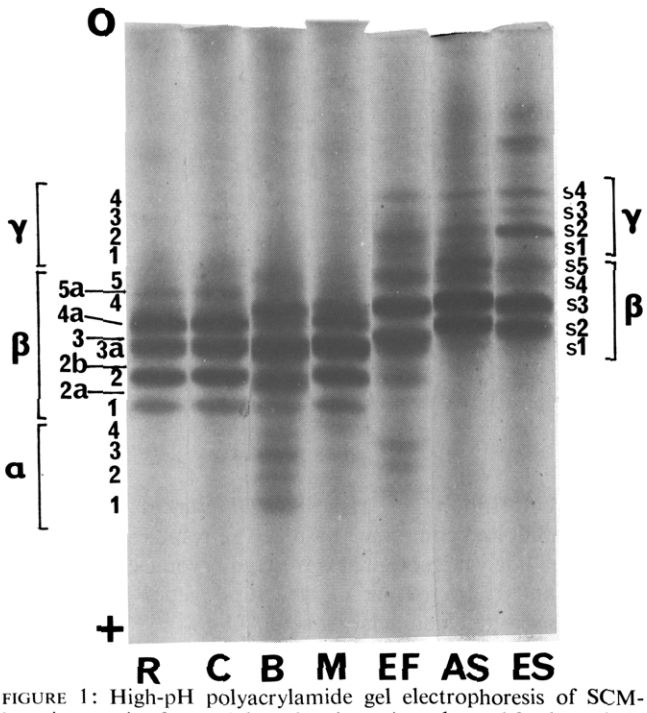

FIGURE 1: High-pH polyacrylamide gel electrophoresis of SCM-keratin proteins from adult and embryonic scales end feathers. 10% acrylamide gels containing 5 M urea, 8 cm long were used. 50 μg of protein/gel was loaded. Electrophoresis was performed at 2 mA/gel until the Bromophenol Blue marker reached the end of the gel. Bands were stained with Coomassie Blue. The division of bands into groups α, β, and γ and the nomenclature for all bands is shown. α1–γ4 refer to the feather proteins. βS1–γS4 refer to the scale proteins. R, rachis proteins; C, calamus proteins; B, barbs proteins; M, medulla proteins; EF, embryonic feather proteins; AS, adult scale proteins; ES, embryonic scale proteins; O, origin; +, anode.

ing material. The spectral properties of these protein preparations indicated that they were not contaminated by nucleic acids or other uv-absorbing material. All preparations have been repeated several times, with similar results.

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis at high pH (pH 9.5) using 10% polyacrylamide gels was performed as described by Canal Industrial Corp., Bethesda, Md., with 5 M urea in the gels and loading buffer. Polyacrylamide gel electrophoresis at pH 2.7 in 2.5 M urea was performed as described by Panyim and Chalkely (1969). Protein bands were stained with Coomassie Brilliant Blue (G. T. Gurr, London) in 10% trichloroacetic acid, and recorded with a photovolt densitometer.

Preparative Polyacrylamide Gel Electrophoresis. Protein fractions were prepared from the adult feather rachis SCM-proteins by high-pH polyacrylamide gel electrophoresis as described above, using a large-scale (1-l. gel capacity) cooled slab-electrophoresis apparatus, essentially similar to that described by Raymond (1964). After a run the gel slab was placed in 10% trichloroacetic acid to precipitate the protein bands. The regions of gel containing visible precipitated protein bands were cut out of the slab with a razor blade and homogenized briefly, using a Lourdes homogenizer, in 20 volumes of a solution containing 4 M urea, 0.005 M EDTA, and 1 M Tris-HCl (pH 9.0). The homogenized gel was extracted with at least four changes of this solution for a minimum of 24 hr. Gel particles were removed by filtration and centrifugation and the extracted protein was recovered by freeze-drying or rotary evaporating the extract after dialysis against glass-distilled water. Three successive purifications were required to obtain a sufficient degree of purity. Yields were approximately 25% for each cycle.

Isoelectric Focusing. Gel isoelectric focusing on the extracted proteins was performed by the technique of Wrigley (1968), using pH 3–5 ampholytes (LKB Produkter, Stockholm), with or without 2 M urea.

