SOME NEW KEYS TO COSMETIC CHEMISTRY —1956.

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By PAUL G. I. LAUFFER*

COSMETIC CHEMISTRY is a vast province with the vaguest of boundaries. Its vastness makes impossible a comprehensive review in tabloid form, and necessitates a critical approach whereby only the more significant advances are mentioned. Its vague demarcation justifies the use of some judgment in selecting the areas to be included.

In 1955, the writer attempted, in an article entitled "The Emerging Cosmetic Chemistry"[1]†, to describe and delineate the body of cosmetic chemistry that had taken form in the previous ten years. This is a review of chemical advances made in late 1955 and in 1956 which may be expected to result in improved cosmetic technology. It will follow the thesis put forward in the 1955 article, that the first concern of cosmetic chemists is with knowledge of the structure, composition, and functioning of the skin, and that all exact data and concepts regarding these subjects come within the purview of cosmetic chemistry.

SKIN

A wealth of up-to-date information, with stress upon the anatomical features, was made available by the publication of Montagna's "The Structure and Function of the Skin,"[2] which complements effectively Rothman's earlier "Physiology and Biochemistry of the Skin."[3]

Newer methods of investigation added to our knowledge of the skin surface and its maintenance. Examination by the stripping method[4] revealed that normal adults had from 0·66 to 1·39 million non-nucleated keratin cells per square centimetre on the forearm, and about 50 per cent less on shoulder and thigh; it was estimated that keratin cells make up over 10 per cent of the total cells of human epidermis, and that reported mitotic rates are sufficient to account for the normal loss of surface cells. Polarised light studies[5] of serial sections cut parallel to the surface of human callus showed that beneath the surface grooves keratin is disposed with the long axis of its chain molecules along the grooves; while beneath the ridges, the keratin molecules lie crosswise, and around the sweat ducts they form rings. Blank's earlier demonstration[6] that water content is the major factor influencing flexibility and softness of the outer skin has led to attempts

* House of Tangee.

† Title appeared erroneously as "The Emerging Cosmetic Industry."

to evaluate various cosmetic ingredients in terms of their ability to increase or maintain water content, and has led to the use of the Shore Durometer for assessing skin softness.[7] Another study[8] indicated that: (1) oil-in-water emulsions are more effective in hydrating keratin than water-in-oil; (2) high-viscosity emulsions are superior to those of low viscosity; (3) water-miscible adjuvants increased water loss, even if they increased viscosity; (4) hygroscopic substances did not increase water absorption by dried keratin.

Administration of cortisone or ACTH increased the resistance of skin capillaries in humans or in rabbits; while that of testosterone to females or of progesterone to males decreased the resistance. The capillary resistance in females was increased by estrone in small doses and by progesterone; it was decreased by large doses of estrone.[9]

Polarigraphic measurement of oxygen in human skin[10] disclosed wide changes in oxygen tension with variations in circulation and metabolism. The rate of carbon dioxide elimination from human skin was reported[11] to be increased by 15 to 20 per cent within an hour after drinking tea, indicating increase in skin metabolism under control of the cerebral cortex. Methionine was added[12] to the list of amino-acids previously found on the skin.

Extraction of human epidermis with 6M urea failed to yield a[13,15] protein similar to the "epidermin" similarly extracted[14] from cow snout epidermis. A hydrogen-bond breaker, 75 per cent lithium bromide solution, did, however, extract from human epidermis a fibrous protein different from any previously obtained and postulated to be a keratin precursor.

Rats fed a diet containing only C-8, C-10, and C-12 fatty acid glycerides had fat from skin and subcutaneous tissue with higher saponification number and lower iodine number than similar fat from controls, indicating that the medium-weight fats can be deposited in the skin.[16] Incubation of skin with acetate-1-C^{14} gave results leading to the hypothesis[17] that the main site of sterol synthesis is the keratinising epidermis, while the main site of squalene synthesis is the sebaceous gland.

Electron-microscopic studies[18] have greatly expanded our knowledge of cellular components in the epidermal layers, and have confirmed the existence of tonofibrils crossing cell boundaries. The observations suggest that the tonofilaments are synthesised in the basal layer and transformed in the stratum granulosum into a non-filamentous but densely packed material which is presumably keratin. At this Society's 1956 Seminar, a symposium on keratinisation included reviews[19,20] of the biochemical, physiological, and pathological aspects of keratinisation.

New observations have demonstrated the presence and/or the location of the enzymes cholinesterase[21,22], monoamine oxidase[23], guanine deaminase[24] (in rat skin but not in human skin), and phosphomonoesterase.[25]

Ultraviolet light and, in the presence of photosensitisers, daylight, inhibit the activity of H-transferring enzymes in the skin by oxidising their SH groups.[26] Human skin *in vitro* synthesised phospholipid from inorganic labelled $P^{32}O_4$ salts.[27]

Effects of various substances on the skin or its components were studied. Sodium dodecyl sulphate combined[28] with alpha-keratose of molecular weight 48,000 in two ways: (1) in firm combination, with the positive groups on the protein; (2) in larger amounts held by looser forces. Alkaline shaving soaps kept the skin surface alkaline for as much as four hours after use,[29] but use of a slightly acid after-shaving lotion counteracted the effect. The nerve fibres of the peripheral nervous system of the skin of humans and cats were specifically affected by applications of acetone.[30] Neutral ointments containing 0·1M concentration of salts of Al, Ca, Cr, Fe, Mn, Ni, or Zn, rubbed on dehaired guinea pig flanks daily for ten days, caused slight thickening of the epidermis.[31] A similar application of copper salts thickened the epidermis threefold. At non-toxic concentrations (0·01M or 0·001M) copper doubled the epidermal thickness. The phagocytic activity of endothelial cells was increased[32] by topical application of coal tar, Ti tannate, and Ti salicylate, and to a lesser extent by many other substances; the effects were greater from an ointment containing the drug in an ethanolamine base, than from one with a petrolatum base. Soaking of the skin in solutions of soap or detergent reduced its water-holding capacity slightly more than did soaking in water;[33] detergents did not seem to differ significantly from soaps in this respect. A method for assay of succinic dehydrogenase and cytrochrome oxidase activities in epidermis homogenates was developed,[34] with the hope that changes in enzyme activity might be correlated with functional and structural abnormalities.

