Biochemistry of the Skin: 50 Years in *The Journal of Investigative Dermatology*

Lowell A. Goldsmith, M.D.

This article reviews part of the history of biochemistry in this journal. I have looked into the past in an attempt to appreciate the difficulty of the scientific and laboratory problems that were being approached. Investigators are products of the ideas, theories, hypotheses, and technologies of their time; they certainly appreciate many of the limitations of their techniques and theories. The written record of science is a highly selected and formalized version of what really happened (e.g., we have no idea which papers were modified, rejected, or sent to other journals), and only begins to describe the actual analytical and synthetic processes in the investigator's mind.

The journal was my primary source. I have concentrated on subjects with which I have worked for two decades and have some perspective. I have been selective; every relevant article could not be included. Articles from the first 25 years of the journals are emphasized because an additional 25 years allowed further perspective on that research.

Investigators today know that on the hundredth anniversary of our society the science of today will fall under analysis and scrutiny as does the science of the past. Our failure to appreciate the complexity of some of the subjects we study will be obvious from that new perspective. This discussion aims to celebrate our beginnings, to determine our progress, to demonstrate the difficulty of some of the problems that were being addressed, and to give encouragement to those who will be investigating biochemistry problems in the skin in the decades ahead.

THE BEGINNINGS: THE FIRST DECADE

In the first editorial in the journal [1] contributions from and material of interest to, the immunologist, allergist, biochemist, etc., were solicited. Early editorial boards had an advisory editorial board including editors on biological chemistry and physics.

The first article in the journal by Fritz Schaaf (translated by Rudolf L. Baer) was a classical, nutritional, biochemical study: the experimental production of xanthomas in laboratory animals [2]. Rabbits were fed a diet with additional lanolin and the nape of their neck was fixed tightly in a clamp. Other animals received daily injections of colin-HCl into the posterior left flank. The experiments continued for almost 1 year and free and total cholesterol, phosphatide-P, total fatty acids, and total cholesterol were determined. Lipid was deposited in the clamped site and the injection site. The conclusions from these experiments relate to the loss of liver regulation of fat-emulsifying agents with the tendency for fats to fall out of emulsion in the blood. There was extensive discussion, relatively little data, and techniques and methods sections that were very brief; quite different from the journal of today.

Lipid metabolism was a continuing interest in the journal: the clinical effect of lipocaic (a pancreatic extract) on psoriasis [3] and the effect of lecithin feeding on the lipid metabolism in psoriatics were studied [4]. Xanthomas have been of long-term interest, extending throughout the entire history of the journal [5–7].

Nutrition Nutrition, especially vitamins, were a major interest in American biochemistry, and because many of the nutritional deficiencies have cutaneous signs it is understandable that the journal had a significant number of articles related to this subject. Sullivan, from the Department of Dermatology at Johns Hopkins University, was a significant contributor to the nutritional literature. He extensively described the skin lesions of B6 deficiency in the rat, which was also called rat acrodynia because of similarities to the human disorder. The scaling on the dorsal of the paws, edema of the paws, facial and submental dermatitis, and priapism were illustrated and extensive histopathology was presented [8] by Sullivan and Nicholls. Gross reported [9] that unsaturated fatty acids in wheat germ oil could delay but not prevent the signs of B6 deficiency. It was known that unsaturated palmitic acid could not be used for the production of linoleic acid from published deuterium experiments. Gangrene and spontaneous amputation of the digits was reported in B6 deficient rats [10]. Riboflavin deficiency [11] and the effects of combined deficiencies of the B vitamins were reported [12] as was the precipitation of pantothenic acid deficiency by chronic zinc chloride poisoning [13].

Skin lesions from patients with pellagra and from rats with experimental pellagra were studied and had hyperplasia and plugging of the sebaceous glands in people and atrophy of the sebaceous glands in rats [14].

Epidermal Proteins Epidermal proteins were to become of major interest in investigative dermatology during the second and subsequent decades of the journal, but there was only one publication on keratins in the first decade. The stability of keratins to alkali [15] was studied. The experiments were described in detail and measured cystine content of nail clippings after alkali exposure to determine if alkali exposure could be an explanation for clinically observed damaged nails. An idea that still has validity. The alkali binding of nail was similar to that found in wool. The chemistry is detailed and discussed relative to the current ideas of protein structure.

pH Irvin Blank, whose investigative career has spanned the entire history of this society, and who is honored by the annual Irvin H. Blank forum, published several articles on the pH of the skin. The glass electrode and the ion tube potentiometer were one of the new instruments of the day. General methodology [16], pH in normal skin at various sites [17], pH in childhood [18], and variation in normal females were reported [19]. With smaller electrodes, Draize studied the pH of the skin of common laboratory animals [20], The effect of pH on flora was studied [21,22], as was the acidity of the scalp in fungal infections [23].

University of Rochester School of Medicine and Dentistry, Rochester, New York, U.S.A.

Publication #63 of the Department of Dermatology, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, New York 14642.

Reprint requests to: Lowell A. Goldsmith, M.D., University of Rochester School of Medicine and Dentistry, Department of Dermatology, 601 Elmwood Avenue, P.O. Box 697, Rochester, NY 14642.

0022-202X/89/$03.50

Disease A classical approach to studying the mechanism of disease is through biochemical studies of diseased tissues. The study of the mechanisms of blistering diseases is one of the major themes in investigative dermatology, and, not surprisingly, there were extensive studies of pemphigus during the first decade of the journal. Lever and his co-workers [24] did determinations of the serum electrolytes and protein in pemphigus, including the response of pemphigus to adrenal cortex extract. Thirty-four cases with extensive clinical and chemical detail were described. Continued studies by Lever's group on the electrolyte content and permeability of the erythrocytes were published [25], as well as the electrolyte content of the blister fluid [26].

The Macht-Pels test for pemphigus was described in the journal in 1947 [27]. The test is toxicity induced in lupinus seedlings by serum. If irradiation with x-ray detoxified the serum, the diagnosis of pemphigus was thought to be firmly established. This test, which goes back to 1927, showed a specificity of 90–95% for pemphigus. It should be remembered that bullous pemphigoid was included within pemphigus at this time, so its specificity may have been even higher. Whether the test was responding to the pemphigus antibody has not been restudied to my knowledge.