Peptide Mapping. Protein samples were digested at 10-mg/ml concentration in 0.2 M *N*-ethylmorpholine acetate buffer (pH 8.3) for 2 hr at 25° using trypsin treated with *N*-tosylphenylalanine choromethyl ketone (Worthington) at 1% w/w enzyme–substrate ratio. The peptides were subjected to electrophoresis in pyridine–acetate buffer (pH 4.7) for 1 hr at 1000 V on cellulose thin-layer chromatography plates (Eastman Organic Chemicals).

Amino Acid Analysis. Samples were hydrolyzed in 6 N HCl at 110° for the indicated times. Hydrolysates were analyzed by the method of Piez and Morris (1960).

Immunological Techniques. Antisera to SCM-keratins from whole adult feathers were prepared in rabbits and the γ-globulin fraction obtained from these sera using methods employed for guinea pig hair follicle SCM-proteins (Kemp and Rogers, 1970). Immunodiffusion was performed by the method of Ouchterlony (1962) using 1% agar gels in 0.01 M sodium phosphate buffer (pH 7.1).

Aggregation. Protein samples (10 mg) were dissolved in 1 ml of 0.2 M *N*-ethylmorpholine acetate (pH 8.3) and incubated at 37° for 24 hr. They were then examined by electron microscopy (using negative staining) and stored at 2–4° to allow gel formation.

Results

The soluble SCM-keratin proteins from the morphological parts of adult feather (*i.e.*, rachis, calamus, barbs, and medulla), embryonic feather, and adult and embryonic scales were prepared as described in the Methods section. The protein species present in all these preparations were examined by the various analytical procedures as outlined below, in order to investigate the number of component proteins in each tissue and to determine whether or not the component proteins of each tissue are identical.

High-pH Polyacrylamide Gel Electrophoresis. Samples of each preparation were subjected to high-pH polyacrylamide gel electrophoresis (Figure 1). In each case, considerable fractionation was obtained. Three groups of bands were apparent. The fastest moving (α) group of these, designated bands α1–α4 occurred in small amounts in barbs, medulla, and embryonic feather. The second (β) group, of intermediate mobility, designated bands β1–β5 contained the major proteins of each tissue although the distribution of these varied greatly from tissue to tissue. The third group (γ), that are the slowest moving bands and designated γ1–γ4, occurred in embryonic feather and in both adult and embryonic scales but were not observed in adult feather.

The differences between the β group of bands of each tissue are demonstrated more clearly by densitometer tracings of the gels (Figure 2). Bands β1–β4 of rachis and calamus appeared as symmetrical peaks, but bands β3 and β4 of barbs and medulla quite clearly each contained at least two components, designated β3 and β3a, and β4 and β4a, respectively. Embryonic feather contained a distinctly different pattern. However, coelectrophoresis of embryonic feather proteins with rachis proteins (Figure 2, R + EF) demonstrated that the major proteins of embryonic feather were in bands β3 and β4.

