X-RAY STUDIES OF THE DISTRIBUTION OF PROTEIN CHAIN TYPES IN THE VERTEBRATE EPIDERMIS

by

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In X-ray studies of a number of different tissues of epidermal origin considerable information on the occurrence of the various forms of polypeptide chains has accumulated. Such studies give a view of the variation that occurs in one molecular species — the α-keratin type. They also show the distribution of the feather keratin type and its alternation with or replacement by α-keratin.

The main properties of the hard keratin of mammalian hair, horn, etc. have been recorded in a series of papers[1], while principal features of the soft keratin of the mammalian epidermis have been described recently[2]. A renewed interest in the unusual fibrous protein 'feather' keratin is aroused by recent studies on the muscle protein 'actin'[3]. We describe below the principal molecular configurations which have been found in the vertebrate epidermis. Useful reviews of the histology of these tissues have been made by STUDNICKA[4] and BIEDERMANN[5], while more specialised histological studies are referred to in the subsequent sections.

The X-ray photographs were taken using copper Kα radiation and specimen to film distances which were near to 4 cm except for Fig. 4 (4.83 cm) and Figs. 12 and 13 (5 cm).

I. CYCLOSTOME AND TELEOST EPIDERMIS

Histological details of the former have been given by BLOMFIELD[6] and of the latter by KANN[7]. By scraping the surface of the lamprey, *Petromyzon fluviatilis*, epidermal cells together with mucus were obtained. The mucus was largely removed by washing in EDSALL's solution and centrifuging to collect the remaining cells. These were dried as thin films on glass plate, and X-ray photographs revealed the α-type of pattern characteristic of keratin and myosin (Fig. 1). In addition there is a diffuse pattern which may represent residual mucus or other ill-defined components. In the case of the hard, horny teeth of the lamprey the constituent fibrous protein is of the well defined α-type. We have not examined the thread-like bodies secreted in the slime. These have been named 'mitin' by FERRY, while COREY has indicated that they show a new type of diffraction pattern[8].

Small pieces of epidermis were obtained from a freshwater perch (*Perca fluviatilis*): they showed clearly defined diffraction patterns of the α-type similar to those of myosin films, for example[9]. Thus in these samples of cyclostome and fish epidermis the predominating fibrous protein is of the α-type, though it cannot be excluded that there may be

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present other types of protein chain configuration. However, such additional constituents must occur in small quantity, or in a relatively 'non-crystalline' condition.

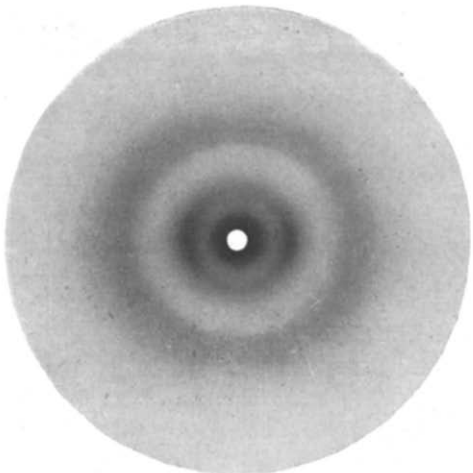

Fig. 1. Epidermal cells of a Cyclostome

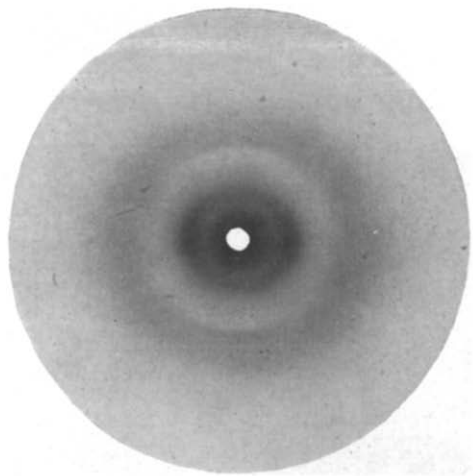

Fig. 2. Shed stratum corneum of a Urodele

II. AMPHIBIA

The epidermis of Amphibia is generally composed of two main cell layers, an outer stratum corneum and an inner stratum germinativum. The thickness of these two strata varies in different members of the class, some typical measurements being given by STEINBACH[10]. Material for X-ray studies is readily available in the shed stratum corneum, and sources used were *Triturus pyrrhogaster* and *Amblystoma tigrinum*. Epidermis was also dissected from the frog and toad. The diffraction pattern obtained from the stratum corneum of *Amblystoma tigrinum* is shown in Fig. 2, where the beam was directed parallel to the surface of a bundle of epidermal layers. It shows a well defined structure of the α-protein type. A defined diffraction pattern of any other protein material was not detected. Similar results were obtained with the other amphibian epidermis samples, whether stratum corneum or stratum germinativum.