Hair

Until recently it was believed that adult epidermis could not create new hair follicles. In 1954, however, new hair follicles and sebaceous glands were shown to be produced in scar tissue of rabbits,[35] and last year the formation of hair follicles from the reconstituted epidermis in abraded areas of the facial skin was observed in five adult humans.[36]

Restricted food intake inhibited hair growth in mice, to degrees dependent on phase of growth in which diet was reduced.[37] The fat content of the skin was found to vary little during the hair growth cycle in mice, although the adipose layer is at least twice as thick during late phases of the cycle, due largely to the bulbs of the hair follicles, which grow into the adipose only in late phases.[38]

Regrowth of hair on shaved skin of rats was greatly accelerated by removal of the pituitary or the adrenals,[39] and this growth was inhibited by injection of hypophyseal adrenocorticotropin, or by chorionic gonadotropins, but not

by hypophyseal growth hormone ; gonadectomy did not alter rate of hair growth in hypophysectomised rats. Cortisone given orally in large doses temporarily restored hair growth in human alopecia, and intradermal injection of hydrocortisone acetate produced local growth of hair in a case of universal alopecia due to financial stress.[40] The uptake of cobalt and nickel by various organs of mice was reported[41] to be correlated with hair colour, more cobalt being absorbed by black mice, more nickel by white mice.

Sweat

The appearance of a new and greatly expanded edition of Kuno's *Human Perspiration*[42] provides an up-to-date compendium on the mechanism of sweating. A review[43] with 221 references on the pharmacology of sweating appeared in 1955. A study[44] of the *p*H of sweat produced by various forms of stimulation disclosed that any changes in *p*H obtained on the skin in response to a stimulus was in the direction of an increase. The *p*H changes observed were attributed primarily to eccrine, not apocrine sweat. Fatigue of the sweat glands was produced by repeated intradermal injections of mecholyl ; sweat production gradually declined until the glands became refractory to further stimulation either by mecholyl or by heat.[49]

The histology of apocrine sweat glands[45] and their innervation[46] was more adequately reported than before, and hormones of wide variety were tested[47] topically and systemically, and found to be without observable effect, although hormonal control is strongly indicated by the fact that apocrine glands start functioning at puberty. Examination of the wax-producing glands of the human ear canal led to the conclusion that they should be classed as apocrine glands.[48]

Shelly's earlier conclusions as to the mechanism of axillary odour production[50] were supported by new studies[51] which showed that apocrine sweat and sebum both remained free of unpleasant odour while sterile ; bacteria of various species were collected from human axillæ, and pure cultures of all but one of the species collected were found to produce bad odours in apocrine sweat ; it was concluded that the benefit of aluminium salts should not be attributed to suppression of eccrine sweating, since they are also antibacterial, and may decrease the bacterial population of the axillæ.

Sebum

Thiamine deficiency produced marked atrophy and loss of lipid in sebaceous glands of mice.[52] Mitochrondria in the cells of the sebaceous glands appeared to play a directive part in the synthesis of sebaceous lipids, but not to undergo direct transformation into lipids.[53] The rate of sebaceous secretion in the forehead in ten elderly persons averaged 31 micrograms per

cm.^{2} in the first 20 minutes after cleansing of the area with ether, and dwindled to 20 micrograms per cm.^{2} in 20 minutes after repeated cleansing over a six-hour period.[54] Human sebum was reported to contain twelve aliphatic alcohols of 14 to 24 carbon atoms, straight and branched, saturated and unsaturated ;[55] in the free acid fraction 31 fatty acids of seven to 18 carbon atoms were detected and estimated by gas-liquid chromatography,[56] including some highly branched and some mono- and di-unsaturated acids.

Cells

Improved methods for locating substances within cells and estimating their concentration include a method for determining dry weight within a cell body[57] which is more practical than the interference method ; and methods for determining four enzymes in single large cell bodies ;[58] the method for malic dehydrogenase requires only 10^{-5} micrograms of dry material, or one two-thousandth the mass of a single cell body.

A summary of factors identified as essential for growth of a mouse fibroblast in tissue culture lists 27 substances.[59] About 70 of the chemical elements are claimed to be plant nutrients in concentrations of 10^{-3} to 10^{-11}, and are postulated to constitute integral elements of the cell, indispensable in the chain of enzymic reactions.[60] A U.S. patent[61] was issued covering the use of metallic complexes as stimulants of cellular respiration, in cosmetics and topical pharmaceuticals.