Adult and embryonic scale proteins appeared identical with

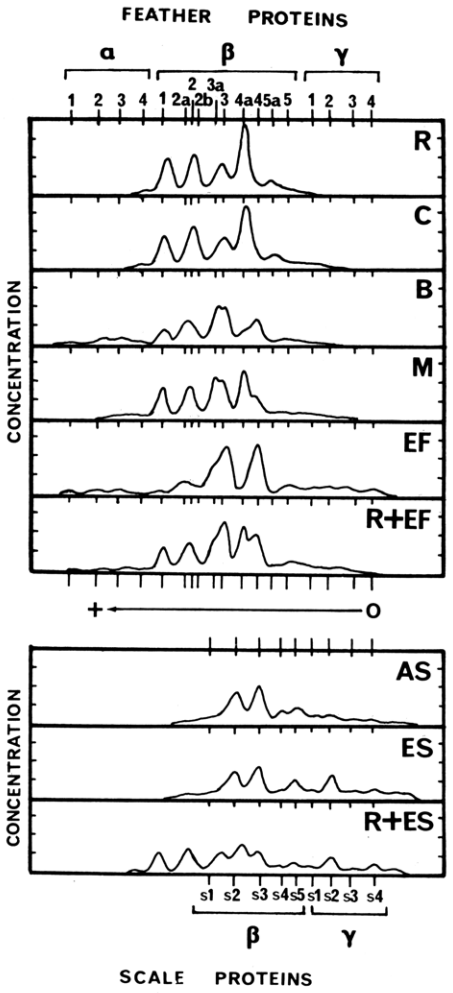

FIGURE 2: Densitometer traces of the protein-containing regions of the high-pH gels shown in Figure 1. R, C, etc., as in Figure 1. R + EF, coelectrophoresis of rachis proteins (25 μg) with embryonic feather proteins (25 μg). R + ES, coelectrophoresis of rachis proteins (25 μg) with embryonic scale proteins (25 μg). The direction of migration is indicated by the arrow. Concentration is in arbitrary units and refers to the concentrations of Coomassie Blue dye bound.

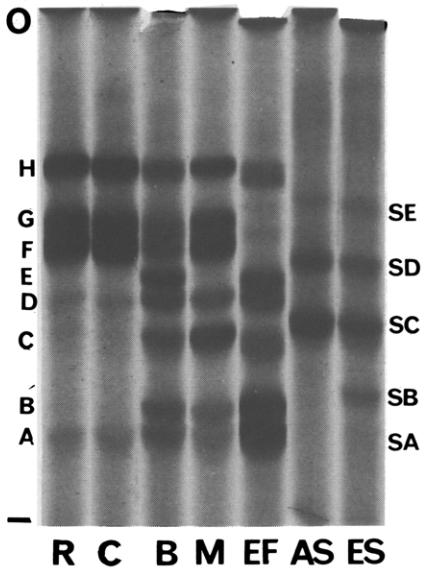

FIGURE 3: Low-pH polyacrylamide gel electrophoresis of SCM-keratin proteins from adult and embryonic feathers and scales. 50 μg of protein/gel was loaded. 15% gels containing 2.5 M urea, 8 cm long were used. Electrophoresis was performed at 2 mA/gel for 4 hr. Bands were stained with Coomassie Blue. R, C, etc., as in Figure 1; −, cathode; 0, origin. Feather protein bands and scale protein bands are designated A–H and SA–SE, respectively.

each other. Coelectrophoresis of embryonic scale proteins with adult rachis proteins (Figure 2, R + ES) and of embryonic scale proteins with embryonic feather proteins (not shown) demonstrated that the major bands of the β region of feather proteins have different mobilities to those of scale proteins.

Polyacrylamide gel electrophoresis at pH 7.5 (Williams and Reisfeld, 1964) gave band patterns essentially identical with those obtained in the high-pH system for all tissues but the bands were not as sharp.

Low-pH Polyacrylamide Gel Electrophoresis. Using the 8-cm, low-pH gel system (Figure 3) a fractionation of the feather proteins was obtained. A maximum of eight major bands was observed for the barb and medulla parts of adult feather and for the total proteins from embryonic feather. These bands are designated A–H. It was subsequently found that on 20-cm gels (Figures 4 and 5) most of these bands could be further resolved, into additional ones designated A1–H2. The major protein bands of embryonic feather (A1–E) were also observed in barbs and medulla whereas only traces were present in rachis and calamus. The groups of bands designated F and G derived from proteins of rachis, calamus, barbs, and medulla of adult feather were absent in embryonic feather although there were traces of similar bands in this region. Band E of embryonic feather was not detectable in rachis, calamus, or medulla.

An additional band (not shown) was present in embryonic feather but not in the other tissues. This component had a mobility much greater than the others, and consequently migrated off the end of gels run for the times shown in Figures 3 and 4.

The protein bands of adult and embryonic scales were qualitatively identical to each other (Figure 3) but they differed in their relative amounts. All the bands of scale (SA–SE) had different mobilities from those of feather, with the possible exception of band SC (Figure 3) which may have a mobility identical with that of feather band C′ (Figure 3).