Pigmentation The biochemistry of pigmentation is a major theme throughout the 50 years of the journal and will be discussed in more detail elsewhere in this issue. The first paper on the biochemistry of pigmentation was by one of the founders of the society, Samuel Peck. Peck and Sobotka [28] fed oral monobenzyl ether of hydroquinone (MBH) to guinea pigs over 5 months. This did not cause depigmentation, although local application to the animals did. Tyrosinase was prepared from potatoes or mealworms and in the presence of MBH there was a decrease in the production of the pink intermediate produced from the tyrosine substrate. There was no quantitation of the results of pigment formation throughout the paper by Peck and Sobotka.

In the next year (1942) the papers by Rothman on pigmentation had a very modern tone and character. In two papers Rothman, as the sole author, described the oxidation of tyrosine by ultraviolet irradiation [29] and then the effect of ascorbic acid on the oxidation of tyrosine by ultraviolet irradiation [30]. A tyrosine solution was irradiated with a cold quartz lamp, while evaporation and pH were controlled. Tyrosine and dopa were estimated in a photoelectric colorimeter. Light was measured in biologic units of threshold erythema doses. Limits of error of the technique were estimated to be around 5%, which was 20–100 times less than the differences reported. Ferrous salts accelerated the formation of dopa.

Ascorbic acid, functioning as a reducing agent, decreased melanin significantly more than a non-reducing control acid [30]. Rothman noted that he was able to decrease the hyperpigmentation of Addison's disease with oral ascorbic acid. In the discussion of his paper, Rothman commented, ''tyrosine in the epidermis behaves like tyrosine in the test tube if irradiated similarly.'' Here Rothman addressed one of the central questions of experimental science, which is the question of whether the *in vitro* experiment has any *in vivo* significance.

In the same year Rothman and Rubin reported on sunburn and photoprotection by p-aminobenzoic acid [31]. There was extensive experimental detail, including action spectra, absorption spectra, and extinction coefficients, and photographs of spectro-photometric analysis of the compounds carried out in the Physics Department at the University of Chicago were included.

Perspective How different was the scientific world in those days? How many basic scientists were ready to approach the problems of diseased or even normal skin? There was little biochemistry of the skin being studied in basic science departments or published in the standard biochemical literature. The mass of excellently trained biochemical researchers that would become productive in the postwar era of research did not yet exist. Training grants, the NIH, and the general acceleration of science as more technology became available for the study of normal and diseased skin, was embryonic. The seeds were there. Rothman from the record of the first decade was a biochemical researcher par excellence, and Lever displayed unbelievable energy and insight in his approach to the problem of blistering disease.

Visions by Early leaders—Post War Predictions and Predicaments At the tenth meeting of the Society for Investigative Dermatology in 1949, Stephen Rothman gave his presidential address [32]. He addressed the basic research in dermatology and the contradictions between the descriptive strengths of the dermatologist and his reluctance to use experimental techniques. The problem of heterogeneity in the skin is addressed as well as the difficulty of transferring from animal models to humans and difficulties in research funding to ''offer sufficient inducement to talented young creative dermatologists with genuine scientific curiosity to stay in research.'' Nothing too new under the sun. Although he does not address the details for progress in research, he sees twelve groups in the United States and three to four groups in Europe with the potential to develop new scientific knowledge.

Cornbleet in his 1953 presidential address [33] predicted useful research endeavors for dermatologic researchers. Polarization optics, synthetic analogues of natural metabolites that may produce biologic antagonism, chelation, and mucopolysacchandes have prominence in his litany. The intermediate metabolism of metabolites with the use of radioisotopes was predicted to play a role in the future of research.

THE SECOND DECADE

During the second decade of the *JID* one saw a journal in which biochemistry had a much larger role than it did in the first decade. Two topics of major interest during that decade are metabolic pathways and the biochemistry of keratinization.

Metabolic Pathways The study of respiration is a major theme in the history of biochemistry. Respiration in turn is related to the understanding of oxidation and combustion, one of the major themes in the history of chemistry. The Warburg apparatus was the high technology tool for the study of metabolism. Walter and Amersbach studied the oxygen consumption in punch biopsies from chest skin [34] and found that skin from older individuals had lower respiration rates than from younger individuals.

In 1948 a long and detailed study on the metabolism of skin and the effect of vesicant agents [35] by Barron, Meyer, and Miller was published in the journal. The studies were relevant to war-time research on mustard gases, which could affect the skin, and were performed from 1942 to 1943. The respirometer was the major research instrument and samples of rat and human skin were studied. There was complete appreciation of the problems related to tissue heterogeneity and attention was taken to remove subcutaneous tissue. The inability to separate dermis from epidermis was lamented. In human fetal skin, oxidative respiration was only slightly increased compared with adult skin, but anaerobic glycolysis was increased 2.5-fold. A similar study in rats varying between birth and 51 d had similar results with a sixfold decrease in oxygen uptake. There was no evidence of carbohydrate synthesis from pyruvate as occurred in kidney and liver, but pyruvate utilization was sixfold higher in fetal skin. The effects of vesicants and inhibitors of glycolysis were studied. Nitrogen mustard was found to inhibit both respiration and glycolysis in both rat and human skin, more in the former. Citrate and alpha-ketoglutarate did not increase respiration. Leibsohn and co-workers [36] did extensive studies on humin skin in disease, showed that the epidermis was the major source of oxygen uptake, and calculated that the epidermis was responsible for 4% of the body's total oxygen consumption.

A logical continuation of tbese studies was the research on intermediary metabolism of carbohydrates in rat skin by Griesmer and Gould [37]. The implications of intermediate metabolism for disease and wound repair were discussed as were methodologic problems related to separation and homogenization and the reference standard for the experimental measurement. The authors asked, ''Whether the soluble protein, DNA, wet weight is the appropriate measure to use to standardize measurements between different samples or different experiments?'' This is still a very common operational problem in dermatologic research. Studies to optimize the mixture in terms of pH and salts were performed, and the ability of various substrates for the citric acid to increase the consumption of oxygen (QO_2) was measured. His optimized system showed stimulation of respiration by succinate, oxaloacetate, alpha-ketoglutarate, malate, and fumarate. Citrate and glutamate did not stimulate. For those substrates that increased respiration, there were presumptive enzymes in the skin; for those that did not, there was presumptive evidence for their absence.

The culmination of the studies in the enzymology in the epidermis was the series of detailed cytochemical studies by Montagna and his co-workers [38–40].

