THE EPIDERMIS

STEPHEN ROTHMAN

THE EPIDERMIS

EDITED BY

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Dedication

This book is dedicated to Stephen Rothman, who directly or indirectly sired much of what is new in modern dermatology.

Those of us who have known Dr. Stephen Rothman are uniquely privileged. He was a great teacher, scientist, and physician, an ornament to the human race and a patron of mankind. He zealously guarded everyone's freedom in science, and championed and encouraged the young. He respected authority, but never revered it. His keen intellect, encyclopedic knowledge and great wisdom gave him unrivaled critical judgment. His criticisms of the faults of others, however, were always tempered by his great heart. As a speaker he was eloquent and witty. As a teacher, he had no equal; he coaxed and cajoled his pupils to capture their attention and engage their genius. A matchless raconteur, with a delectable sense of humor, he enlivened even the most savagely serious biochemical discussions with sprightly, appropriate anecdotes. As a scientist, he had a voracious appetite and was competent in morphology, physiology, biochemistry, and clinical sciences. He had matchless acumen.

To be with Stephen Rothman was always a memorable experience. No word can convey his vibrant personality and dynamism. At ease in scientific matters, medicine, the letters and the arts, he was also an accomplished pianist. He performed his beloved Mozart, Chopin, Schubert, and Schumann with the adroitness and understanding of a professional musician. The many of us who have had the joy of knowing him say, "Thank you, Stephen, for enriching our lives." The world of science may well say, "Thank you, Dr. Rothman, for the milestones which you erected in science and in medicine."

In the annals of dermatology, this is the era of Rothman.

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Preface

This symposium, the first of a contemplated series on the biology of the skin, was motivated by the growing needs of those active in the areas of dermatologic research to familiarize themselves with the rapidly accumulating knowledge from many and varied disciplines. The desire for this type of symposium was expressed in 1960 in two separate governmental advisory committees, the General Medical Study Section, Division of Research Grants, U.S. Public Health Service, and the Committee on the Cutaneous System of the National Research Council. Members of these groups met informally in 1961, expressed their mutual views and invited the Dermatology Training Committee of the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service, to join them in planning such a symposium. It was decided that the symposium should not duplicate any other conference media in dermatology, and that it should present a program dealing with a specific fundamental aspect of the biology of the skin. The intention of the symposium was to explore fully the discoveries from many disciplines utilizing the talents of those scientists, domestic and foreign, who were recognized authorities. In order to foster discussions it was necessary to have a closed meeting in an attractive and remote area, conducive to and organized for scientists and scientific interchange. It was decided that the first symposium should explore in detail the fundamental aspects of the epidermis and the still poorly understood process of keratinization. The Division of Dermatology, University Extension and the School of Medicine of the University of California at Los Angeles agreed to sponsor the conference and offered the University's Residential Conference Center at Lake Arrowhead for the meeting place. The proceedings of this symposium are published herein. This volume is a source book of basic dermatologic thought and information. More than a book of dermatology, this volume makes a singular contribution to our knowledge of keratinization. The symposium and the published proceedings were made possible by Grant No. AM-06747-01 and 02 from the Public Health Service (National Institutes of Health).

It is evident that, even with this massive amount of data, opinions differ on the precise mechanisms of keratinization. In spite of this, however, much progress has been made. Never before has so much information been gathered on this subject.

W. MONTAGNA

WALTER C. LOBITZ, JR.