Isoelectric Focusing. Preliminary experiments using wide-range ampholytes (pH 3–10) indicated that all detectable proteins in the various preparations had isoelectric points in the region of pH 3–4. In further esperiments therefore, ampholytes in the range of pH 3–5 were used. The number and pattern of bands was found to be dependent on the time the current was applied although reproducible at a given time (Frater, 1970). The clearest pattern was given at 5 hr (Figure 6).

It can be seen that the different parts of adult feather have some protein bands in common and that some of these are present in embryonic feather. However, in the high-pH region some of the protein components are completely absent from rachis and calamus. Isoelectric focusing of embryonic feather proteins demonstrated that this tissue has a large proportion of protein components that have isoelectric points that are in the higher region of the pH range 3–4. This result is consistent with the observations made with low-pH gels and by amino acid analysis.

The proteins of scales did not focus under these conditions, but precipitated in the gels throughout their length. The addition of 2 M urea to the gels prevented this precipitation

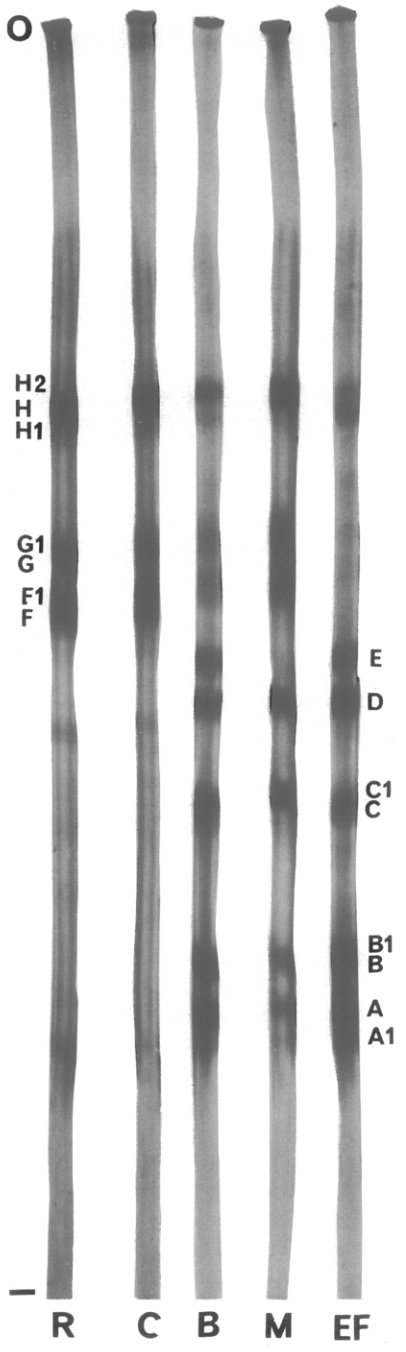

FIGURE 4: Low-pH polyacrylamide gel electrophoresis of SCM-keratin proteins from adult and embryonic feathers. 100 μg of protein/gel was loaded. 15% gels containing 2.5 M urea, 20 cm long were used. Electrophoresis was performed at 500 V for 24 hr. Bands were stained with Coomassie Blue. R, C, etc., as in Figure 1; –, cathode; 0, origin. Bands are designated A1–H2.

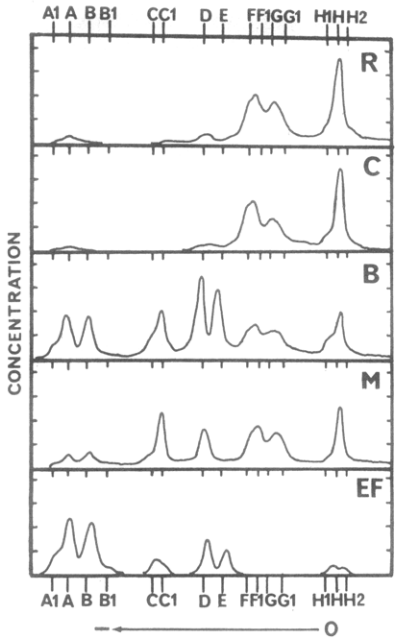

FIGURE 5: Densitometer traces of the low-pH gels shown in Figure 4. Details as in Figure 2. Feather protein bands A1–H2 are indicated. It is clear from densitometry that in the F–G region, particularly in the case of calamus, there are minor components at the leading and trailing edges. However, for simplicity of discussion, these will be ignored.

but the pH gradient did not extend below pH 3.45 after a 5-hr run. The resulting patterns for both feathers and scales were not reproducible and did not allow any firm conclusions to be drawn.