The Structural Proteins of the Epidermis Although keratinocyte proteins were a minor topic of the journal in the first decade, they became a major feature in the second and succeeding decades (Fig 1). Rothman has reviewed some of the early history of keratinization [41–43]. The chemistry of keratins was of economic interest to the wool chemist, and much of the earlier work on keratinization was related to wool chemistry. The annual reviews of biochemistry have been taken as measures of what is new and accepted in the biochemistry of keratins. In 1936 [44] there was a reference to a paper by Block and Vickery which defined keratin as a protein resistant to digestion with trypsin and pepsin, and insoluble in dilute acid, alkali, and organic solvents, which yielded the amino acids in a ratio of 1:4:12 for histidine, lysine, and arginine. Material from the epidermis of the foot or exfoliated dermatitis was studied, and Block found a higher cystine content than in other described keratins. Basic amino acid data was accumulated, and Rimington, a reviewer, suggested the protein model in which there was a central basic nucleus characteristic of any type of biologic protein around which the remaining amino acids were united (the view of Kossel). Hemoglobins were thought to fall into another group of proteins with a different ratio of basic amino acids.

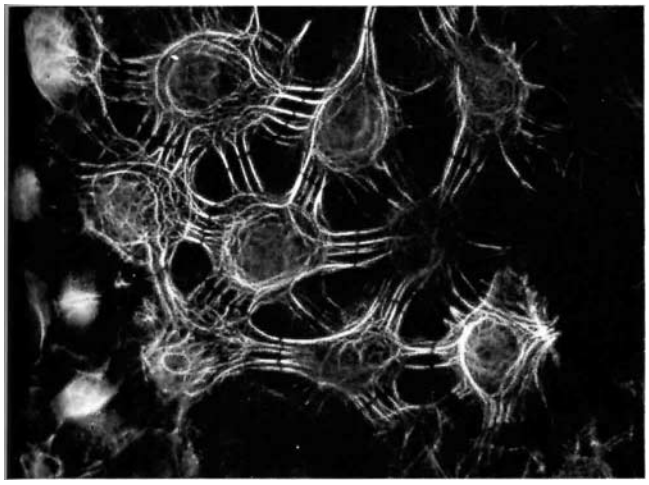


Figure 1. The keratinocyte and its structural proteins are a major interest in cell biology. This photo from the author's laboratory shows a cyotkeratin network in a group of epithelial cells. The desmosomal plate shows up as a *dark line* between portions of adjacent cells. The delicate nature of the cyto-skeleton, its interactions, and its role in a variety of cellular processes contribute to the high levels of interest in these molecules. Stained with monoclonal antibody Kab-1.

X-ray diffraction analysis was to play an important role in the early understanding of the structure of the epidermal keratins. For several decades the keratins would prove to be difficult to analyze because of their insolubility in the usual solvents and their heterogeneity. These problems were to plague protein chemists of the late 1940s, the 1950s, and most of the 1960s. It required denaturing electrophoresis and chromatography in denaturing solvents to begin to answer the questions concerning the nature of the epidermal structural proteins.

Keratins were a natural subject for x-ray diffraction because the wool or hair fiber with its high degree of orientation was able to form very clear x-ray diffraction patterns. This was a time when it was not yet unequivocally accepted that proteins were polypeptide chain systems. The concept that protein might have residues in fixed ratios and limited numbers of molecular weight groups was a common idea mentioned in an article by Astbury [45]. The x ray would prove to be important in the early 1950s, when Rudall showed that epidermal keratins had an alpha-keratin x-ray diffraction pattern [46].

In 1948, Meirowsky and Behr reviewed some aspects of the physiology and pathology of cornification [47]. One of the problems discussed in this paper was the relationship of keratin to keratohyalin; two products that the histologist was able to easily distinguish. Several authorities thought keratohyalin was a prokeratin, and Unna thought it was a by-product of keratin. Using histochermcal staining their studies concluded that keratohyalin was a prokeratin and not a by-product of keratins.

We now understand the matter more clearly. Over these four decades we have begun to catalog the macromolecules of the epidermis and understand their function and physiology. We know keratins and keratohyalin are proteins derived from individual genes. All data must be interpreted within the theories and the dogma of the time. It is interesting and adds a bit of intrigue and uncertainty to the understanding of the epidermis to know that there is at least one common antigenic determinant between epidermal keratins and keratohyalin. Several years would follow before more papers on epidermal proteins would be in the journal, but these would become part of a continuous and growing sea of information on the epidermal structural proteins and the control of their formation.

In addition to the increased emphasis on the structural proteins, the application of electron microscopy to the study of the process of keratinization was beginning. In 1952 there were two papers on the electron microscopy in normal human skin. There was much concern about technical artifacts in the preparation of tissue for electron microscopy. Laden, Erickson, and Armen [48] concluded that intercellular bridges were tubes of cytoplasm connecting the cytoplasm of adjoining cells; thus the cells of the epidermis were a syncitum. Gray, Blank, and Rake [49] did not think the cells were connected. This discussion has more than historical interest because recent work by Diaz and his colleagues once again has seriously raised the question of predominantly intracellular proteins passing between cells at the intercellular bridges.

In 1952 Rudall published a major review on the proteins of the mammalian epidermis using proteins obtained from the thick, almost hair-free cow snout [46]. Many investigators used this material through the years because of the large amounts of both incompletely and completely keratinized proteins that could be extracted from this source.

Matoltsy, a major contributor to the understanding of keratinization and an awardee of the Rothman medal by the Society for Investigative Dermatology, described the pulverization of the epidermis of human plantar skin, the extraction of soluble proteins in phosphate buffer, and

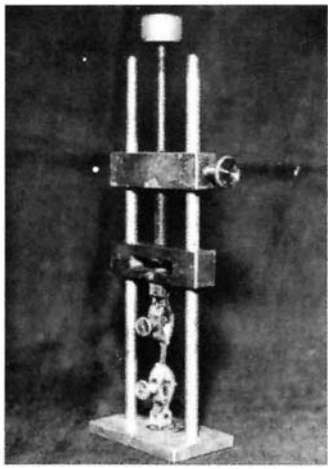


Figure 2. A clamp used to stretch and orientate isolated epidermal fibrous proteins [55].

the separation of this fraction into dialyzable and nondialyzable protein. The non-dialyzable components were called soluble epidermal keratins [50]. These soluble fractions were 21% of the dry weight of the starting callus. The residue from this extraction was solubilized in alkaline solutions with a pH of 12 and sodium sulfide or thioglycolic acid. This was called the ''epidermal kerateine derivative.'' A residue with wrinkled and thick cell membranes thought to be the highly resistant protective system of the stratum corneum remained. It was not known by the authors whether the proteins soluble in neutral buffer, which were characterized by their mobility in free and continuous zone electrophoresis, were precursors or degradation products of the keratins. Similar studies were performed on human abdominal skin [51]. Much of the next 30 years of epidermal research was to be spent on the characterizing of proteins in these various components of the epidermis. The degree of heterogeneity in these preparations could only be imagined by the initial researchers.