June, 1964

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CHAPTER I

Keratinization in Historical Perspective

*Stephen Rothman**

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I. INTRODUCTION

It is true that I was the first to write (in collaboration with Schaaf) a comprehensive review of the chemistry of keratinization (Rothman and Schaaf, 1929) and since this was thirty-five years ago, I am a kind of a veteran in the field. It may even be true that there were a few good points in that ancient review, such as for instance, the statement that the resistance of keratins to proteolytic enzymes and to acid and alkaline hydrolysis must be based on the formation of stabilizing bonds between polypeptide chains because the "keratin peptones" are as vulnerable to hydrolysis as any other "peptones." But in those days the disulfide bridges were not yet known to exist—not to speak of hydrogen bonds—and my speculation on the nature of these stabilizing bonds could only be rather amateurish. I speculated whether the solidification might come about by a loss of water from between the polypeptide chains, or by some mild acidification of the protein, so that a denaturation occurs and so on. In retrospect, this chapter now appears so naive as to disqualify its author forever as an expert in keratinization.

In an introductory chapter on keratinization one must consider that the problem has many facets. Among others, anatomists, zoologists, em-

* Dr. Rothman died on August 31, 1963.

[1] Operated by the University of Chicago for the United States Atomic Energy Commission.

bryologists, tissue culturists, geneticists, electron microscopists, physiologists, biochemists, dermatologists, endocrinologists, wool textile manufacturers, and even furriers are interested in varying aspects of keratinization. For this talk, I must confine myself to just a few facets, and this is not easy because all of them are close to my heart. I have, therefore, selected three topics which fascinate me: the history of the keratohyalin granules, the role of lipids in the orderly keratinization of the epidermis, and the desquamation process.

II. KERATOHYALIN GRANULES

No matter which aspect of keratinization is studied, from the historical point of view one has to go back into the 19th century. Perhaps the younger readers believe that the world started with the atomic model of Bohr, and have never heard of that century. But it did exist and it was a great century, even if a slow one!

In those days there were no typewriters, no telephones, no telegraph, no automobiles, and no airplanes. The advent of the steam locomotive with a top speed of 30 kilometers per hour was regarded as risky for human health. Nevertheless, it seems that this very lack of time-saving devices gave men plenty of time for everything, particularly for conversation, for contemplation, and for detailed and precise observation.

It was in that serene, peace-loving atmosphere of wax candles and horse carriages that the existence of granules in the granular layer was first observed and recorded by Auffhammer (1869), who wrote: "If one prepares a fresh section through the palma manus which previously was hardened in alcohol, and if one places the section for a short time in dilute acetic acid (1:400), stains it diffusely with carmin and decolorizes it with water containing a few drops of NH_3 so that the carmin loosens, one observes below the stratum lucidum or, more correctly, below the horny layer (because these two strata are so interwoven that one cannot notice a borderline) 1 to 3 rows of clearly *granulated* cells which at this site have already taken up a spindle shape and which are clearly delineated against the stratum lucidum, the elements of the latter being not or only weakly stained, and not showing any trace of granulation . . . ." A long sentence but a good observation!

A few years later the granules were also recognized by Langerhans (1873) who stated that a granular layer ("Körnerschicht") occurs constantly everywhere in the epidermis (Fig. 1). Waldeyer (1882) christened these granules with the now familar name of "keratohyalin." He stated that they resembled v. Recklinghausen's hyalin but also emphasized that they were

not identical with that material, and assumed that they must have something to do with keratinization; therefore, he chose the name "keratohyalin."

I believe this completes the history of keratohyalin granules, because this is about all we know about them even today. And if somebody contradicts me, and calls to my attention the fact that electron microscopists have found that the granules are conglomerates of tonofibrils, I must reply that this, too, is a 19th century discovery: Kromayer (1897), who was then a young dermatologist in Halle, claimed the same thing, and his