Peptide Mapping. One-dimensional electrophoresis (not shown) of the tryptic peptides prepared from the proteins of each tissue gave results consistent with the above observations. Barbs, medulla, and embryonic feather gave patterns that were similar and were more complex than those of rachis and calamus. Adult and embryonic scales were again similar to each other, but contained some peptides not present in the feather preparations. The same results were obtained when the peptides were fractionated by paper electrophoresis at pH 4.7, 3.7, and 6.5 in pyridine–acetate buffers.

Immunodiffusion. Double diffusion of the various preparations against antisera prepared to the SCM-proteins of whole adult feathers (see the Methods section) is shown in Figure 7. Rachis and calamus were immunologically identical at all antigen concentrations tested. At the lower concentrations, all other feather preparations contained at least one additional antigen (precipitin line) not present in rachis and calamus. Scale proteins contained at least two antigens common to feather.

These reactions were not prevented by the incorporation of 0.025 M SCM-cysteine (a potential hapten inhibitor) into the agar, indicating that the reactions were not determined by the *S*-carboxymethyl groups. Also, no reaction was obtained with SCM-protein from guinea pig hair, further indicating that hapten effects were not involved.

Aggregation. Harrap and Woods (1967) found that SCM-(feather) keratin preparations from adult rachis aggregated into a gel if allowed to stand for some time in borate buffer and that long fibrils were formed during this process (Filshie *et al.*, 1964). Consequently, samples of the proteins from adult feather, rachis, calamus, barbs, and medulla, from embryonic feather and scales and from adult scales were tested for their ability to aggregate. All formed apparently identical fibrils within 24 hr, as determined by electron microscopy and all eventually gelled.

Isolation of Feather Protein Bands from High-pH Gels. Purified samples of bands α4, β1, β2, β3, and β4a from rachis were prepared by preparative high-pH polyacrylamide gel electrophoresis as described (see the Methods section). Densitometer traces of these fractions rerun on high-pH gels are shown in Figure 8. Identity of the fractions was estab-

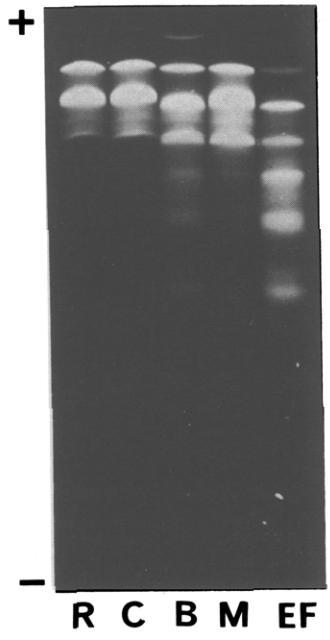

FIGURE 6: Isoelectric focusing in polyacrylamide gels, in the absence of urea, for 5 hr, using pH 3–5 ampholytes. 50 μg of protein/gel was loaded. Bands were precipitated with 10% trichloroacetic acid and photographed unstained, using darkfield illumination. R, C, etc.; as in Figure 1; +, anode (low pH) end; −, cathode (high pH) end.

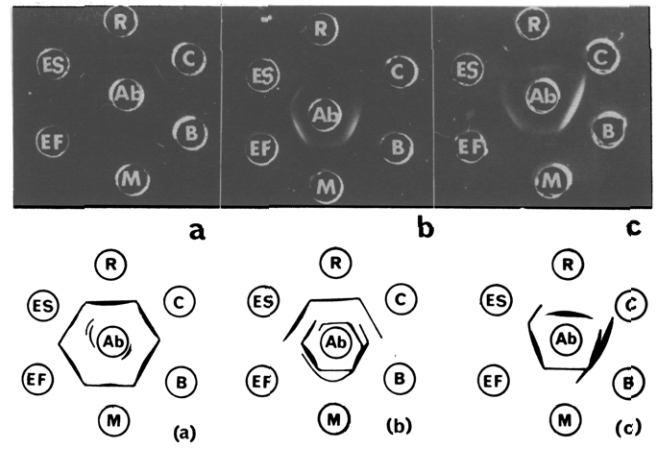

FIGURE 7: Immunodiffusion of keratin proteins from adult and embryonic feathers and scales against rabbit antifeather SCM-proteins serum. Protein (antigen) concentration: (a) 10 mg/ml, (b) 1.25 mg/ml, and (c) 0.31 mg/ml. R, C, etc., as in Figure 1; Ab, antiserum. The unstained gels were photographed using darkfield illumination.