The action of colchicine in blocking mitosis was found to be inhibited by adrenocortical hormones, which favour mitosis.[62] A substance was isolated from deoxyribonucleic acid preparations which markedly promotes cell division in concentrations as low as 1 microgram per litre ; the promoter was named kinetin, and was shown to be 6-furfurylpurine.[63] Analogues with sidechains of similar size to furfuryl were also active, and showed marked regulatory effects on developmental processes in plants, and on growth in animal tissues.[64] The molecular basis of cell division in yeasts was concluded to be reshaping of the protein comprising the inner cell wall by reduction of the S-S linkages, and protein disulphide reductase was characterised as the "division enzyme."[65] Glutathione was reported to play a metabolic role in mitosis.[66]

On the yeast cell, evidence indicated the presence of at least two species of sites for binding cations, tentatively identified as phosphoryl and carboxyl groups ; these are on the periphery of the cell, isolated from endogenous cations by a permeability barrier.[67] Inside the liver cell, quinacrine was seen to be bound by numerous cell constituents, chiefly in its micellar form.[68]

It has been observed that in the mitochondria of house-fly muscle cells, there are enzymes which dephosphorylate the triphosphates not only of adenosine but also of guanosine, inosine, uridine, and cytidine, and there appeared to be a separate enzyme for each nucleotide ; calcium

appeared to inhibit these enzymes, and magnesium or manganese to activate them.[69]

Mutation was induced at 1,000 times the natural rate by addition of 5-bromouracil and sulphanilamide to virus culture media.[70] Strong evidence has been presented that human cells do not contain 48 chromosomes, as so long believed, but 46.[71]

Protein Biosynthesis

Protein synthesis was observed in isolated cell nuclei from calf thymus, the participation of deoxyribonucleic acid being essential, and the high energy requirements being supplied by intranuclear systems which generate energy-rich triphosphates.[72] A specific surface and available energy were found indispensable for formation and orientation of peptide chains, and high-pressure resynthesis of proteins in the presence of proteolytic enzymes disproved the need of different specific catalysts for each peptide bond.[73] New models and hypotheses were offered to explain the transfer of specificity from the deoxyribonucleic acid of the gene, through the specific ribonucleic acid, to the enzymes being synthesised,[74,75] and additional statistical evidence was offered to support an earlier hypothesis that the 20 amino-acids found in proteins are disposed in the new protein molecule to match the 20 possible triplets of nucleotide residues which can be formed from four different nucleotides, disregarding order.[76] A chemical manufacturer announced the opening of a pilot plant which will be capable of producing all the essential amino acids except threonine.[77]

Protein Structure

The complete amino-acid sequence of alpha-corticotropin was established,[78] as was that of beta-corticotropin ;[79] the latter contains 39 amino acids in its chain, but a 24-unit portion of the chain retains the hormonal activity. A new model was proposed for feather keratin, with 64 amino-acid residues in the 190A unit of pitch, and ten helixes coaxially aggregated by lateral H-bonds.[80] It was shown that a primary valence bond in a protein may have more apparent stability than the corresponding bond in a low molecular weight compound because of support by neighbouring H-bonds, which may account for some observed differences in the behaviour of proteolytic enzymes toward native and denatured protein substrates.[81] A new method was devised for measuring distances between protein sidechains, based on the absorption spectra of their compounds with molecules containing two groups separated by known distances and capable of binding the two protein sidechains.[82]

Melanin, keratin, and collagen formed crystalline substances under mild influences such as ultrasonic waves, hydrogen peroxide, or various diseased

conditions.[83] Denaturation was considered to involve extensive disturbance of weak bonds formed by the side chains, without alteration of many intra-chain bonds.[84] The conditions for the helix-to-random-coil transition involved in denaturation were more narrowly defined, and methods were devised for inducing the controlled degradation of nucleic acid necessary for study of its structure.[85] Ribonucleic acid[86] and deoxyribonucleic acid[87] were synthesised, albeit by enzymatic methods which shed little light on the structure. A theory was advanced to explain elastic mechanisms in fibrous proteins on the basis of melting of their crystalline regions,[88] such melting possibly occurring as the result of an agent which combines more readily with the liquid protein, thus shifting the equilibrium.[89]

Collagen and Elastin

Recent books bearing upon collagen include *Fibrous Proteins and Their Biological Significance*,[90] originating in the Society for Experimental Biology, and *The Chemistry and Reactivity of Collagen*[91] by K. H. Gustavson. Numerous investigators have contributed to the cracking of the tough problem of collagen structure, but a working model acceptable to all parties is still lacking. There seems to be general agreement[92,93] that collagen possesses a three-chain coiled-coil structure with right-handed major and left-handed minor helix,[94,95] with individual chains ending at staggered positions. The chains are most probably of the poly-L-proline type, and may not all have the same amino-acid sequence.[96,97] Acid-stable cross links of at least two varieties are present in varying degree in different collagens.[98] Two typical building-stones of collagen, delta-hydroxylysine and 4-hydroxyproline, have attracted considerable study, since each has 16 possible stereoisomers.[99,100] A study using glycine-C^{14} led to the conclusion that albumins are the predecessors of skin collagens.[101] X-ray studies under small incidence angles indicated that the cross striations of collagen are not results of the protein structure, but are caused by polysaccharides held to the protein chains by forces weaker than covalent bonds.[102] Collagens from different tissues showed varied degrees of resistance to cleavage because of different surface films or coatings.[103] Chlorinated naphthalene, which produced hyperkeratosis in calves, also depressed plasma levels of vitamin A and ascorbic acid suggesting that the pathological changes in skin and connective tissues might be connected with those substances.[104]

The amino-acid composition of elastoidin from the ceratotrichia of a shark justified its inclusion in the collagen group of proteins, but its high tyrosine and cystine content were cited as the possible basis of its peculiar hydrothermal behaviour.[105] Collagen treated with enzymes, acids, or alkalis formed networks of fibres resembling elastin.[106,107] Rats fed a diet containing 50 per cent *Lathyrus odoratus* meal developed aneurysms of the aorta, as a result of general lysis of elastic fibres.[108]