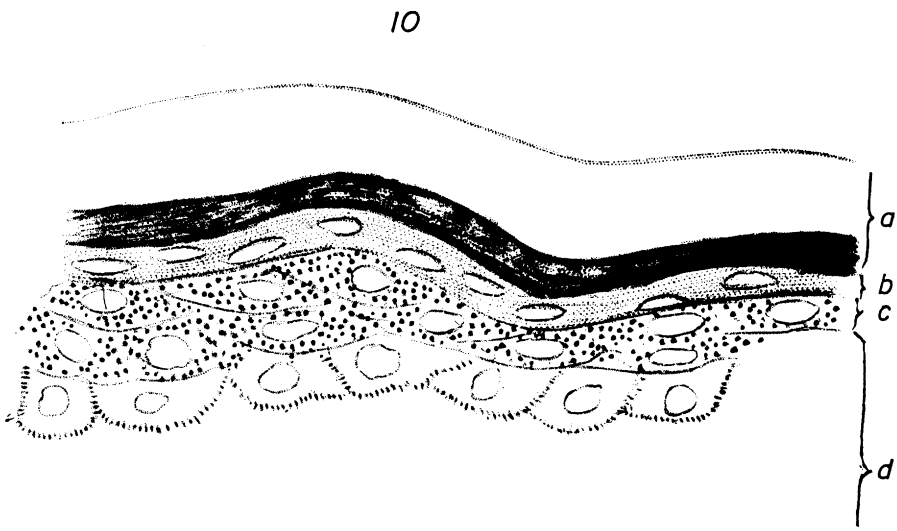

FIG. 1. Section through the epidermis of a 2½-month-old infant, 1100: 1. a = stratum corneum, b = stratum of Oehl, and c = upper part of rete (d). [Reproduced from Langerhans (1873). Hand-drawing.]

evidence was at least as good as is that of the electron microscopists.[2] The nice keratohyalin granules seen in the light microscope (Fig. 1) have become ugly big black blotches under the electron microscope, but all that we still know about them is that they are neither hyalin nor keratin, and, therefore, we call them keratohyalin granules.

We should not, however, leave the story of keratohyalin granules without mentioning two towering figures in the early history of keratinization: L. Ranvier and P. G. Unna. Ranvier (1879), who first described "eleidin,"

[2] Originally, Kromayer (1890) claimed that the intracellular fibrils "decompose" to form keratohyalin. He later (1897) modified this view but insisted that the tonofibrils contribute to the formation of keratohyalin granules.

and wrote a great deal about this "oily liquid," decided after the publication of Waldeyer that Waldeyer's granules represented granular eleidin (". . . les granulations d'éléidine colorées . . .") (Ranvier, 1884). Unna, as Waldeyer had pointed out, was the only one to take early notice of Auffhammer's observation, to coin the term "stratum granulosum," and to speak of a "traditional layer between cornified and non-cornified epidermis" (Unna, 1876). Immediately after Waldeyer's publication, Unna accepted the name "keratohyalin" (Unna, 1882). As we still shall see, no other person's imagination was spurred as passionately and uninhibitedly by the mysteries of keratinization as was Unna's.

III. THE ROLE OF LIPIDS IN KERATINIZATION

The story of the possible role of lipids in orderly keratinization, just like the story of the keratohyalin granules, does not start with Swanbeck (1959) and his beautiful X-ray diffraction studies, but goes back into the 19th century.

It started in the 1870's with eleidin, as already mentioned, which Ranvier suspected was an essential oil representing a pre-keratin (Ranvier, 1879). In a more sophisticated manner, Liebreich (1890) assumed that the keratinization process is intimately connected with the esterification of cholesterol. He regarded keratohyalin and eleidin as precursors of cholesterol esters. He was strongly supported by diverse authors who, by means of the Liebermann-Burchard reaction, claimed to have demonstrated histochemically the presence of cholesterol or cholesterol esters in the granular and horny layers, but not below (for literature, see Rothman and Schaaf, 1929). The great wizard, Unna (see Unna and Golodetz, 1913), even claimed that protein in the granular cell is converted into glycogen in the infrabasal horny cell, and that glycogen, in turn, is converted into oleic acid in the basal horny cell. Unna thought that this fatty transformation was an important facet of keratinization.

In this respect we have made real progress in the 20th century. We know that keratins are proteins, made up of amino acids just like other proteins, and that fats are fats and lipids are lipids, made of activated 2-carbon fragments. But we still believe that the transformation of soft cellular proteins into the more resistant keratins, and the synthesis of fatty substances at the same time, are closely interwoven events. Why do we think so?