lished by coelectrophoresis with unfractionated rachis proteins in each case (Figure 8). Three unsuccessful attempts were made to run these fractions on low-pH gels. Most of the protein did not enter the gel and the remainder formed several bands, none of which had the same mobility as any band in the control gels. Most probably some solubilized polyacrylamide contaminating the preparations interacted with the proteins at the low pH causing the anomalous behavior.

Amino Acid Analyses. The unfractionated proteins from each tissue and the purified protein components obtained from rachis by high-pH polyacrylamide gel electrophoresis were subjected to amino acid analysis. Control experiments showed that the presence of solubilized polyacrylamide in the rachis fractions did not affect the analyses providing hydrolysis was carried out in the presence of phenol, to protect tyrosine. SCM-cysteine was destroyed to a varying extent.

Table I presents analyses of the unfractionated proteins from each tissue and Table II presents analyses of the purified components.

The analyses of unfractionated adult feather proteins agree well with those of Harrap and Woods (1964a). Embryonic feather proteins were similar to adult. Adult and embryonic scale proteins, although almost identical with each other, were significantly different from feather proteins in their lower content of SCM-cysteine and serine and their increased content of glycine, methionine, and tyrosine. The analysis of each purified fraction was typical of feather keratin and indicates the existence of a family of closely related protein species.

Discussion

Heterogeneity of Feather Proteins. This work was aimed at providing an analytical basis for the study of specific gene action in the development of keratinizing tissues in the chicken. Quantitative densitometry of the extracted SCM-proteins on acrylamide gels (Figures 2 and 5) provides a suitable technique for measuring the mobility and the amount of each band. However, the question of whether each band resolved by acrylamide gels contains one gene product or more is obviously important. The heterogeneity of keratin proteins from other keratinizing tissues (see, for example, O'Donnell and Thompson, 1968) suggests the need for caution in such interpretation.

The possibility that any of the bands are aggregates can be ruled out because the same protein species occur in the various feather tissues in different amounts. The bands on high-pH

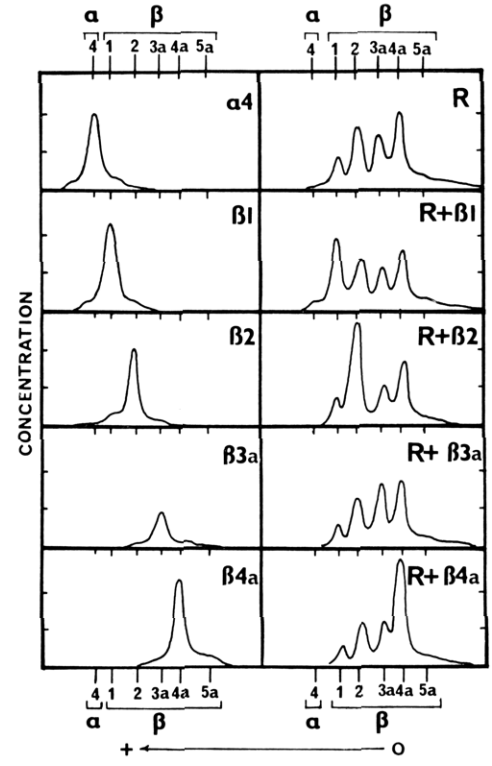

FIGURE 8: Densitometer traces of bands purified by high-pH polyacrylamide gel electrophoresis. Details as in Figure 2. R + β1, etc., coelectrophoresis of rachis and band β1, etc.

TABLE I: Amino Acid Analyses of the Unfractionated Keratin Proteins from Various Tissues (Moles/100 Moles).