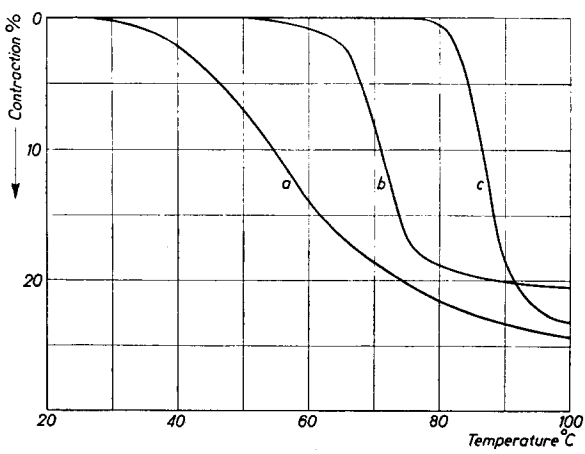

Fig. 3. Thermal contraction curves of strips of films; a. myosin; b. Triturus stratum corneum; c. human leg stratum corneum

A study was made of thermal contraction in the epidermis of a urodele. Strips of stratum corneum shed from *Triturus pyrrhogaster* were mounted in adjustable frames and measurements of length changes were made following the same procedure as described for mammalian epidermis[2]. A curve showing the relationship between length and

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temperature is given in Fig. 3b, there being no difference between the results for normal and soxhletted (ether 12 hours) material. The curve for Triturus stratum corneum is similar to the curve for human leg stratum corneum, except that the main thermal contraction takes place at the lower temperature range of 65–75° C. In terms of the temperature of transformation the Triturus epidermis occupies a position intermediate between that of mammalian epidermis(c) and myosin(a)[9]. When the thermal contraction of Triturus epidermis was followed by X-rays, an incipient change was observed at 67.5° C, while after immersion in water at 71° C for 1 minute there was a considerable amount of β-protein produced. In similar tests of mammalian stratum corneum (upper lip of cow) the β-protein pattern did not begin to appear until the temperature was over 80° C. These facts indicate that the molecular chains in the mammalian stratum corneum are more stably linked together than in the amphibian stratum corneum.

III. REPTILIA

MARWICK[11] has already made a preliminary study of the horny shields of the common tortoise. The diffraction pattern was apparently identical with that of the feather keratin of goose and seagull quill, but was relatively poor because of lack of parallel orientation. Thus a detailed comparison of 'avian' and 'reptilian' keratin was not immediately possible.

More studies have been made on snake epidermal material than in other Reptilian groups. In Squamata the external surface is covered by a special cuticle originating from the two layers of shedding cells, while the epidermis and scales alike are divided into an outer 'compact' and an inner 'loose' horny layer. In the epidermis between the scales the compact horny layer (which is specially characteristic of the scales) is markedly reduced. In a long series of papers SCHMIDT[12] has described the principal histological features of Reptilian epidermal structures.

From unidentified snake skins obtained from the London Zoo, the large ventral scales were removed and soxhletted in ether for 24 hours. After this treatment they