The cell envelope demonstrated in these studies was shown to consist of crosslinked proteins and their component proteins, the transglutaminases, which can crosslink these proteins. The regulation of this cross-linking was to become an important topic in the 1970s and 1980s [52].

Carruthers and co-workers [53] detailed protein preparation from the cow snout and from mouse skin. Proteins were fractionated into fibrous and non-fibrous fractions and extensive electrophoretic mobility studies were performed. The effects of detergent (sodium lauryl sulfate) were studied with significant changes in protein mobility at high detergent concentrations. The proteins extracted by detergent appeared to be unrelated to the proteins extracted by urea on the basis of mobility, but there was still not appreciation of the problems of heterogeneity and aggregation of the epidermal proteins. Matoltsy and Herbst studied the urea extractable proteins of human abdominal skin and characterized them by paper electrophoresis [54]. Their 6 M urea extraction was 28% of the material and not dissimilar in amount to what was extracted with phosphate buffer or acetate buffer from the epidermis. The phosphate content and the absorption spectra of the extracts were similar. The authors suggested that the urea extract was not a specific extractant for epidermal proteins. It was recognized that many components of the epidermal proteins could be migrating as a single component in the electric field.

Roe studied a fibrous protein precursor from human epidermis [55]. Although a fibrous protein could not be extracted from callus or abdominal skin with urea when skin was extracted with 75% lithium bromide protein, which could be stretched and oriented and could

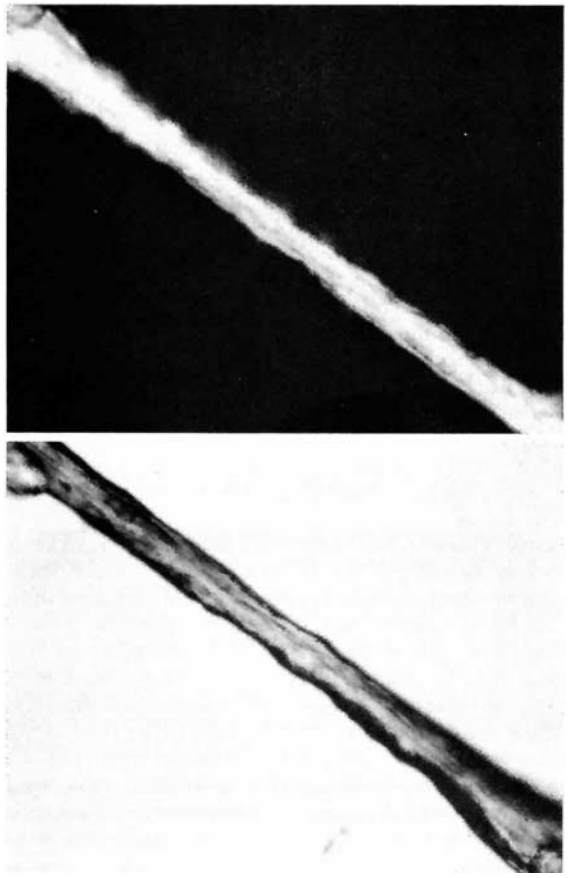


Figure 3. Using the clamp (Fig 2), an orientated film of keratinized material was formed *(top).* When examined with polarized light *(bottom),* the orientation of the material could be demonstrated by birefringence [55].

show birefringence, an alpha x-ray diffraction pattern could be obtained (Figs 2 and 3). With heating the protein contracted, and it was concluded that the substance was probably a prekeratin and the relationship of this protein to the keratins was studied by analyzing the histologic changes that occurred after extraction of skin with 75% LiBr [56].

The changes of keratinization in embryonic skin were studied at different stages of embryonic skin in the chicken embryo by Matoltsy [57], who demonstrated an increase of sulfhydryl rich proteins toward the end of the embryonic period of development.

Over the next two and one-half decades there would be techniques for solubilizing and characterizing the epidermal proteins into classes separated by molecular weight and isoelectric points; forming of monoclonal antibodies to individual keratins; cloning of keratin genes; and questions about the controlling elements of these genes in development and in acquired and neoplastic diseases.

Keratohyalin Rothman [43] and Meirowsky [47] discussed the history of keratohyalin. Early electron microscopic studies published in the journal [58] suggested that the keratohyalin granules had the general appearance of keratin fibers and appeared to be directly transforming into the fibrous proteins. Much more detailed biochemistry and immunoelectronmicroscopy would be required to settle some of these issues.

Over the 25 years, there was significant progress in the understanding of keratohyalin. Keratohyalin was the major product labeled with radioactive histidine [59] (Fig 4). This labeling was increased in the granular layer of the skin and occurred rapidly after injection of the

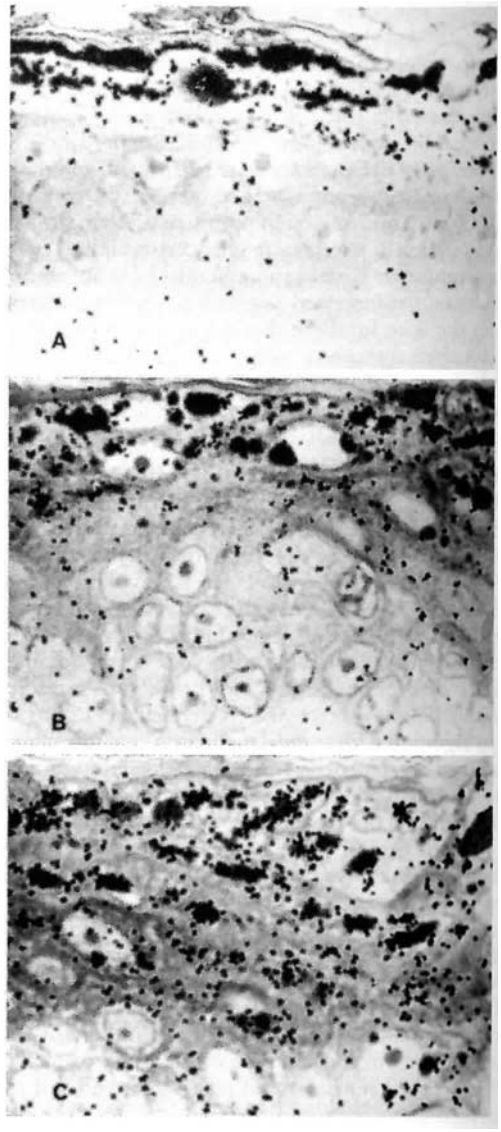


Figure 4. Results from an experiment in which radioactive histidine labeling was used to label newborn rat epidermis [59]. When skin samples were biopsied after different time intervals there was a higher concentration of labeling over the keratohyalin granules. Histidine labeling of keratohyalin granules still remains an important marker of the histidine containing protein of the keratohyalin granules filaggrin. A is 15 minutes after labeling, B 3 hours and C 6 hours.

histidine. Histidine labeling in psoriasis and other diseases with deficient keratohyalin granules was decreased [60,61]. Filaggrin was shown to be a major component of keratohyalin, filaggrin and profilaggrin were characterized, and their genes were cloned. With these data, it has now become possible to ask precise questions about the changes that may be occurring in ichthyosis vulgaris, a disease in which there is a deficiency of filaggrin [62] (Fig 5).