Tissue	Rachis[a]	Calamus[b]	Barbs[b]	Medulla[b]	Embryonic[b] Feather	Adult[b] Scale	Embryonic[b] Scale
CM-cysteine	8.0	7.8	7.7	7.9	8.1	5.4	5.8
Aspartic acid	5.7	5.5	5.7	5.9	6.0	4.2	4.2
Threonine	4.1	4.2	5.2	4.1	4.0	3.9	3.7
Serine	14.7	13.4	14.9	13.9	12.0	9.4	9.5
Glutamic acid	6.8	7.2	8.2	7.8	7.6	5.8	5.8
Proline	11.7	9.8	10.7	9.7	11.3	6.9	7.7
Glycine	13.6	14.8	11.8	13.6	12.5	26.3	25.8
Alanine	8.5	9.1	5.4	7.3	4.5	6.5	7.9
Half-cystine	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Valine	8.0	7.9	8.2	7.7	8.4	6.5	6.4
Methionine	0.0	0.0	0.0	0.1	0.0	0.7	0.6
Isoleucine	2.9	3.2	4.4	3.6	4.6	2.7	2.6
Leucine	7.8	8.4	7.1	8.1	7.5	6.3	5.8
Tyrosine	1.3	1.6	1.6	1.5	2.5	6.6	6.2
Phenylalanine	3.1	3.2	3.7	3.8	4.2	3.0	2.6
Lysine	0.2	0.2	0.2	0.5	0.3	0.9	1.0
Histidine	0.0	0.1	0.1	0.2	1.0	0.7	0.6
Arginine	3.6	3.7	4.7	4.4	5.5	4.3	3.9

[a] Average of three analyses, on 20-, 44-, and 72-hr hydrolysates. [b] One analysis, on 20-hr hydrolysate.

gels were all isolated and these reran as single bands on high-pH gels, further ruling out the possibility that some bands are aggregates. Amino acid analyses of the unfractionated proteins from each tissue rule out in a similar way the possibility that any of the major bands are artefacts due to incomplete reduction or carboxymethylation.

TABLE II: Amino Acid Analyses of Feather Protein Fractions (Moles/100 Moles).

Major Band Isolated	$\alpha 4^a$	$\beta 1^b$	$\beta 2^a$	$\beta 3a^a$	$\beta 4a^b$
CM-cysteine	6.7	6.6	5.0	3.6	5.5
Aspartic acid	6.0	6.5	6.6	5.3	5.0
Threonine	4.2	3.9	4.2	4.5	4.7
Serine	14.9	14.7	15.6	12.7	14.2
Glutamic acid	9.0	8.9	8.8	6.8	5.4
Proline	10.2	10.0	10.2	9.4	10.9
Glycine	13.2	13.0	12.7	17.5	15.9
Alanine	8.1	7.9	8.0	13.8	10.9
Half-cystine	0.0	0.0	0.0	0.0	0.0
Valine	8.3	9.3	9.2	6.7	7.2
Methionine	0.0	0.0	0.0	0.0	0.0
Isoleucine	3.4	3.2	3.9	3.1	3.2
Leucine	6.7	6.5	6.8	8.4	10.4
Tyrosine	1.3	1.4	1.1	1.3	1.2
Phenylalanine	4.1	4.2	4.1	3.0	2.3
Lysine	0.3	0.0	0.0	1.0	0.0
Histidine	0.0	0.0	0.0	0.2	0.0
Arginine	3.7	4.0	3.7	2.7	3.0

[a] One analysis on 20-hr hydrolysate. [b] Two analyses, on 20- and 72-hr hydrolysates.

The results from isoelectric focusing, peptide maps, and immunodiffusion are entirely consistent with the conclusion from polyacrylamide gel electrophoresis that the various feather tissues share many of the different polypeptide chain species but in different amounts. The results of these experiments do not, however, allow any further conclusions as to the number of polypeptide chain species present. In further studies in progress in this laboratory (I. D. Walker and G. E. Rogers, in preparation) the number of polypeptide chain species is being investigated by a combination of chromatographic isolation, polyacrylamide gel electrophoresis, and the peptide mapping of major components.