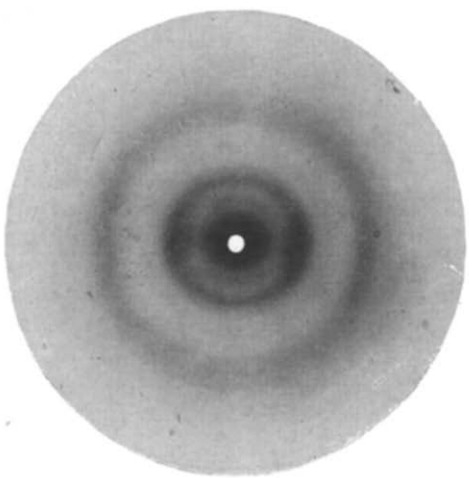

Fig. 4. Outer compact layers of snake scale

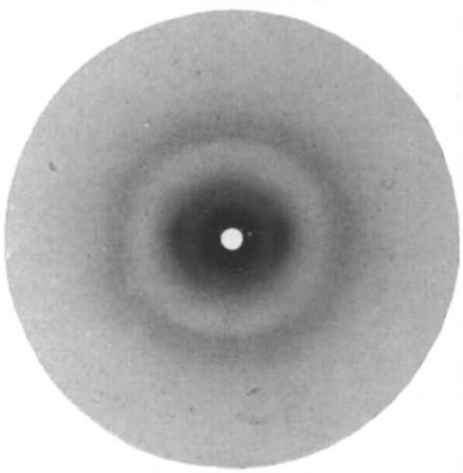

Fig. 5. Inner loose layers of snake scale

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could be divided easily into an upper compact and transparent layer and a lower, softer and opaque layer. Furthermore, the thinner postero-ventral part of the scale divided into an outer cuticle-like structure and an inner part consisting of compact and translucent cell layers. An X-ray examination was made of the layers so obtained. The outer compact layers of the scale proper gave a diffraction pattern of the feather keratin type (Fig. 4). The molecular chains lie approximately parallel to the surface of the scale but show no preferred orientation with reference to an axis in the surface of the scale. The principal spacings are listed in Table I. In contrast to the outer compact layers, the inner loose layers give a diffraction pattern of the α-keratin type without any sign of the feather keratin pattern (Fig. 5). This same α-pattern is given also by the lower layers of the postero-ventral part of the scale, while the outer thin 'cuticle' of this region gives a poorly defined β-type of pattern which is more related to the feather keratin structure. The whole epidermis from the 'between scale' regions gave the α-type of pattern, cuticle and compact horny layers being present in insufficient quantity to give rise to a detectable feather keratin pattern. The distribution of the two kinds of diffraction pattern in the snake scale is summarised in Fig. 6.

Fig. 6. Diagram of snake scale; – – – – feather keratin; . . . . . α-keratin

The flat sheets of cells as in the outer layers of the snake scale do not possess a high degree of internal orientation. Many attempts to improve the orientation by stretching have been unsuccessful. With difficulty about 30% extension may be obtained but without much improvement in the alignment of the protein chains. A comparatively high degree of orientation occurs naturally in the claws of the lizard *Varanus niloticus* — a circumstance probably connected with the restricted nature of the germinal matrix[13]. In the Varanus claw the chains lie roughly parallel to the long axis of the claw and closely parallel to its surface. Photographs with the X-ray beam parallel to the surface and perpendicular to the long axis of the claw give fine patterns of the feather keratin type (Fig. 12). The principal spacings are listed in Table 1. The largest observed meridional spacing is 24.7 A, and all the meridian (or near meridian) spacings taken together indicate a fundamental period of 98.5 A.

An important concern at the moment is to obtain a sufficiently high quality diffraction pattern of a reptilian keratin for comparison with avian keratin. This comparison is considered below in dealing with feather keratin.

Other reptilian materials examined were epidermis, scales and claws from the crocodile *Gavialis gangeticus*, and ventral scales from a young African crocodile. The crocodile material differs from the epidermis and scales of Squamata in that it is not divided into 'compact' and 'loose' horny layers, and it is not shed periodically but is gradually replaced as it wears away. In the Gavialis material the epidermis from between the scales gives the typical α-type pattern together with a strong lipoid pattern. By contrast the horny material of the scales and claws give ill-defined diffraction patterns showing neither the α- nor the feather keratin pattern in a well defined form. In some samples a moderately distinct meridional arc of spacing 5.1 A suggests the presence of α-keratin; in others such an arc is not apparent. In all samples a diffuse equatorial reflection at 4.6 A and a recognisable meridional reflection at c. 23 A indicate the presence

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of the feather keratin type. The ventral scales of the African crocodile showed a poorly defined pattern of the feather keratin type but no definite evidence of the presence of α-keratin.

IV. AVIA

General studies of the histological structure of the epidermis in birds have been made by GRESCHIK[14] and FREUND[15]. In our present X-ray studies we are concerned with the thinner epidermis of the main feather-bearing areas and the thick epidermis that occurs between the toes and on the ventral surface of the feet. This latter type (after soxhletting in benzene) gives a very well defined α-type pattern (Fig. 7) and a fairly strong meridional reflection at 23 A.. There are also reflections from residual lipoids. Epidermis from other regions of the body was examined in a number of birds. In the case of the guinea-fowl a comparison was made of epidermis from the featherless regions of the neck and from the feather tracts of the back. The neck epidermis showed a defined α-protein structure superposed on a diffuse pattern such as might be expected from amorphous material. By contrast, the epidermis from the dorsal areas does not give the defined α-type pattern, but instead an ill-defined β-type probably related to feather keratin. This is illustrated in Fig. 8 where lipoids not removed by prolonged soxhletting in benzene are also visible. A very faint meridional reflection at c. 23 A is detectable in the original negative. The ill-defined pattern of Fig. 8 is shown by many of the samples of bird epidermis studied. It cannot be distinguished with certainty from the background or amorphous pattern in epidermis from other vertebrate classes. Studies of the epidermis in the main feather bearing areas is difficult. It is thin, very abundantly impregnated with lipoids which are difficult to remove, and the protein diffraction pattern is inherently ill-defined. Thinness of cell layers and lack of strong development of intracellular fibrous proteins may be associated with poor blood supply to the skin surface[15].