Once more, I must go back into the 19th century to introduce to you a remarkable German chemist, Ernst Schulze, who wanted to oblige both the wool industry and the dermatologists who needed a good ointment base; so he studied wool fat. In a painstaking study, he isolated a hitherto un-

known material from the unsaponifiable fraction of wool fat, a gelatinous flock which could be separated from cholesterol as the benzoic acid ester (Schulze, 1872). He considered this new material to be closely related to cholesterol and called it "isocholesterol" (Schulze, 1875). Almost 60 years later, after much work had been done on isocholesterol all over the world, Windaus and Tschesche (1930) showed that this is not a chemical entity, but a mixture, its main constituents being lanosterol, a C_{30} compound, and as such regarded as a cyclized triterpenoid built from six isoprene units.

Now we may jump again in time, but this time only about two decades, when one nice spring day an issue of the *Journal of Investigative Dermatology* came out with a communication by Sobel (1949) in New York which reported that human skin surface fat contains squalene. As I heard later, Victor Wheatley made the same discovery simultaneously and independently in London.

For some reason or other, I became really excited over this finding and ran over across the inner court of the campus at the University of Chicago to the biochemistry department to see the great cholesterol biochemist, Konrad Bloch. I told him that if it was true that the human skin surface contains both squalene and cholesterol, it certainly looked very much as if they were right who in the past had claimed that squalene was an intermediate in cholesterol synthesis. Dr. Bloch looked at me as one looks at a dangerous amateur, and patiently tried to explain that if squalene is cyclized it will form a triterpenoid and not a sterol ring structure. This was just a short time before Ruzicka in Zurich did his beautiful work showing that lanosterol can be regarded as a sterol as well as a triterpenoid compound (Voser *et al.*, 1952).

I was stubborn and went over to see Konrad Bloch day after day, until finally I managed to convince him that the presence of squalene in the skin surface might have something to do with cholesterol synthesis. Soon afterwards, in a brilliant series of isotope experiments, Konrad Bloch demonstrated beyond doubt that—starting with acetic acid—squalene is an intermediary product of cholesterol synthesis (1957). This was just one of the several instances when dermatology fertilized biochemistry.

Now the questions arose, "What sense does it make that the human skin manufactures both the end-product—cholesterol—and an intermediate product—squalene? Once the skin has all the enzymes to carry on the synthesis from acetic acid to cholesterol, why does it happen that in some instances the synthesis gets stuck in the squalene stage?" Having formulated the problem in this form, it occurred to me that, considering the great heterogeneity of skin tissue, it is possible that some skin cells have the complete enzyme sequence necessary for the manufacture of cholesterol, and

others do not. There was quite a bit of evidence, going back to Salkowski (1910a, b), that epidermal cells manufacture cholesterol with great ease, and from this I conceived the idea that, in contrast, perhaps the human sebaceous gland cells do not have the complete enzyme system, and so in these glands the synthesis gets stuck in the squalene stage.

That the source of squalene is indeed the sebaceous gland was then proved to be true by Nicolaides in our laboratory (Nicolaides and Rothman, 1955) as shown in Table I. Different layers of different skin regions of a freshly amputated human arm were incubated with labeled acetic acid, and it was found that only those layers of the skin which contained sebaceous glands manufactured squalene in substantial amounts.

TABLE I
DISTRIBUTION OF RADIOACTIVITY IN THE LIPID CONSTITUENTS OF VARIOUS HUMAN SKIN SPECIMENS AFTER INCUBATION WITH ACETATE-1-C^{14} [a]

Kind of skin	Fatty acids (%)	Sterols (%)	Squalene (%)	Total (%)
Forearm epidermis	66	23	1.1	90
Forearm corium	43	20	32	95
Palmar epidermis plus upper corium	52	40	1.7	94
Palmar lower corium	80	17	2.3	99
Scalp (total skin)	52	2.6	40	94

[a] Reproduced from Nicolaides and Rothman (1955). The data on total skin from scalp are taken from Nicolaides *et al.* (1955).