Enzyme Biosynthesis and Structure

A useful review on enzymes as units of biological structure and function was published in 1956 as the result of a symposium held in 1955.[109] In another brief review of induced synthesis of enzymes the enzyme-forming system was visualised as a complex between ribonucleic acid, inducer, and enzyme, with simple components, probably amino acids, being drawn from a metabolic pool for synthesis on the template, which may be ribonucleic acid.[110] Liver cell nucleoli were isolated and found to have properties suggesting that they furnish the templates for synthesis of the enzymes which govern mitosis.[111] Cytochrome C was studied by isolating the portion of the apoenzyme bound to the porphyrin ; two cysteine residues were found to be joined to the porphyrin by sulphide bridges, and a histidine residue next to one of the cysteines was believed to be bound to the iron through its imidazole ring.[112]

Enzyme Mechanism

In a preliminary sketch of the principal features of a theory of catalysis[113] it was pointed out that all catalytic reactions are caused and directed by free valencies, with the catalyst playing a role not unlike that of a free radical. The fatty acid cycle was duplicated, using model compounds containing the structural elements considered important in coenzyme A activity.[121] A possible analogue of enzyme action was seen in the "chain effect" of polysarcosine dimethylamide in accelerating the polymerization of phenylalanine-N-carbonic anhydride ; the accelerating effect was proportional to the number of units in the polysarcosine molecules used.[115]

The transport of electrons over long chains of atoms appears to be involved in enzyme activity, and in macromolecules a distant transfer of ionisation is possible, not by a small displacement of a multitude of elementary charges, but by a large displacement of relatively few charges along the molecules.[116] The electron transport systems of cell mitochondria are conceived to be quasi-conducting continua in which electrons originating in reduced diphosphopyridine nucleotide and succinate are transferred ultimately to molecular oxygen. The various enzymes of this semi-conductor system are linked to one another by bonds which permit resonance interaction throughout the entire structure in a manner not describable by classical kinetics. The many non-hem iron atoms present may act as conducting and structural links between the different oxidation-reduction units of the system.[117]

Progress has been made in determining methods of attachment of enzymes to coenzymes and substrates. Kinetic studies supported the idea that primary amino groups of the old yellow enzyme serve as binding sites for the phosphoric acid residue of flavine mono-nucleotide.[118] It was concluded that two carboxyl groups of glutamic acid combine with NH^2 or NH radicals

of glutamic dehydrogenase protein.[119] The SH group of muscle protein was shown to be essential for the action of acetylcholine, and in addition to the N atom of the latter, the ester group also reacted with active groups of the protein molecule.[120] Methylation of lysozyme did not inactivate it, showing that the groups active in catalysis are not those active in the enzyme-substrate union.[121]

Molecular geometry was studied in connection with the cholinesterase surface, and it was found that in the betaine amino alcohols, those with hydroxyl trans to the carboxyl inhibited the ester, but similar cis compounds showed no inhibition.[122] Evidence was cited that precise orientation of catalytic groups at the point of bond formation and cleavage is critical for enzyme activity, implying that there is no necessity for high molecular weight in the enzyme molecule.[123] In a brief review of mode of action of oxidation enzymes, Theorell[124] pointed out that so far we know the structure of only the most easily accessible one-sixth of the smallest enzyme molecule, cytochrome C.

An antidote for nerve gas was developed as a by-product of basic research on cholinesterase; the antidote, 2-pyridine aldoxime, fits into a specific active spot on the cholinesterase surface, preventing the nerve gas from becoming attached there through its phosphorous-containing group.[125] The enzymatic activity of grain seeds was increased, and the germination and growth processes were stimulated, by ultrasonic treatment of the seeds.[126]

Structure-Activity

The edema producing potency, measured by increase in water content of a part of the conjunctiva of rabbits' eyes, increased with increasing molecular weight in the normal alcohols methyl to amyl, and among the butyl alcohols the potency decreased in passing from primary to secondary to tertiary.[127] A chemical model of drug action using diethylaminoethanol in a two-layer solvent system was selected because of its containing two active groups at the same distance, 5½A, as is typical of a striking number of drugs with a great variety of effects; the tendencies of various amino acids to become bound to this model were measured.[128] The activities of 47 thyroxine analogues were correlated to the abilities of their side chains to release electrons.[129] A study of competitive photohalogenation of cyclohexane and toluene led to the conclusion that to account for the effect of structure on the selective rates of free radical reactions involving C–H bonds it is necessary to consider (1) bond dissociation energies, (2) the extent of bond breaking in the transition state, and (3) polar effects in both reacting molecules.[130]

A review of inclusion compounds suggested division of clathrates into three types: *channel*, like urea clathrates; *cage*, like water molecules;

and *layer lattices*, like montmorillonite.[131] The twisting or stretching of C–C double bonds due to steric hindrance increases the activity of the bound atoms.[132] Cold-working of long chain ethers and ketones increased their dielectric loss, probably by causing dislocations in the crystal lattice.[133] Under extremely high pressures, 50,000 to 120,000 atmospheres, an electronic isomerisation is believed to occur, pushing electrons from higher orbitals into unfilled higher-energy lower orbitals, and under still higher pressures the atoms are postulated to exist in a "universal metallic state."[134]

The complex formation of over 100 systems in various solvents was followed in order to characterise H-bonding behaviour of alcohols and ketones.[135] A new type of H-bond, reported to exist between the single hydrogen and three of the four carbonyls of cobalt carbonyl hydride, may throw new light on catalysis mechanisms.[136] The new technique of nuclear magnetic resonance promises to yield information on fine details of molecular activity which should assist in clearing up many structure-activity problems.[137]