Of the special epidermal structures the most instructive for study is the feather and its follicle sheaths. The histology and manner of growth of feathers have been studied by DAVIES[16], STRONG[17], HOSKER[18], and particularly on the experimental side by LILLIE and co-workers[19]. At present the molecular structure of products of the three principal cell layers will be considered:

A. The outer layer of the feather sheath and the calamus sheath.

B. The products of the stratum cylindricum — the series of feather caps or quill cones.

C. The products of the stratum intermedium — barbules, barbs, rachis and calamus; the inner layers of the feather sheath.

The diffraction patterns obtained from the cell layers A and B are characterised by:

1. strongly developed diffractions from lipoid material;
2. a very well defined pattern of the α-keratin type; and
3. distinct meridional reflections of spacing about 23 A.

The diffraction pattern obtained from calamus sheath soxhletted in benzene is shown in Fig. 9.

A brief description of feather keratin as it occurs in seagull calamus has been given by ASTBURY and MARWICK[20]. The molecular configuration corresponds to a not quite fully extended β-keratin structure. The relative inextensibility of the feather keratin

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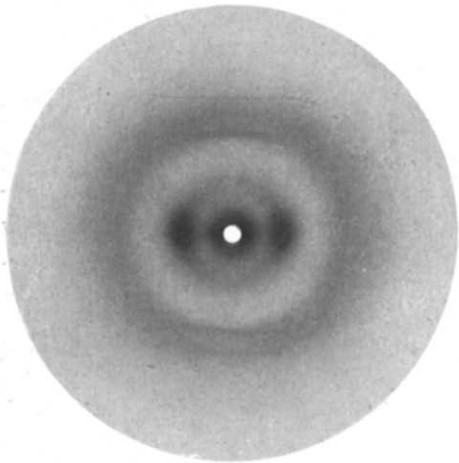

Fig. 7. Thick epidermis from ventral surface of bird's foot

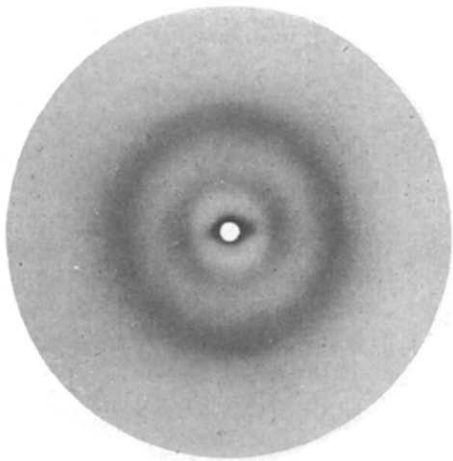

Fig. 8. Thin eperdermis from ventral surface of bird's foot

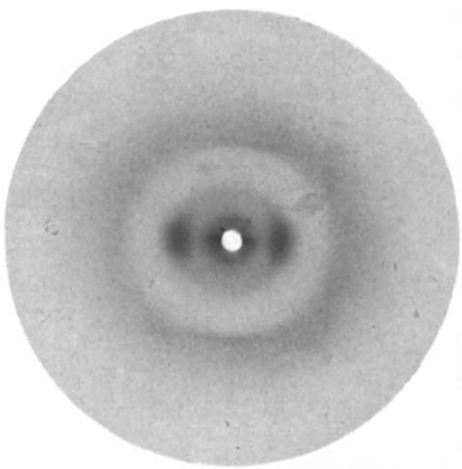

Fig. 9. Calamus sheath of goose quill

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pattern explains the similar low extensibility of a piece of the feather, just as the $\alpha \rightleftharpoons \beta$ transformation explains the long-range reversible extensions obtainable with mammalian hairs. Further consideration of the feather keratin pattern and its relation to corpuscular proteins has been made by ASTBURY and LOMAX[21].

In the present work it has been established that fundamentally the same pattern (e.g. Fig. 13 B) is given by the barbules, barbs, rachis, calamus and feather medulla. All these structures are produced from the 'intermediate cells'; the inner layers of the feather sheath which have a similar origin likewise give the feather keratin pattern. We conclude from these studies that feather keratin is synthesised in the products of the intermediate celles, the α-keratin structure being essentially absent. On the other hand, α-keratin is formed in the products of the stratum cylindricum, in the outer sheath of the growing feather, and in the calamus sheath. In these α-keratin structures the distinct meridional reflection at 23 A suggests the possible presence of a small amount also of the feather keratin structure. It is not known whether this possible feather keratin is confined to a few adhering cells of the stratum intermedium or is generally distributed in α-keratin producing cells.

In Fig. 10 a diagrammatic representation of the growing feather calamus shows parts of α-keratin and feather keratin structure shaded differently. (Studies of the follicle wall sheath have shown that it possesses a well defined α-type structure). In the collar parts which are presumed to give rise to the two kinds of keratin are likewise shaded. In histological studies of feather growth there is probably some uncertainty as to the exact definition of an intermediate cell. DAVIES[16] gives no reason for distinguishing cylindrical cells from intermediate cells other than that of shape, which he admits is not always a satisfactory criterion. STRONG[17] tells us that except for the weaker staining and the blunt basal ends of the cylindrical cells they are not otherwise distinguishable from the intermediate cells.