The idea that some cellular elements in the skin of mammals may have an incomplete set of the enzymes serving cholesterol synthesis proved to be fruitful, and had ever broadening consequences. We suddenly were able to interpret the presence of different compounds as intermediary products of cholesterol synthesis in the skin of mammals. These products are shown in Fig. 2.

Squalene occurs in human skin surface fat, identified by Sobel (1949); lanosterol and dihydrolanosterol, identified in wool fat by Windaus and Tschesche (1930) and by Ruzicka *et al.* (1944), respectively; lathosterol or Δ^7-cholestenol in rodent skin, identified by Idler and Baumann (1952); and finally 7-dehydrocholesterol, the precursor of vitamin D in human epidermis, identified by Reinertson and Wheatley (1959) in our laboratory.

My associates, Lorincz and Wheatley, then had the idea that if all these compounds occur because some of the skin cells, particularly sebaceous cells,

have enzyme systems, which are incomplete to different degrees (for instance, human sebaceous glands carry the synthesis only to squalene, sheep sebaceous gland cells to lanosterol and dihydrolanosterol) then this series of compounds may present a *pathway of cholesterol synthesis in the skin*—a pathway which is quite substantially different from that established for the liver by

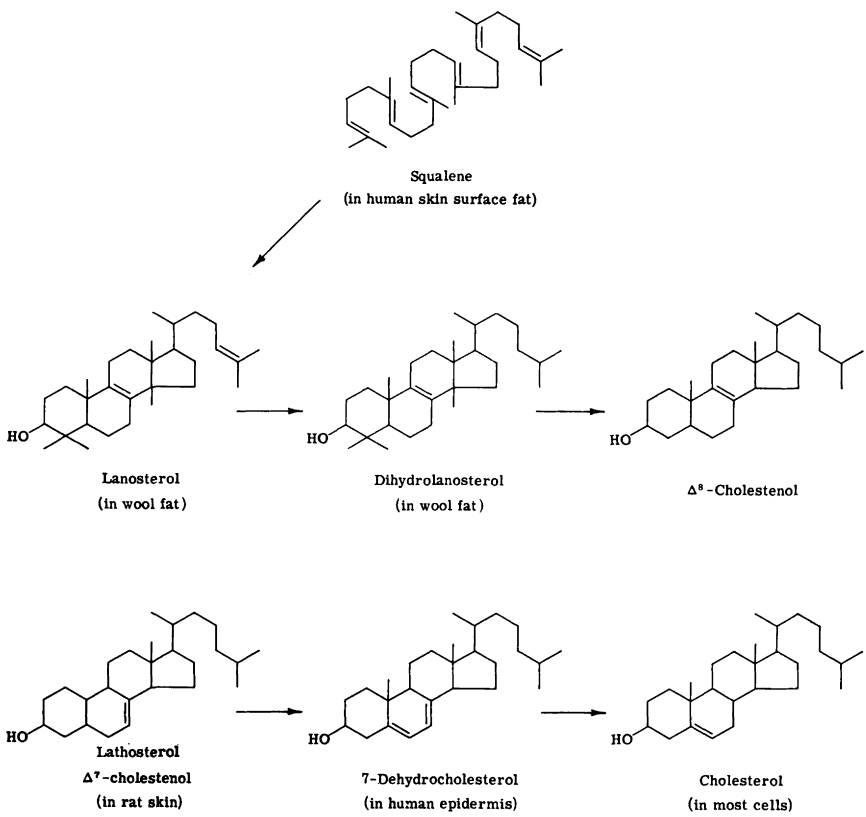

FIG. 2. Intermediary products of cholesterol synthesis in skin and skin excretions. [Reproduced from Rothman (1959).]