Differences in the Proteins of Feathers and Scales. The results from polyacrylamide gel electrophoresis demonstrate that the major β group of protein species of scales are different from those of feathers. Besides the developmental significance, this observation serves as an excellent experimental control to show that all the proteins are in fact tissue specific. They are not, for example, ribosomal proteins or other cellular proteins which would be expected to be common to each tissue. The amino acid composition of each feather protein fraction further suggests each component is a keratin protein.

Microheterogeneity and Relationship to Keratin Structure. The results from polyacrylamide gel electrophoresis, the immunological cross-reactions between feather and scale proteins and the ability of these proteins to fibrillate and gel *in vitro*, suggest that they consist of a family of closely related protein chains. Moreover, the similarity in molecular weight (Harrap and Woods, 1964b; Jeffrey, 1970) and amino acid composition (present results) of all the β-protein species suggests that they have all arisen by duplications and subsequent mutational divergence from a common ancestral gene. This mode of origin is generally accepted for the diversity of hemoglobin chains and other protein families (Zuckerkandl and Pauling, 1965).

Various models have been proposed for the structure of

feather keratin based on X-ray diffraction (Fraser *et al.*, 1971) and electron microscopic evidence (Rogers and Filshie, 1963). It is likely that each type of protein chain is a structurally equivalent monomer in the keratin quaternary structure and the filament unit (microfibril) of avian keratins could be built from any one of them.

Significance in Relation to Development. Embryonic feathers and scales have been shown to contain some different species of protein chains. Thus, different structural genes are expressed in the epidermal-derived cells as a direct or indirect result of the dermal "inductive" influence. The expression of these genes represents the dominant synthetic activity of the cells after a defined temporal point in the development of each tissue.

Studies on recombination of dermis and epidermis from various regions (Rawles, 1963) have clearly shown that feather and scale morphogenesis from epidermal cells is determined to some extent at least by dermis by as yet poorly understood processes. The studies of Dhouailly (1967) have demonstrated that the morphology of feathers and hence control of cell proliferation, is controlled by the nature of the dermis. Cytological studies have shown the presence of different cell types in embryonic feathers, for example, the flattened sheath cells and elongated cylindrical cells of the barbules. The studies of Kato (1969) have shown that at least one of these cell types (scale cells) can be induced by scale dermis to differentiate from chorionic epithelial cells which in the normal state do not differentiate in such a manner.

The present study shows that in the differentiation of morphologically distinct derivatives of the embryonic epidermis such as feathers and scales, distinct groups of proteins chains are produced. These observations lead to the conclusion that there exists a major set of structural genes for all of the different protein chains of feather keratin and that a particular set of these genes that is expressed in the cells of feather is entirely different from the one expressed in scale cells. Similarly, the group of proteins (bands A–E) synthesized by barb and barbule cells which are the predominant cells in embryonic feathers (Bell and Thathachari, 1963) is distinct from the proteins (bands F–H) synthesized by the rachis cells of the adult feather. Here again, one concludes that the gene set selected for expression is distinct in two different populations of cells.

It is seen from an examination of the variety of protein chains present in the different parts of adult feather, the rachis, calamus, barbs, and the medulla (Figure 5) that within experimental error the amounts of the proteins represented by bands F, G, and H remain constant relative to one another. The proteins of bands A and B from each of these tissues also show a similar relationship. In contrast, no constant relationship exists between the protein bands of A and H or those of bands C, D, and E. A possible explanation of the coordinated behavior among different groups of proteins is that several unique populations of cells are present within the tissue; each population might exclusively synthesize a single group of proteins, thus yielding coordination within a group. The apparent lack of coordination between groups of proteins might then result from the presence of varying numbers of each of the discrete cell types within the tissue, each type having its distinct set of active genes.

It is not known how selection of these genes for expression in each cell type is controlled. The present results provide an analytical approach which should be readily applicable to the study of such mechanisms in the developing tissues. Use of the acrylamide gel systems described here will allow unambiguous identification of the keratin proteins at the earliest stage of development at which they are present and densitometry will allow their quantitation. Preliminary results indicate that the keratin proteins can first be detected in the developing embryonic feather at about 13-days incubation.

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