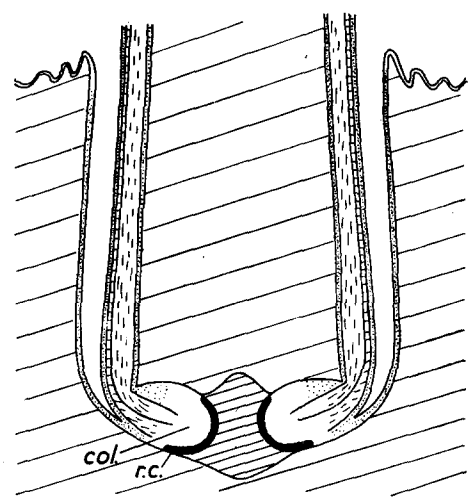

Fig. 10. Diagram of growing feather calamus; – – – – feather keratin; . . . . α-keratin; col. = collar; r.c. = regeneration cells

LILLIE and WANG[19] describe special regeneration cells which are totipotent as regards feather production. They give rise to a mass of cells in the collar, the more centrally placed being intermediate cells, while those on the pulp side are cylindrical layer cells and those on the follicle wall side are presumptive sheath cells (stratum corneum) and follicle wall cells. In part Fig. 10 is based on the diagrams of LILLIE and WANG. It seems probable that from the division of the totipotent regeneration cells, two different kinds of cell are produced, namely, α-keratin producing cells which occupy the more peripheral parts of the collar and feather-keratin producing cells which occupy the more central parts of the collar. It is conceivable that different factors for protein synthesis could be segregated in the stratum intermedium on the one hand and in the outermost layers of the stratum corneum and in the stratum cylindricum on the other, the factors being present together

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in the regeneration cells. Further work is required to define satisfactorily the relationship of the various cells in the collar region.

V. SCALES, CLAWS AND BEAK OF BIRDS

The X-ray diffraction patterns of these structures show the feather keratin diagram but are unsuitable for detailed study owing to the imperfect orientation. We are anxious to decide the point whether these structures contain α-keratin besides feather keratin. In the beak there are regions of a predominantly α-type structure. These lie at the proximal regions adjoining the epidermis of the head and extend for some distance on to the outer surface of the beak. They are of softer consistency than the beak proper. The softer internal surface of the beak lining the roof of the mouth similarly has a well defined α-type structure. Some diffraction patterns of samples of the bird's beak have suggested that the α-type and feather-type patterns occur together. We cannot conclude from this that the two fibrous proteins occur together in the same cell. Where defined cell layers can be separated in the beak it is clear that one or the other pattern is wholly predominant. More information on the molecular structure of claws and beak may be obtained by studying the keratin layers developed from the terminal matrix and basal matrix as defined by LE GROS CLARK[22].

TABLE I

MERIDIONAL AND EQUATORIAL SPACINGS OF VARIOUS FEATHER-TYPE KERATINS

Snake scale (outer)			Feather (calamus, rachis)			Varanus claw		
Meridional								
d.obs.	Int.	Order of 92.8 A	d.obs.	Int.	Order of 94.6 A	d.obs.	Int.	Order of 98.5 A
23.2	s	4	23.6	s	4	24.7	ms	4
14.5*	m	6	11.9	w	8	12.36	s	8
9.23	s	10	10.45	w	9	8.23	w	12
6.18	m	15	6.30	s	15	6.51	m	15
						6.18	w	16
5.52	m	17	5.53	w	17	5.73	w	17
5.1	m	18	4.98	s	19	5.16	m	19
4.63	wm	20	4.45	m	21	4.61	m	21
						4.11	m	24
			3.54	w	27	3.86	w	26
3.1	w	30	3.29	w	29	3.51	w	28
2.99	w	31	3.08	m	31	3.24	m	31
			2.94	w	32	3.08	wm	32
			2.74	w	35			
Equatorial								
10.7	m		34	s		29.8	s	
9.3	m		17.6	w		15	w	
4.6	m		11.3	m		9.8	m	
			8.8	m		4.6	m	
s = strong; m = medium; w = weak			5.8	w				
			4.5	s				

* non-axial

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VI. COMPARISON OF REPTILIAN AND AVIAN KERATIN

In feather keratin BEAR[23] has found a fundamental period of 94.6 A, and Table I gives the various meridional spacings recorded by ASTBURY and BELL[24] and by BEAR[23]. The data given by the snake scale are less precise but the observed spacings fit in well with a slightly smaller fundamental period of 92.8 A. In the snake scale, therefore, the keratin is possibly in an even more contracted form than in feather. Another principal difference is the apparent enhancement of the 18th order (5.1 A); this reflection is absent in feather while the 19th order is enhanced. The enhancement of the 18th order in the snake scale may be an intrinsic feature of its feather keratin type or it may arise from the presence of an α-type structure — either α-type protein alone, or a combination between α-type protein and atomic groupings distributed at the 18th order levels along the feather-type chain.