Free Amino Acids in the Stratum Corneum Water soluble components comprise 21% of callus [50]. Flesch and Esoda found 7% water extractable components in psoriatic scale compared with 24% in post-ultraviolet light (UVL) scaling [63]. They also noted decreased water binding ability of the psoriatic scale. Cornish and coworkers [64] found no difference between the free amino acid composition of psoriatic scale and post-UVL scale. They noted that the amino acid composition differed from that of keratin. The high free amino acid composition of guinea pig epidermis was noted by Tabachnick [65]. Rothman [42] had postulated that the free amino acids in the epidermis are of cellular origin and related to protein hydrolysis.

The high urocanic acids in stratum corneum were thought to relate to histidine deamination, but the source of the histidine was not known. Over the next 25 years it became clearer that the histidine was released from filaggrin (stratum corneum basic protein, keratohyalin), and that, although urocanic acid was not a major natural sunscreen, it might play a role in the immunosuppression induced by ultraviolet light [66].

Structural Proteins in Psoriasis The study of tissues from those with cutaneous diseases is a major project for research dermatology. The disorders of keratinization, especially psoriasis, have been extensively studied.

Psoriatics and normals were given oral doses of radioactive L-methionine [67]. Skin was scanned directly with a Geiger counter. The counts in psoriatic skin were four to five times higher than those in control skin. The radioactive counts stayed elevated for 7 d at a time when control skin or psoriasis-free skin had no detectable radioactivity. The workers concluded that there was a defect in sulfur-containing compounds in psoriasis. That methionine might be a marker for protein metabolism *per se* was not discussed.

Radioactive glycine incorporation into psoriatics and controls (patients with chronic myelogenous leukemia) were reported in 1962 [68]. The epidermal proteins were defatted, dialyzed to remove free amino acids, and hydrolyzed. The glycine was isolated by chromatography and electrophoresis, weighed, radioactivity quantitated, and its specific activity was determined (Fig 6).

Glycine reached the uppermost layers of the stratum corneum 13–14 d after incorporation in normals and reached its peak at 26–28 d. In psoriasis the peak was 3–4 d, although the specific activity of glycine was lower. In the discussion of these results it was recognized that different proteins in the epidermis had different turnover times.

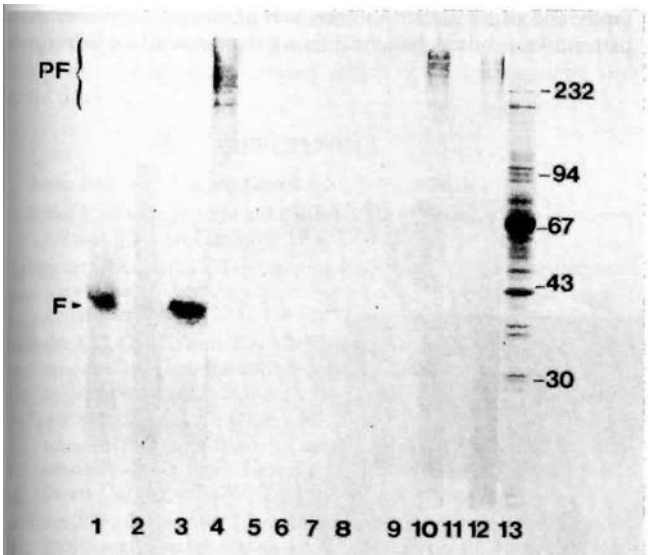
Using another biochemical approach, Rothberg [69] extracted scales from patients with psoriasis and those with ichthyosis (type unspecified). These extracts were digested with trypsin and chymotrypsin and then two-dimensional mapping with paper chromatography and paper electrophoresis was performed. Different peptide patterns were noted, but considering the tremendous heterogeneity of the starting materials they were difficult to interpret. Further studies in the guinea pig by Baden and Freedberg measured the incorporation of amino acids into soluble and insoluble fractions of guinea pig epidermis and separated those proteins by the mobility in starch block [70,71] (Fig 7).

BIOCHEMISTS: RECOGNITION BY THE SOCIETY

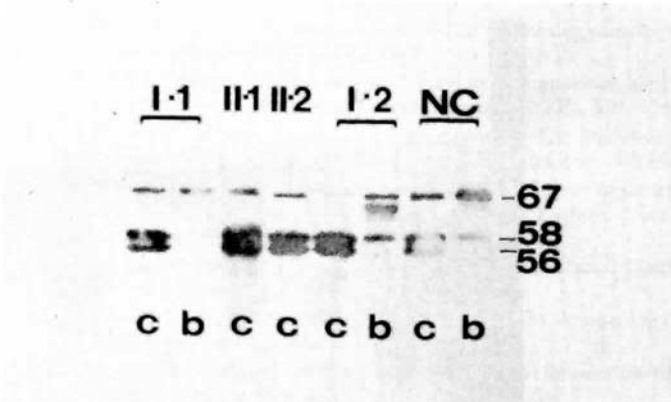
Biochemistry's contributions to the research efforts of the SID have been recognized in several ways. One way has been that several awardees of the Stephen Rothman Award, the society's highest honor, have made major contributions to biochemistry: Thomas B. Fitzpatrick, 1970, the biochemistry of melanization; Aaron B. Lerner, 1971, the biochemistry of pigmentation and the discovery of MSH and melatonin; Eugene Van Scott, 1975, the biochemistry of keratinization; Irvin Blank, 1977, the biology of percutaneous absorption; Isador Bernstein, 1981, the biochemistry of keratohyalin; and A. Gedeon Matoltsy, 1984, the biochemistry of keratinization. Biochemists have been the editors of the journal over the past 15 years: Irwin Freedberg (1972–77), Ruth Freinkel (1977–82), and Howard Baden (1982–87).