Permeability

The factors governing the permeability of the skin and of cell membranes received considerable study. Hydration of the horny layer of the skin was found to inhibit sweating on palms and soles.[138] Injection of the antidiuretic hormone pitressin reduced water loss both in normal and in hyperkeratotic skin.[139] In a new method for determining water loss through skin, dry air was passed through a vessel affixed to the skin, and the moisture in the emerging air was determined gravimetrically.[140] By this method, it was found that the water permeability of films of fatty acids or of their triglycerides decreases significantly with increasing chain length.[141] In isolated frog skin, sodium influx followed a pattern similar to a Langmuir adsorption isotherm and an equation was derived fitting the data, on the assumption that influx occurs chiefly by means of a sodium complex.[142]

A study of structural features influencing amino acid transfer into cells led to the conclusion that chelation and Shiff base formation are involved in the transfer.[143] The specific sites responsible for concentrating amino acids in the cell were regarded as catalytic ones in a membrane rather than stoichiometric ones inside the cell.[144] A cycle of permeability changes was shown to follow the penetration of T 2 virus into *E. coli* cells.[145]

Protein Binding

In the binding of small molecules to proteins, hydroxylation was found to play an important role in determining whether a protein-bound metal ion can interact further with a small molecule.[146] In hemerythrin, an oxygen-carrying pigment devoid of porphyrin, the larger portion of the iron

appeared to be attached directly to the protein through its SH groups.[147] When 1,2,5,6,-dibenzanthracene-9,10-C^{14} was applied to mouse skin, at least part of the phenanthrene-dicarboxylic acid formed was bound to the protein of the skin through the diamide or the monoamide of the acid.[148] Azo compounds with a carboxyl group rigidly held 12 to 14 angstroms from a dimethylamino group were bound to human serum albumin by both these groups, the dimethyl amino group binding to a tyrosine hydroxyl and the carboxyl binding to a nearby cationic site on the albumin ; when the terminal groups of the dye were less than 10 or more than 14 angstroms apart, only one bond could be formed, usually by the dye carboxyl.[149] The free energy of binding of crystal violet by bovine serum albumin was calculated to be 8·1 kcal for the first dye molecule.[150] The heat of formation of a peptide H bond was calculated as–1·5 kcal.[151]

Antibodies and Allergy

New studies have at least begun to penetrate the thick maze of immunology and allergy. Kinetic studies indicated that one or more carboxyl groups are involved in the antigen-antibody bond,[152] and the same conclusion was supported by the demonstration that acetylation of anti-bovine serum albumin antibody destroyed nearly all of its activity.[153] In six different antigen-antibody systems evidence was obtained that free amino groups are critically involved in antibody action ;[154] in one system the behaviour pointed to a group with p K of about 9·8, close to that of the epsilon-NH^2 of lysine.[155]

In inflammation, which is a manifestation of severe cellular injury, the exudative liquid contains a factor called leucotaxine, which appeared to be a polypeptide to which an unknown prosthetic group may be attached ; leucotaxine raises capillary permeability and attracts polymorphonuclear leucocytes.[156] Cortisone and similar compounds were found not to inhibit the production of substances which propagate inflammation, but to inhibit tissue reactivity to such substances.[157] Tracer studies showed that entire molecules of protein antigen (not just the haptens) penetrate into the cells and concentrate in the cytoplasmic granules.[158] In eczematous sensitisation, the sensitising agent is believed to form a conjugate with a protein of the body, probably of the epidermis, which causes an alteration in enzymes of cells of the lymphoreticular system, so that when the sensitising agent again is encountered an eczematous reaction develops.[159]

Pigmentation

Synthesis of melanin in tissue cultures of chick embryo skin appeared to be inhibited by addition of phenylalanine ; the inhibition was reversed by addition of tyrosine to the culture medium.[160] Compounds such as

8-methoxypsoralen continued to receive study as means of restoring pigmentation in limited skin areas.[161]

Antibacterial Action

In connection with the reported tendency of nonionic surfactants to lower the efficacy of many antibacterial agents, it was observed that various phenols react with surface-active polyethers to produce an insoluble oil which may be responsible for problems such as irregular release of antibacterial agents and breakage of emulsions.[162] Microelectrophoretic studies indicated that the exterior of E. coli cells is a polysaccharide, possibly an arabate.[163] A review of bacteriophage described the prophage as occupying a definite site on the bacterial chromosome, dividing with the nucleus, and occasionally leaving the host to form free phage particles with tadpole-like structure, the head consisting of deoxyribonucleic acid, the tail and the covering of the head of protein; the tail punctures the membrance of a bacterial cell, and the DNA of the head is injected into the bacterium, to attach to the chromosome and to synthesise viral DNA from the bacterial nucleic acid.[164]

Effects of Vitamins on Skin

On a diet free of vitamin A, mice had hair-growth cycles as long as 24 days, instead of 21 days on normal diets.[165] The methyl ether or the palmitate of vitamin A, applied in 95 per cent alcohol to the backs of plucked mice, caused irritation which was greater in regions of resting follicles than in regions of follicle growth.[166] Hyperkeratosis produced in animals by chlorinated naphthalene or chlorinated phenols was accompanied by low vitamin A levels in the serum.[167] Capillary resistance in rabbits was decreased by reducing their diet to $\frac{1}{3}$ to $\frac{1}{4}$ the normal amount; administration of vitamins B^2, B^6, C. P, and K increased the capillary resistance but vitamin B^1 had no effect.[168]

Ageing

Among the recent books on ageing, *Hormones and the Aging Process*,[169] edited by Engle and Pincus, and *The Biology of Senescence*,[170] by Comfort, may be mentioned. In old persons, milk proteins were found to have a better substitutive value than wheat proteins, reversing the situation found in younger adults.[171] Normal individuals showed no change in basal respiratory rate nor in tidal volume with advancing age, but efficiency of ventilation was reduced about 20 per cent.[172] In men of 65–75 years, corticotropin appears in the blood as an activable form, instead of the active form found in the blood of normal young men.[173]