The meridional spacings of the more perfect pattern obtained from the Varanus claw (Fig. 12) give a fundamental period of 98.5 A. In the keratins of both feather calamus and Varanus claw we have the same odd orders 15, 17, 19, 21, with similar relative intensities which indicate the close relationship between the two structures, but a point of difference is that in the Varanus claw there is also the sequence of related even orders, 4, 8, 12, 16, 24 and 32. Particularly noticeable in Fig. 12 is the rather greater intensity of the 12.36 A reflection and the lesser intensity of the 24.7 A reflection; these are the reverse of what is seen in feather keratin where the 8th order is very much weaker than the 4th order. As regards the equatorial reflections much more detail is shown by the feather keratin. In the Varanus claw a smaller equatorial period (29.8 A) is associated with a larger meridional period (24.7 A).

A number of X-ray photographs of feather calamus and Varanus claw were taken side by side on the same film using the sector holder described by ASTBURY and WOODS (1933). In this way the meridional spacings can be accurately compared, and those of the Varanus claw appeared the larger by 3–4 %, thus agreeing with the measurements of Table 1. Care was taken to relax both specimens in water so that the spacings correspond to equilibrium conditions and not to a possible extended state[20]. A similar comparison is given in Figs. 13 A and B, the two photographs being taken separately and placed side by side after cutting. The feather sample B was treated with 0.2 M mercuric acetate in 0.1 N acetic acid for 3 days at room temperature. It was washed in 0.1 N acetic acid and in water then dried in air. This particular photograph is chosen for the enhancement of the meridional spots at 11.9 A. It is also noteworthy for the apparent change of intensity of the 34 A equatorial reflection and certain of the associated row line reflections. Apart from these intensity changes the meridional spacings do not differ from those listed in Table I. The combination of mercuric acetate with hair keratin has been studied by SPEAKMAN and co-workers[25]. The present studies indicate directions in which heavy atoms may be used to explore the structure and reactivity of fibrous proteins.

VII. MAMMALIAN EPIDERMAL STRUCTURES

The structure of the mammalian epidermis has been examined by X-rays and reported on already[2]. The only well defined protein pattern is that of the α-keratin type. A wide range of structures, viz., hair, spines, scales, horn, hoof, claws and whalebone similarly show the α-keratin pattern in varying degrees of perfection. In the hair follicle

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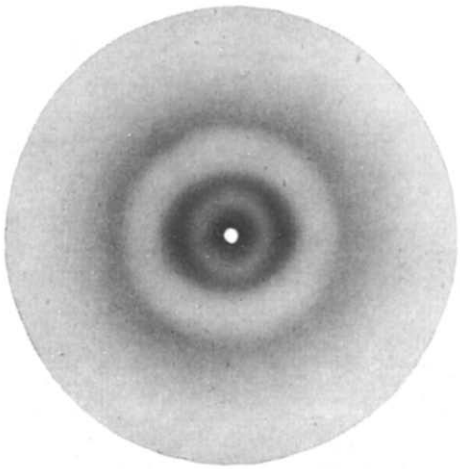


Fig. 11. Porcupine quill medulla. Beam parallel to surface of flattened layers plane of which is vertical.

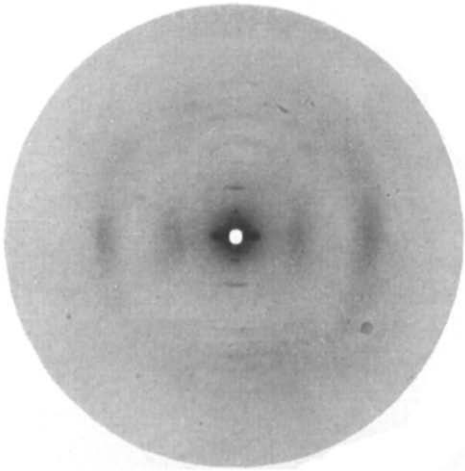
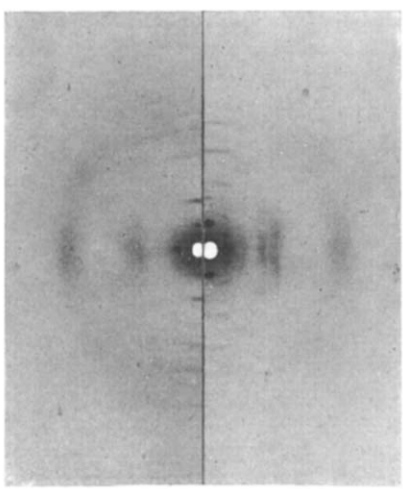