THE FUTURE

It is hard to predict the future in all but the most general of terms, Biochemical skills are now disseminated and relatively common

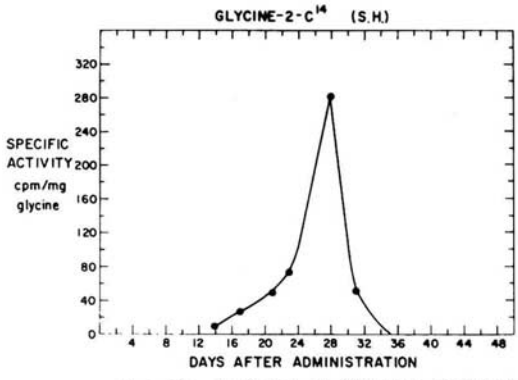


Polyacrylamide gel and immunoblots of profilaggrin and filaggrin from extracts of biopsies and cultured cells of patients with IV. Note that immunoreactive filaggrin (*F*) is decreased in biopsies from A I-1 (*1*) and undetectable in A I-2 (*2*) when compared with normal foreskin (*3*). Likewise, immunoreactive profilaggrin (*PF*) is decreased in extracts of cells cultured from A I-1 (*5*) and undetectable in A II-1, A II-2, A I-2, B I-2, and C III-1 (*6–8,9,11*) when compared with cells cultured from normal control (*4*) or unaffected family members B I-1 (*10*) and C II-1 (*12*). *1–3*, immunoblots of equal protein loadings [20] of extracts from skin biopsies; *4–12*, immunoblots of equal protein loadings [20] of high salt extracts of confluent secondary (Family A) and tertiary (Families B and C) cultured cells; *13*, Coomassie blue-stained gel of an extract of A I-1 (stained polyacrylamide gels of all culture extracts were identical). Numbers to the left are M_r of standards (kD).

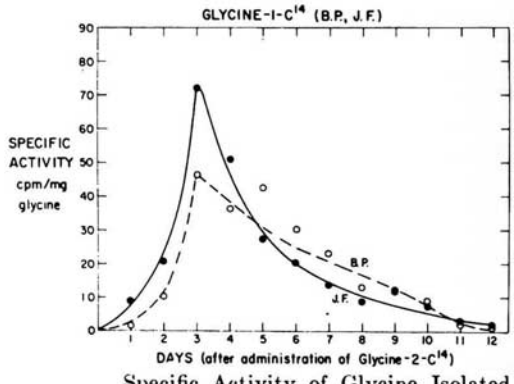


Immunoblots of keratins from extracts of biopsies and cultured cells stained with antikeratin antibody AE3. Confluent tertiary cultures from Family A. Note that the high-molecular-weight, differentiation-specific 67 kD keratin (keratin #1 [25]) and the 58 kD keratin (keratin #5 [25]), expressed by all stratified squamous epithelia [27] are expressed in both skin and cultured skin cells. The 56 kD keratin (#6 [25]) associated with hyperproliferative epithelia [28] is found only in cultured cells. The keratin profiles for affected and control skin biopsies are identical, as are those for cultured cells. *c*, immunoblots of equal protein loadings [20] of extracts from cultured cells; *b*, immunoblots of equal protein loadings [20] of extracts from biopsies. *NC*, tissue from normal control; *I-1, II-1, II-2, and I-2*, tissue from members of Family A. Numbers to the left are M_r of standards (kD).

Figure 5. In the past decade the ability to study disease from both tissue biopsies and cultured epidermis has been a major advance. Using such techniques Mein can be separated by electrophoresis and examined for profilaggrin and filaggrin, shown in the gel on the *left;* and for keratins as shown in the gel on the *right* [62].



Specific Activity of Glycine Isolated from Normal Epidermal Proteins After Administration of 100 μc Glycine-2-C14 (S.H.)



Specific Activity of Glycine Isolated from Proteins of Psoriatic Scale After Administration of 10 μc Glycine-1-C14 (B.P., J.F.)

Figure 6. Early studies administering glycine to a control patient, labeled epidermal proteins began to appear after 14 d and reached peak specific activity after 28 d (left). In the *right figure* two patients with psoriasis have a much more rapid incorporation of glycine into epidermal proteins [68].

throughout the research dermatology community. The lines between molecular biology and biochemistry have already been blurred, and the lines between cell biology and biochemistry will become less distinct with time. Molecular biology will be yielding new information; new probes for the individual molecules of the cell; and information about the control of their synthesis, their movement within the cell, and their degradation. How molecules are arranged in arrays of varying composition and how those arrays interact to produce