Bloch (1957) and others. The main difference is this: In the postulated skin pathway, as described by Wheatley (1959) and by myself (Rothman, 1959, 1960), the double bond in the side chain between C-24 and C-25 is saturated early in the lanosterol stage, while in the liver pathway the saturation of this double bond is the very last step in the conversion from desmosterol to cholesterol, as shown in Fig. 3.

In 1958 it became clear that if our postulated pathway works at all, the classical pathway must work too, because Stokes *et al.* (1958) showed that desmosterol *is* present in substantial amounts in rat skin. Soon afterwards, Kandutsch and Russell (1960a) demonstrated that in a transplantable tumor of the preputial gland (modified sebaceous gland) of the mouse, our theoretical pathway does work. They demonstrated the presence of 24,25-dihydrolanosterol, Δ^7-cholestenol, 7-dehydrocholesterol (Kandutsch and Russell (1960b), and also, the 4-methyl derivative of Δ^8-cholestenol, which we theoretically postulated without finding it anywhere (Kandutsch and Russell, 1960a).

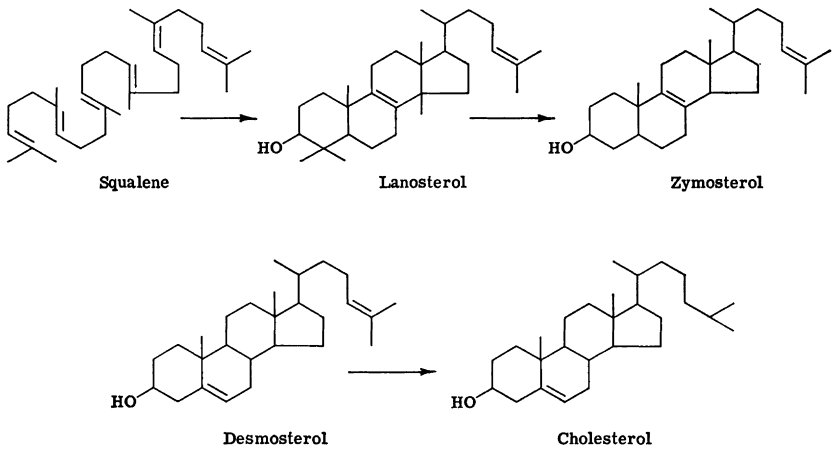

FIG. 3. Regular pathway of cholesterol synthesis in liver.

Since these publications, we call this pathway the Kandutsch-Russell pathway, and I hope we shall hear more about it from Dr. Kandutsch in this conference.

Last year Wilson (1963) in Texas showed that this pathway operates also in the *normal skin*, and in *normal preputial glands* of rats. This has been a happy conclusion to all of our enthusiastic speculation. The University of Chicago had the ideas but the dirty work was done by others.

What does all this have to do with keratinization? The answer can be found in the work of Swanbeck (1959) who made it highly probable that Liebreich in the 1880's was right in saying that the orderly formation of an epidermal horny layer requires an orderly arrangement of lipids. Swanbeck (1959) found that the protein fibril of the horny layer from normal skin (which has a diameter of 250 Å) must be surrounded—to remain normal—

by a 80-Å thick lipid layer. Furthermore, Swanbeck and Thyresson (1962) have shown that in pathological keratinization this lipid layer behaves abnormally.