Squalene was synthesised from synthetic and from natural nerolidol, and was utilised to the same extent as natural squalene for cholesterol synthesis by rat liver cells.[174] In passing from squalene to cholesterol, the oxygen added to form the intermediate, lanosterol, was shown to come from molecular oxygen, not from water, and the oxidocyclase system involved was believed to be a metalloenzyme similar to oxidases working upon various aromatic and hydroaromatic compounds.[175] The last links are being furnished in the series of steps involved in synthesising cholesterol from acetate,[176] and the data obtained may hasten the control of ageing in arteries, connective tissues, etc. Evidence was presented indicating that a deficiency of vitamin B^6 may influence development of atherosclerosis.[177]

In human cartilage, uronic acid was found to decline with age, and hexosamine to increase in relative concentration, up to maturity.[178] Examination of individual collagen fibres and bundles with the phase contrast microscope revealed variant fibres with chemical and physical behaviour differing from that of collagen, with the proportion of the variant fibres apparently related to age.[179] Observation of the greatly increased amounts of work needed to bring the collagen fibres of rats from the stretched inelastic state to the contracted elastic state, as compared with fibres from young rats, led to the conclusion that forces between the collagen molecules change with age of animal.[180] A study of cross-linking mechanism in polymers such as polyethylene has yielded some basic data which may be applicable to similar processes which possibly are involved in the changes of connective tissue fibres with age.[181]

The conjugated lipides of connective tissue were reported to increase with age in the rat.[182] In human skin succinic dehydrogenase concentration was found to be lower in older than in younger persons.[183]

Analytical Methods

Improved analytical methods have continued to make the cosmetic chemists' work more accurate and effective. An improved method for determination of hydrocarbons in cold creams is based on adsorption of other nonvolatile materials on alumina.[184] The mass spectrometer was found to be valuable in relating composition of paraffins to melting point and penetrability, but not to tensile strength.[185]

The separation of mixtures of fatty acids by chromatographic columns has improved, and the saturated fatty acids from C-6 to C-22 have been quantitatively separated by this method.[186] Since the use of gas-liquid partition chromatography was first reported[187] in 1952, it has made possible a tremendously more effective detailed analysis of complex mixtures. Sebum has been shown to contain at least 31 fatty acids,[56] and three branched four- and five-carbon acids have for the first time been shown to be components of animal depot fat.[188] The method has proven extremely valuable in

the analysis of essential oils and other mixtures of odorants,[189] and in the examination of aerosol ingredients and products.[190] A gas analyser measuring sound velocity in gases may find application as the detector unit in the gas chromatograph.[191] By ion-exchange chromatography, commercial adenosine triphosphate was found to contain 8 per cent tetraphosphate and a small amount of pentaphosphate.[192]

Miscellaneous

Biochemistry has progressed far since the time, only 20 or 30 years ago, when "some biologists were still insistent that comparison of the living cell with any physicochemical system was sheer impertinence."[193] Szent-Gyorgyi points out that some biological phenomena may belong to the domain of "quantum biology," and suggests that three new factors may have to be introduced into biological thought : water structures, the electromagnetic field, and triplets or some other unusual form of excitation made possible by water structures.[194] The importance of water structures is connected with the likelihood that cells contain very little random water, but do contain ice, or more exactly water which has acquired an ordered structure around surfaces. Thus water forms cubic lattices around nonpolar substances.[195] Electropolar groups on surfaces may also induce order in the adjacent water.[196]

The biochemical use of radioactive tracers may be expanded by Wilzbach's report that a wide variety of organic compounds can be labelled (more or less at random) by warming them for a few days with tritium in sealed tubes.[197]

The Pace of Progress

As is apparent from the above selective review, 1956 was a year of extremely rapid progress in the life sciences. The rate of progress is likely to continue to accelerate at least for the duration of the current period of prosperity. The government grants which sustain about 60 per cent of the research in this country[198] continue to be available. Of the Federal grants for unclassified research in the life sciences, at least 143 subjects in the report[199] for fiscal 1954 were judged to be of interest to the cosmetic chemist.[200] For fiscal 1955, 223 subjects were considered of interest.[201] In the important fields of enzyme mechanisms and protein structure, 93 projects were listed in 1955 against 86 in 1954. Federal backing of research and development, which totalled $2.3 billion in fiscal 1955 and $2.4 billion in 1956, is expected to reach $2.7 billion in fiscal 1957.[202] The Federal budget for fiscal 1958 calls for $3.4 billion for research.[203] There appears to be a general impression that U.S. management, both industrial and governmental, has favoured applied over basic research ; it is therefore encouraging

to note that the National Science Foundation budget, which goes largely to fundamental studies, has been doubled in each of the past two fiscal years, to reach $42 million for fiscal 1957.[204] Basic research is estimated to account for about $240 million, or 9 per cent of the fiscal 1957 Federal research budget of $2.7 billion.[205]

Summary

New discoveries in chemical aspects of the life sciences have provided a wealth of new facts, concepts, and techniques which may be applied by the cosmetic chemist to improve the efficacy and attractiveness of his products while maintaining their high standard of safety. As ever, much of last year's new data came from sources outside our own industry, and as the volume of reported work continues to increase, its proper assimilation becomes a growing problem. Continued and increasing support of basic research by public funds promises that many more problems now puzzling our best investigators will be solved in the next few years. Cosmetic chemistry is still a fast-growing child.