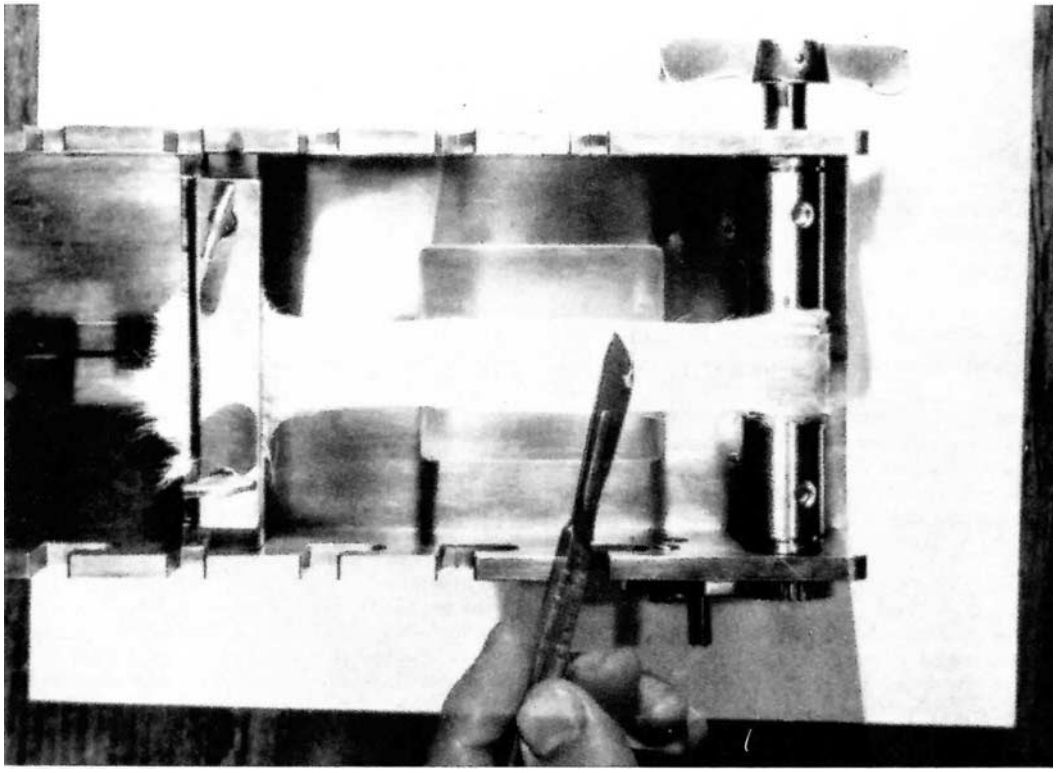


Figure 7. Separation of epidermis from dermis has been a challenging task for research dermatology [70]. A variety of devices have been used to accomplish this task. Even more challenging has been the separation of various epidermal cell populations; such research continues today.

functioning cells and tissues will be elucidated over the next few decades. This further blurring of biochemistry and physiology will yield more understanding of the skin's normal rations and the altered functions which occur with genetic and acquired disease.

REFERENCES

1. Anon: Editorial. J Invest Dermatol 1:5–7, 1938
2. Schaaf F: On the experimental production of xanthomas in laboratory animals. J Invest Dermatol 1:11–30, 1938
3. Stewart CD, Clark DE, Dragstedt LR, Becker SW: The experimental use of lipocaic in the treatment of psoriasis: A preliminary report. J Invest Dermatol 2:219–234, 1939
4. Smith CC, Goldman L, Fox HH: Lecithin feeding in the syndrome of psoriasis. II. Chemical studies of the relationship of lecithin feeding to fat metabolism. J Invest Dermatol 5:321–330, 1942
5. Lever WF, Smith PAJ, Hurley NA: Idiopathic hyperlipemia and primary hypercholesteremic xanthomatosis. III. Effects of intravenously administered heparin on the plasma proteins and lipids. J Invest Dermatol 22:71–87, 1954
6. Lever WF, Lyons ME: Idiopathic hyperlipemia and primary hypercholesteremic xanthomatosis. VIII. Effects of protamine on the electrophoretic and ultracentrifugal changes produced in the serum by heparin. J Invest Dermatol 27:325–334, 1956
7. Hu CH, Ellefson RD, Winkelmann RK: Lipid synthesis in cutaneous xanthoma. J Invest Dermatol 79:80–84, 1982
8. Sullivan M, Nicholls J: The nutritional approach to experimental dermatology. Nutritional dermatoses in the rat. I. Vitamin B6 deficiency. J Invest Dermatol 3:317–335, 1940
9. Gross P: The role of the unsaturated fatty acids in the acrodynia (vitamin B6 deficiency) of the albino rat. J Invest Dermatol 3:505–522, 1940
10. Sullivan M, Nicholls J: Nutritional dermatoses in the rat. III. Gangrene and spontaneous amputation of the digits produced by the combined deficiency of vitamin B6 and the filtrate components. J Invest Dermatol 4:123–133, 1941
11. Sullivan M, Nicholls J. Nutritional dermatoses in the rat. IV. Riboflavin deficiency. J Invest Dermatol 4:181–191, 1941
12. Sullivan M, Nicholls J: The nutritional approach to experimental dermatology. Nutritional dermatoses in the rat. II. Skin changes in rats deficient in the entire vitamin B complex other than thiamin. J Invest Dermatol 3:337–345, 1940
13. Gross P, Harvalik Z, Runne E: Vitamin deficiency syndrome in the albino rat precipitated by chronic zinc chloride poisoning. J Invest Dermatol 4:385–398, 1941
14. Smith SG, Smith DT, Callaway JL: Dysfunction of the sebaceous glands associated with pellagra. J Invest Dermatol 4:23–42, 1941
15. Chiego B, Silver H: The effect of alkalis on the stability of keratins. J Invest Dermatol 5:95–103, 1942
16. Blank IH: Measurement of pH of the skin surface. I. Technique. J Invest Dermatol 2:67–74, 1939
17. Blank IH: Measurement of pH of the skin surface. II. pH of the exposed surfaces of adults with no apparent skin lesions. J Invest Dermatol 2:75–79, 1939
18. Blank IH: Measurement of pH of the skin surface. III. Measurements on the arms of children with no apparent skin lesions. J Invest Dermatol 2:231–234, 1939
19. Blank IH: Measurement of pH of the skin surface. IV. Daily variations for adult females with no apparent skin lesions. J Invest Dermatol 2:235–242, 1939
20. Draize JH: The determination of the pH of the skin of man and common laboratory animals. J Invest Dermatol 5:77–85, 1942