Recently a dangerous poison, called MER-29 or triparanol, was synthesized which has the remarkable property of blocking the reduction of the 24-25 double bond of desmosterol, thus preventing its conversion to cholesterol. Wilson (1963) has shown that in the Kandutsch-Russell pathway

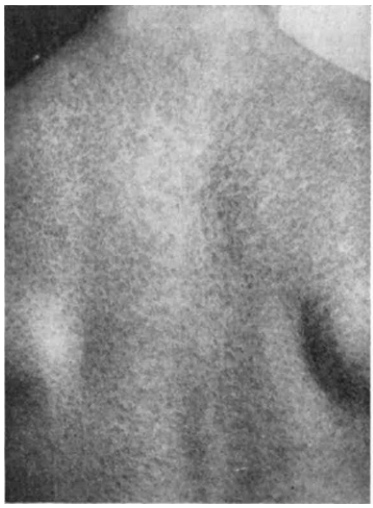

FIG. 4. Ichthyosiform eruption after triparanol administration. [Reproduced from Winkelmann *et al.* (1963).]

FIG. 5. Hair loss, change in hair texture and color after triparanol administration. [Reproduced from Perry *et al.* (1962).]

MER-29 hinders the same saturation of the double bond in the side chain also in the conversion of lanosterol to dihydrolanosterol.

A great propaganda campaign was launched for MER-29, with the claim that it kept down cholesterol synthesis by blocking it in the desmosterol stage, thereby lowering the cholesterol level of the blood, and thus is a marvelous prophylaxis against arteriosclerosis.

The result was the development of a severe ichthyosiform condition, almost identical with the picture of ichthyosis vulgaris (Winkelmann *et al.*, 1963) (Fig. 4), and also severe interference with keratinization of hair (Perry *et al.*, 1962) (Fig. 5).

I believe we do not need more dramatic evidence to demonstrate the intimate connection of keratinization and lipid synthesis than these pictures.

IV. DESQUAMATION

In the last few years, Bullough (1962) has presented impressive evidence that there is a regulatory mechanism controlling the desquamative process in mammals. He showed that under normal circumstances there is a perfect equilibrium in mammalian epidermis whereby proliferation of epidermal cells and desquamation of horny cells maintain a balance based on a sensitive feedback mechanism. This equilibrium may be shifted by hormonal influences. Both proliferation of epidermal cells and shedding of horny cells are accelerated by thyroid hormone and, conversely, retarded by adrenal cortical steroids. The balance of cell gain and cell loss, however, which takes care that the thickness of the epidermis remains constant, is primarily under the control of a tissue-specific local hormone which *inhibits mitosis*. This fine regulatory mechanism determines whether a newly formed cell in the basal layer will divide mitotically or will differentiate into a horny cell which will eventually be shed as a single cell. Thus, Bullough's mitosis-inhibitor acts as a promoter of differentiation. In Bullough's concept it will depend on the production of this water-soluble, heat-labile material in the epidermis, which types of messenger-RNA will be produced by the deoxyribonucleic acid of the chromosome; those which lead to proteins necessary for mitosis, or those which manufacture the usual proteins leading to differentiation and keratinization. When the concentration of the hormone is low, the cells produce the proteins promoting mitosis; when it is high, the cells differentiate.

It might be interesting to look at this regulatory mechanism from the comparative-anatomical point of view.

Zoologists tell us that phylogenetically keratinization came about when the vertebrates ventured to adapt themselves to life on land. In the larval form of Amphibia a tegument is found which is rather similar to that of fishes and has cilia on the surface. In the adult form, however, the Amphibia develop a kind of keratinizing epidermis. The epidermis of reptiles develops an impressive horny layer. As far as shedding goes, mammals and birds have very similar epidermal desquamation processes, but not the Amphibia and reptiles (Biedermann, 1926).

The periodical shedding of the complete horny layer in one piece, or at least in large lamellae all over the body surface, has been observed in snakes and lizards by all zoo-visitors, and it is obvious that this type of shedding must have a different rhythm and different feedback mechanism from that in birds and mammals.