Fig. 12. Varanus claw tip



A B

Fig. 13. Comparison photographs of A, Varanus claw; and B, feather calamus

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system the root sheaths have been shown to have an α-type structure in the case of porcupine quills. Exceptions to the general occurrence of only the defined α-pattern are the hair cuticle and the hair medulla. A number of samples of cuticle scraped from porcupine quills have been examined but no well defined and characterisable pattern was obtained. Some problems connected with this cell layer have been reviewed elsewhere[26].

The medulla has been examined of the porcupine quills of *Erethizon dorsatum*. In the natural condition these cell residues are richly impregnated with lipoid materials. The pulp-like medulla can be readily dissected free from cortex and the lipoids can be extracted by soxhletting in benzene. The fat-free cell residues when lightly pressed give a pattern of the β-keratin type with only a trace of the α-keratin structure (Fig. 11). This β-pattern is oriented after the manner of the cross β-form previously described[2]. In the present case, however, the effect is due to the sidechains being oriented approximately perpendicular to the thin laminae of the cell residues, as is found after the heavy pressing of horn and hair[27] or myosin[9]. When films of medulla residues are stretched they give rise to the parallel β-configuration.

The medulla is of interest in that it corresponds to hair bulb cells in which synthesis of keratin is more or less absent. This accounts for the relative absence of the α-pattern and of cystine[28]. The medulla is largely composed of basic cell substance which, if keratin had been synthesised, would have formed an ordinary cortical cell. In the highly hydrated hair-forming cells around the papilla of the follicle most of the volume is occupied by the nucleus. In the formed hair, where the substance is little hydrated, only a small volume is occupied by the nucleus. The growth of cytoplasmic substance is very large during the formation of the cortical cell. Making the assumption that presumptive medulla cells divide at about the same rate as presumptive cortical cells, it may be estimated from BARRITT and KING's figures[28] that the nucleus and basic cytoplasm comprise about one-tenth of the whole cortical cell. A more satisfactory figure could be obtained by direct measurements of the volume of dry presumptive cortical cells and dry fully formed cortical cells.

VIII. DISCUSSION

In Vertebrates the characteristic structural fibrous protein of the epidermis and its cellular products is of the α-type. In cyclostomes, teleosts, amphibia and mammals the absence of the α-type or the presence of another type is apparently rare. In Reptiles and Birds on the other hand, in addition to the α-type structure there constantly occurs another fibrous protein, described as the feather keratin structure because it was first recognised in parts of the feather system. The close relationship between the molecular structure of the characteristic keratins in Reptiles and Birds gives, at the molecular level, a definition of their affinity. The presence of the α-type of fibrous protein in the epidermis of all vertebrates is likewise a mark of the affinity of all these creatures, while intrinsic variations in the detail of structure of these α-proteins may be regarded as changes that have occurred in the evolution of a molecular species.

The principal features governing the distribution of α- and feather keratin type proteins are best illustrated by the studies on the feather follicle system. In the feather system the intermediate cells produce a great quantity of feather keratin and apparently no α-keratin, while the outermost layers of the stratum corneum and the stratum cylin-

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dricum produce a large quantity of α-keratin and none or only a little of the feather keratin type. The epidermal cells of birds are capable of synthesising two different kinds of fibrous proteins and these kinds tend to be restricted to different cells.

In the snake scale the 'feather' and α-type proteins appear to be produced in series, as if the cells first produced from the stratum germinativum synthesised mainly feather keratin while those produced later synthesised mainly the α-type protein. However, the growth of the scale may be rather similar to the growth of the feather, and we can postulate that the scale germ divides into the equivalent of 'intermediate cells' and 'cylindrical cells' which further increase in number to produce the compact horny layer and the loose horny layer, respectively. This assumes that factors for the synthesis of α- and feather-keratin are segregated in cylindrical and intermediate cells, respectively. Then if the intermediate cells fail to divide secondarily we obtain a predominantly α-keratin structure, and by failure of cylindrical cells to divide secondarily we could reach a predominantly feather keratin structure. Somewhat different views may be taken. We may suppose that all the cells are fundamentally the same, the kind of intracellular protein produced depending on factors operating at the particular sites.