BIBLIOGRAPHY

[1] Lauffer, P. G. I., *J. Soc. Cosm. Chemists*, **7**, 26–37 (1956).
[2] Montagna, Wm., *The Structure and Function of the Skin*, Academic Press, New York, 1956.
[3] Rothman, S., *Physiology and Biochemistry of the Skin*, University of Chicago Press, Chicago, 1954.
[4] Hunter, R., Pinkus, H., and Steele, C. H., *J. Inves. Dermat.*, **27**, 31–34 (1956).
[5] Matoltsy, A. D., and Odland, G. F., *J. Inves. Dermat.*, **26**, 121–6 (1956).
[6] Blank, I. B., *J. Inves. Dermat.*, **18**, 433–440 (1952); **21**, 259–269 (1953); *Proc. Sci. Sect. T.G.A.*, No. 23, 19–23 (May, 1955).
[7] Peck, S. M., and Glick, A. W., *J. Soc. Cosm. Chemists*, **7**, 530–540 (1956).
[8] Shelmire, J. B., *J. Inves. Dermat.*, **26**, 105–109 (1956).
[9] Yokozeki, T., *Shikoku Acta Med.*, **7**, 80–90 (1956).
[10] Montgomery, H., Horwitz, O., and Penneys, R., *Trans. Assoc. Am. Physicians*, **8**, 185–198 (1955).
[11] Petrun, M. M., *Fiziol. Zhur. Akad. Nauk. Ukr. R.S.R.*, **1**, No. 2, 108–112 (1955).
[12] van Heusden, P. L., *Excerpta Med., Sect.* 11, **8**, 482 (1955).
[13] Roe, D. A., *J. Inves. Dermat.*, **27**, 1–8 (1956).
[14] Rudall, K. M., *Advances in Protein Chemistry*, **7**, 253–288 (1952).
[15] Matoltsy, A. G., and Herbst, F. S. M., *J. Inves. Dermat.*, **27**, 263–270 (1956).
[16] Thomas, K., *et al.*, *Arch. Tierernahr.*, Beihefte, No. **5**, 103–109 (1954).
[17] Nicolaides, N., and Rothman, S., *J. Inves. Dermat.*, **24**, 125–129 (1955).
[18] Selby, C. C., *J. Soc. Cosm. Chemists*, **7**, 584–599 (1956).
[19] Flesch, P., *J. Soc. Cosm. Chemists*, **7**, 521–530 (1956).
[20] Rothman, S., *J. Soc. Cosm. Chemists*, **7**, 576–583 (1956).
[21] Aavik, O. R., *J. Invest. Dermat.*, **24**, 103–106 (1955).
[22] Hellman, K., *J. Physiol.*, **129**, 454–463 (1955).
[23] Shelley, W. B., Coehn, S. B., and Koelle, G. B., *J. Inves. Dermat.*, **24**, 561–565 (1955).
[24] Block, W. B., and Johnson, D. V., *J. Biol. Chem.*, **217**, 43–48 (1955).
[25] Spier, H. W., Pascher, G., and Martin, K., *Dermatologia*, **3**, 9–13 (1955).
[26] Repke, H., *Naunyn-Schmiedeberg's Arch. exptl. Pathol. Pharm.*, **228**, 227 (1956).
[27] Tanaka, H., Demura, K., and Kato, H., *Acta Med. et Biol.*, **3**, 101–104 (1955).
[28] O'Donnell, I. J., and Woods, E. F., *Australian J. Chem.*, **9**, 212–221 (1956).
[29] Detlev v. Uexkull, J., *Seifen-Öle-Fette-Wachse*, **81**, 611–612 (1955).
[30] Agureikins, S., *Latvijas P.S.R., Zinatnu Akad. Vestis.* 1955, No. **4**, 127–138.
[31] Paschoud, J. M., *Dermatologica*, **112**, 323–334 (1956).