In this peculiar rhythm the basal layer differentiates in the usual manner until it reaches a certain number of cell layers and a compact horny

layer. This is followed by a complete cessation of mitosis *and* of differentiation; it is like the resting phase in the hair cycle. Suddenly, a burst of activity is resumed, and a new generation of differentiating cells is formed so that we now have two horny layers, the old on top of the new, and the old comes off more or less in one piece. For a while, the new horny layer, which suddenly comes into contact with the outer world, remains somewhat vulnerable, but in the subsequent rest period it hardens fairly rapidly. As shown in Fig. 6,

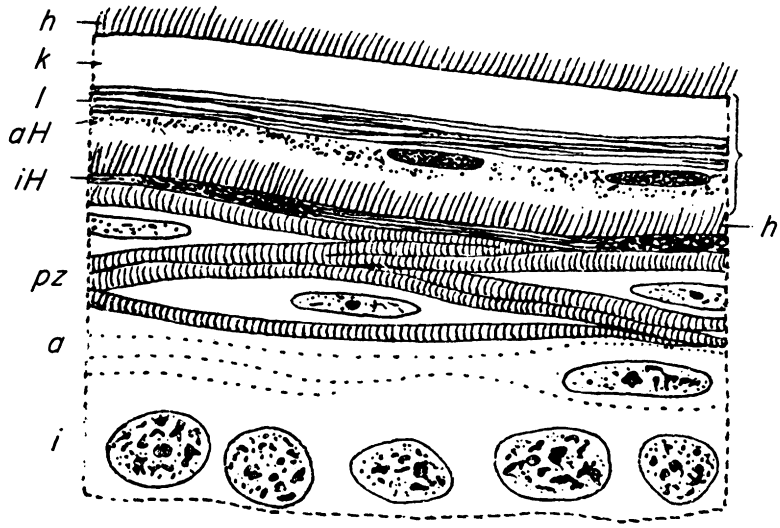

FIG. 6. Section through the epidermis of a young *Tarentola mauritanica* as it appears shortly before shedding. Schematic drawing by W. J. Schmidt. [Reproduced from Biedermann (1926).] i = inner Malpighian layer, a = outer Malpighian layer, pz = flattened cells with incipient keratinization, iH = inner shedding cells, l = loose horny layer, k = compact horny layer, h′ = cuticular hairs of the young epidermal generation, h = cuticular hairs of the old epidermal generation.

there is a remarkable deviation in the structure of the horny layer from what we are accustomed to in mammals: we have *first* a loose horny layer and on top of it a compact horny layer. I wonder if, from this more clumsy mechanism in reptiles, Dr. Bullough could not find out more about his feedback mechanism by studying reptilian skin; if not a boa constrictor, at least a lizard.

While Bullough's work has given us a good idea on the mechanism of the shedding of the horny layer, we still know very little about the intimate nature of the shedding mechanism.

It is certain now that there is a *cementing* substance between the com-

pletely cornified cells of the mammalian epidermis, and it is obvious that some change must occur in this cement when single cells are shed from the surface layer as is the case in normal—insensible—desquamation. But what happens to the cement? Does it shrink because of dehydration or does chemical decomposition of Flesch's mucolipoprotein (Flesch, 1962) or whatever it is, take place? I believe that this is an important question, with clinical implications, because of the so-called superkeratinization phenomenon (Rothman, 1954).

Human skin may protect itself against repeated insults from external injurious agents, such as friction, pressure, ultraviolet rays, thermal and chemical burns, by forming a thicker and harder horny layer than before.

The tentative conclusion was drawn that this reaction is based on a hindrance of shedding. The injurious stimulation brings about a modification in the differentiation process of the Malpighian cells so that they now produce a more compact horny layer with more completely cornified keratin, or a more solidified cement so that the epidermal horny layer now becomes similar to hairs and nails which do not peel off. This kind of subtle change in differentiation may be not only a useful defense reaction, but may also represent the incipient phase of precancerous states, as is the case with some keratoses and leukoplakias. Thus, it appears important to make increased efforts toward a better understanding of the shedding process in mammalian epidermis.

I need not discuss all of the other problems of keratinization, which will be dealt with in the rest of this book; all of the other problems, I am sure, will be as intriguing as the examples I have chosen.

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