The question arises as to what happens if a given cell synthesises both types of keratin. The very poorly defined diffraction patterns of the epidermis from the main dorsal areas of birds may represent a condition where feather and α-keratin are being produced together in the same cell with interaction of the molecular chains or mixed growth of fibrous molecules; and the less well defined patterns from the crocodile claw and scales and other structures could represent a similar condition. While certain kinds of interaction or mixed growth may lead to an irregular pattern, regular kinds of interaction may also be possible. There are good reasons for the view that there are cells in which α-keratin production is suppressed and feather keratin is produced, and other cells in which the converse holds. It is not known whether the two proteins are ever produced in a cell in more or less equal amounts.

In certain regions of the bird epidermis such as between the toes and on the gripping under-surface of the feet, the cell layers are thickened and the α-type pattern is strongly developed. This unusual feature for bird epidermis seems to be associated with the strength and high elasticity of the structure in these regions. The thinner epidermis from the neck shows again the development of the α-type pattern. Among reptiles the α-structure is predominant in the 'between scale' epidermis of snakes and in the 'between scale' epidermis on the digits of the crocodile Gavialis. Thus in birds and reptiles where the two chief fibrous proteins are (a) the feather-type keratin of little elasticity and (b) the α-type protein of high elasticity, the latter type forms the main structure of the areas of the epidermis where high elasticity is clearly required. There are, however, other cell layers in which the α-type structure is strongly developed, viz., follicle wall, feather sheath and stratum cylindricum, but which apparently do not need to be particularly elastic.

Fibrous proteins of the feather keratin type might be widely distributed in all kinds of body cells, as are the α-type proteins [2]; but from the results of the present studies on vertebrate epidermal cells, it is only in reptile and bird structures that the feather keratin type occupies the position of the principal intracellular fibrous protein.

SUMMARY

1. The chief structural protein of the vertebrate epidermis, as observed in X-ray diffraction studies, is of the α-keratin type.

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2. Only in Amniota are lipoid constituents visible in the diffraction patterns of the epidermis: an exception is the parakeratotic epidermis from the upper lip of the cow. These lipoids seem to be associated with the keratohyalin – eleidin system.

3. In Reptiles and Birds fibrous molecules of the feather keratin type are predominant in the hard keratin structures. Detailed studies of the feather follicle system show that the stratum intermedium synthesises feather keratin and apparently no α-keratin, while the outermost layers of the 'stratum corneum' and the stratum cylindricum synthesise mainly the α-keratin type.

4. Reptilian and Avian keratins have many features in common but differ in detail.

5. In regions of the epidermis of reptiles and birds where strength and flexibility are required the α-protein structure is strongly predominant.

RÉSUMÉ

1. La principale protéine de structure de l'épiderme des vertébrés d'après les résultats obtenus par la diffraction des rayons X, appartient au type α-kératine.

2. Le spectre de diffraction de l'épiderme met en évidence des constituants lipoïdiques uniquement dans l'amnios. Seul, fait exception l'épiderme parakératique de la lèvre supérieure de la vache. Ses lipoides semblent être associés au système kérato-hyaline-éléidine.

3. Chez les reptiles et les oiseaux, les molécules du type kératine des plumes prédominent dans la structure de la kératine solide. Des observations détaillées du système du follicule des plumes montre que le "stratum intermedium" synthétise la kératine des plumes et non pas l'α-kératine, alors que les couches externes du "stratum corneum" et du "stratum cylindricum" synthétisent surtout le type α-kératine.

4. Les kératines des reptiles et des oiseaux ont beaucoup de points communs dans leur structure, mais diffèrent dans les détails.

5. Dans les régions de l'épiderme des reptiles et des oiseaux, qui nécessitent de la force et de la flexibilité, c'est la structure α-protéine qui prédomine.

ZUSAMMENFASSUNG

1. Das hauptsächliche Struktureiweiss der Wirbeltierepidermis ist, wie bei Röntgenstrahldiffraktionsuntersuchungen beobachtet wurde, vom α-Keratintyp.

2. Nur bei Amniota sind Lipoidbestandteile in den Beugungsgittern der Epidermis sichtbar; eine Ausnahme bietet die parakeratotische Epidermis der Oberlippe des Rinds. Diese Lipoide scheinen mit dem Keratohyalin-Eleidinsystem assoziiert zu sein.

3. Bei Reptillien und Vögeln sind Fasermoleküle des Federkeratintypes bei den harten Keratinstrukturen vorherrschend. Detaillierte Untersuchungen des Federfollikelsystems zeigen, dass das stratum intermedium Federkeratin und offensichtlich kein α-Keratin synthetisiert, während die äussersten Schichten des "stratum corneum" und das stratum cylindricum hauptsächlich den α-Keratintyp synthetisieren.

4. Reptilien — und Vögelkeratine haben viele Eigenschaften gemeinsam, unterscheiden sich aber in Details.

5. In den Gebieten der Reptil- und Vogelepidermis, wo Stärke und Beugsamkeit benötigt werden, herrscht die α-Eiweissstruktur stark vor.

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