21. Whiccomb FC, Arnold L: The relationship between acid-base equilibrium and endogenous bacterial flora of the skin. J Invest Dermatol 4:317–324, 1941
22. Arnold L: Relationship between certain physico-chemical changes in the cornified layer and the endogenous bacterial flora of the skin. J Invest Dermatol 5:207–223, 1942
23. Herrmann F, Behrendt H, Karp FL: On the acidity of the surface of the scalp and other areas of the skin in children. J Invest Dermatol 7:215–226, 1946
24. Talbott JH, Lever WF, Consolazio WV: Metabolic studies on patients with pemphigus. J Invest Dermatol 3:31–68, 1940
25. Kurnick NB, Lever WF, Talbott JH: Electrolyte content and permeability of the erythrocyte in pemphigus. J Invest Dermatol 4:431–450, 1941
26. Lever WF, Talbott JH: Electrolyte content of blister fluid in pemphigus. J Invest Dermatol 5:303–309, 1942
27. Macht DI, Ostro M: An improved method for diagnosing pemphigus. J Invest Dermatol 8:171–175, 1947
28. Peck SM, Sobotka H: Effect of monobenzyl hydroquinone on oxidase systems *in vivo* and *in vitro*. J Invest Dermatol 4:325–329, 1941
29. Rothman S: *In vitro* studies on pigmentation. I. The oxidation of tyrosine by ulraviolet irradiation. J Invest Dermatol 5:61–67,1942
30. Rothman S: *In vitro* studies on pigmentation. II. Influence of ascorbic acid on oxidation of tyrosine by ultraviolet irradiation. J Invest Dermatol 5:67–75, 1942
31. Rothman S, Rubin J: Sunburn and para-aminobenzoic acid. J Invest Dermatol 5:445–457, 1942
32. Rothman S: Presidential Address: Basic research in dermatology. J Invest Dermatol 13:165–170, 1949
33. Cornbleet T: Dermatology of tomorrow: Some areas for dermatologic research. J Invest Dermatol 21:215–221, 1953
34. Walter EM, Amersbach JC: Correlation of age and respiration of skin from the human female. J Invest Dermatol 10:11–14, 1948
35. Barron ESG, Meyer J, Miller ZB: The metabolism of skin. Effect of vesicant agents. J Invest Dermatol 11:97–118, 1948
36. Leibsohn E, Appel B, Ullrick WC, Tye MJ: Respiration of human skin. Normal values, pathological changes and effect of certain agents on oxygen uptake. J Invest Dermatol 30:1–8, 1958
37. Griesemer RD, Gould E: A method for the study of the intermediary carbohydrate metabolism of epidermis. I. Oxidation of acids of the citric acid cycle. J Invest Dermatol 22:299–315, 1954
38. Montagna W, Chase HB, Lobitz WC: Histology and cytochemistry of human skin. IV. The eccrine sweat glands. J Invest Dermatol 20:415–423,1953
39. Montagna W: Histology and cytochemistry of human skin. VIII. Mitochondria in the sebaceous glands. J Invest Dermatol 25:117–119, 1955
40. Montagna W, Ellis R: Histology and cytochemistry of human skin. XII. Cholinesterases in the hair follicles of the scalp. J Invest Dermatol 29:151–157, 1957
41. Rothman S: Epidermal proteins. In: Rothman S (ed.). The Physiology and Biochemistry of the Skin 1954. Univ of Chicago Press, Chicago, pp 343–365
42. Rothman S: The keratinization process. In: Rothman S (ed.). The Physiology and Biochemistry of the Skin 1954. Univ of Chicago Press, Chicago, pp 366–390
43. Rothman S: Keratinization in historical perspective. In: Montagna W, Lobitz WE, Jr. (eds.). The Epidermis 1964. Academic Press, New York, pp 1–14
44. Rimington C: The chemistry of the proteins and amino acids. Ann Review Biochem 5:117–158, 1936
45. Astbury WT: X-ray studies of the structure of compounds of biological interest. Ann Review Biochem 8:113–132, 1939
46. Rudall KM: The proteins of mammalian epidermis. Adv Protein Chem 7:253–259, 1952
47. Meirowsky E, Behr G: Some aspects of the physiology and pathology of cornification. J Invest Dermatol 10:343–361, 1948
48. Laden EL, Erickson JO, Armen D: Electron microscopic study of epidermal prickle cells. J Invest Dermatol 19:211–215, 1952
49. Gray M, Blank H, Rake G: Electron microscopy of normal human skin. J Invest Dermatol 19:449–457, 1952
50. Matoltsy AG, Balsamo CA: Preliminary and short reports. The components of the cornined epithelium of the human skin. J Invest Dermatol 25:71–74, 1955
51. Matoltsy AG, Herbst FSM: Preliminary and short reports. A study of human epidermal proteins. J Invest Dermatol 26:339–342, 1956
52. Goldsmith LA: The epidermal cell periphery. In: Goldsmith LA (ed.). Biochemistry and Physiology of the Skin 1983. Oxford University Press, New York, pp 184–196
53. Carruthers C, Woernley DL, Baumler A, Kress B: Proteins of mammalian epidermis. J Invest Dermatol 25:89–101, 1955
54. Matoltsy AG, Herbst FSM: Observations on urea-extractable substance of the human abdominal epidermis. J Invest Dermatol 27:263–271, 1956
55. Roe DA: A fibrous keratin precursor from the human epidermis. I. The extraction and physical properties of a fibrous protein found in the human epidermis. J Invest Dermatol 27:1–8, 1956
56. Roe DA: Further studies of a fibrous keratin precursor from the human epidermis. J Invest Dermatol 27:319–324, 1956
57. Matoltsy AG: Keratinization of embryonic skin. J Invest Dermatol 31:343–346, 1958
58. Laden EL, Gethner P, Erickson JO: Preliminary and short report. Electron microscopic study of keratohyalin in the formation of keratin. J Invest Dermatol 28:325–327, 1957
59. Fukuyama K, Epstein WL: Ultrastructural autoradiographic studies of keratohyalin granule formation. J Invest Dermatol 49:595–604, 1967
60. Voorhees JJ, Chakrabarti SG, Bernstein IA: The metabolism of ''histidine-rich'' protein in normal and psoriatic keratinization. J Invest Dermatol 51:344–354, 1968
61. Baden HP, Roth SI, Goldsmith LA, Baden SB, Lee LD: Keratohyalin protein in disorders of keratinization. J Invest Dermatol 62:411–414, 1974
62. Fleckman P, Holbrook KA, Dale BA, Sybert VP: Keratinocytes cultured from subjects with ichthyosis vulgaris are phenotypically abnormal. J Invest Dermatol 88:640–645, 1987
63. Flesch P, Esoda ECJ: Deficient water-binding in pathologic horny layers. J Invest Dermatol 28:5–13, 1957
64. Cornish HH, Block WD, Lea WA: Distribution of lipids and free amino acids in psoriatic scale. J Invest Dermatol 32:43–47,1959
65. Tabachnick J: Studies on the biochemistry of epidermis. I. The fret amino acids, ammonia, urocanic acid and nucleic acid content of normal albino guinea pig epidermis. J Invest Dermatol 32:563–568,1959
66. Ross JA, Howie SEM, Norval M, Maingay J, Simpson TJ: Ultraviolet-irradiated urocanic acid suppresses delayed-type hypersensitivity to herpes simplex virus in mice. J Invest Dermatol 87:630–633,1986
67. Lipnik MJ, Levy SH: Altered L-methionine S^{35} utilization in psoriasis. J Invest Dermatol 32:519–524, 1959
68. Rothberg S, Crounse RG, Lee JL: Glycine-C^{14} incorporation into the proteins of normal stratum corneum and the abnormal stratum corneum of psoriasis. J Invest Dermatol 37:497–505, 1961
69. Rothberg S: The evaluation of keratin fractions in normal and abnormal epidermis. J Invest Dermatol 34:197–206, 1960
70. Freedberg IM, Baden HP: Studies of epidermal protein metabolism. I. Incorporation of amino acids *in vivo*. J Invest Dermatol 39:339–345, 1962
71. Baden HP, Freedberg IM: Studies of epidermal protein metabolism. II. Soluble epidermal proteins. J Invest Dermatol 39:401–408, 1962