[32] Kato, L., and Gozsy, B., *Can. Med. Assoc. J.*, **73**, 31–34 (1955).
[33] Blank, I. B., and Shappirio, E. B., *J. Inves. Dermatol.*, **25**, 391–401 (1955).
[34] Griesmer, R. D., and Gould, E., *J. Inves. Dermatol.*, **25**, 383–389 (1955).
[35] Breedis, C., *Cancer Research*, **14**, 575 (1954).
[36] Kligman, A. M., and Strauss, J. S., *J. Inves. Dermatol.*, **27**, 19–23 (1956).
[37] Loewenthal, L. A., and Montagna, W., *J. Inves. Dermatol*, **24**, 429–433 (1955).
[38] Borodach, G. N., and Montagna, W., *J. Inves. Dermatol.*, **26**, 229–232 (1956).
[39] Houssay, A. B., Penhos, J. C., and Foglia, V. G., *Rev. Soc. Argentina Biol.*, **31**, 7–21 (1955).
[40] Rony, H. G., and Cohen, D. M., *J. Inves. Dermatol.*, **25**, 285–287 (1955).
[41] Abe, K., *Med. J. Osaka Univ.*, **6**, 605–617 (1955).
[42] Kuno, Yas, *Human Perspiration*, Charles C. Thomas, Springfield, Ill. (1956).
[43] Randall, W. C., and Kimura, K. K., *Pharmacol. Reviews*, **7**, 365–397 (1955).
[44] Herrmann, F., and Mandol, L., *J. Inves. Dermatol.*, **24**, 225–246 (1955).
[45] Shelley, W. B., and Levy, E. J., *J. Inves. Dermatol.*, **25**, 249–263 (1955).
[46] Cahn, M. M., and Shelley, W. B., *J. Inves. Dermatol.*, **25**, 63–66 (1955).
[47] Shelley, W. B., and Cahn, M. M., *J. Inves. Dermatol.*, **25**, 127–131 (1955).
[48] Perry, E. T., and Shelley, W. B., *J. Inves. Dermatol.*, **25**, 439–451 (1955).
[49] Thaysen, J. H., and Schwartz, I. L., *J. Clin. Inves.*, **34**, 1719–1725 (1955).
[50] Shelley, W. B., Hurley, H. J., and Nichols, A. C., *Arch. Derm. and Syph.*, **68**, 430–466 (1953).
[51] Strauss, J. S., and Kligman, A. M., *J. Inves. Dermatol.*, **27**, 67–71 (1956).
[52] Argyris, T. S., *Science*, **123**, 634–635 (1956).
[53] Montagna, W., *J. Inves. Dermatol.*, **25**, 117–119 (1955).
[54] Kirk, J. E., and Chieffi, M., *J. Inves. Dermatol.*, **27**, 15–17 (1956).
[55] Hougen, F. W., *Biochem. J.*, **59**, 302–309 (1955).
[56] James, A. T., and Wheatley, V. R., *Biochem. J.*, **63**, 269–273 (1956).
[57] Meyer-Arendt, J., *Science*, **123**, 1176–1177 (1956).
[58] Lowry, O. H., *et al.*, *J. Biol. Chem.*, **222**, 97–107 (1956).
[59] Eagle, H., *Science*, **122**, 501–504 (1955).
[60] Schweigert, H., *Rev. fermentations et inds. aliment.*, **11**, 63–66 (1956).
[61] Cook, E. S., and Kreke, C. W., U.S. Patent 2,719,811, Oct. 4, 1955.
[62] DeHarven, E., *Bull. classe sci., Acad. roy. Belg.*, **42**, 499–505 (1956).
[63] Miller, C. O., *et al.*, *J. Amer. Chem. Soc.*, **78**, 1375–1380 (1956).
[64] Strong, F. M., Miller, C. O., and Skoog, F., *Abstracts Biol. Chem. Div., A.C.S. Meeting, Sept.* 1956, No. 29.
[65] Nickerson, W. J., *ibid.*, No. 32.
[66] Stern, H., *Science*, **124**, 1292–1293 (1956).
[67] Rothstein, A., and Hayes, A. D., *Arch. Biochem. and Biophys.*, **63**, 87–99 (1956).
[68] Reiner, L., *et al.*, *J. Pharmacol. Exptl. Therap.*, **117**, 52–61 (1956).
[69] Hassett, C. C., *Armed Forces Chem. J.*, **11**, No. 1, 26–28 (1957).
[70] Anon., *Chem. and Eng. News*, **33**, 6372 (1956).
[71] Anon., *Science*, **124**, 576 (1956).
[72] Allfrey, V. G., Mirsky, A. E., and Osawa, S., *Nature*, **176**, 1042–1049 (1955).
[73] Straub, F. B., *Priroda*, **45**, No. 2, 38–43 (1956).
[74] Lockingen, L. S., and De Busk, A. G., *Proc. Natl. Acad. Sci. U.S.*, **41**, 925–934 (1955).
[75] Bloch, D. P., *Proc. Natl. Acad. Sci. U.S.*, **41**, 1058–1064 (1955).
[76] Gamow, G., and Yeas, M., *Proc. Natl. Acad. Sci. U.S.*, **41**, 1011–1019 (1955).
[77] Anon., *Chem. and Eng. News*, Jan. 7, 1957, p. 106; Shepherd, R. G., *et al.*, *J. Amer. Chem. Soc.*, **78**, 5051–5078 (1956).
[78] Li, C. H., *et al.*, *Nature*, **176**, 687–689 (1955).
[79] Anon., *Mfg. Chemist*, **25**, 433 (1955).
[80] Krimm, S., and Schor, R., *J. Chem. Phys.*, **24**, 922–3 (1956).
[81] Laskowski, M., and Sheraga, H. A., *J. Amer. Chem. Soc.*, **78**, 5793–5798 (1956).
[82] Peticolas, W. L., and Klotz, I. M., *J. Amer. Chem. Soc.*, **78**, 5257–5262 (1956).
[83] Meirowsky, E., *J. Inves. Dermatol.*, **26**, 95–104 (1956).
[84] Belicer, V. A., *Congr. Intern. Biochem., Resumes Communs.*, 3e *Congr. Brussels* **1955**, 19.
[85] Doty, P., *Abstracts Biol. Chem. Div., A.C.S. Meeting*, Apr. 1956, No. 15.
[86] Grunberg-Manago, M., Ortiz, P. J., and Ochoa, S., *Science*, **122**, 907–910 (1955).
[87] Kornberg, A., Lehman, I. R., Bessman, M. J., and Simms, E. S., *Biochim. et Biophys. Acta*, **21**, 197–198 (1956).
[88] Flory, P. J., *J. Amer. Chem. Soc.*, **78**, 5222–5235 (1956).
[89] Flory, P. J., *Science*, **124**, 53–60 (1956).

[90] Symposia of Society of Exptl. Biol., No. 9, *Fibrous Proteins and Their Biological Significance*, Academic Press, New York, 1955.
[91] Gustavson, K. H., *The Chemistry and Reactivity of Collagen*, Academic Press, New York, 1956.
[92] Rich, A., and Crick, F. H. C., *Nature*, **176**, 915–916 (1955).
[93] Boedtker, H., and Doty, P., *J. Amer. Chem. Soc.*, **78**, 4267–4280 (1956).
[94] Ramachandran, G. N., and Gopinarth, K., *Nature*, **176**, 593–595 (1955).
[95] Ramachandran, G. N., *Nature*, **177**, 710–711 (1956).
[96] Cowan, P. M., McGavin, S., and North, A. C. T., *Nature*, **176**, 1062–1064 (1955).
[97] Reed, R., Wood, M. J., and Keech, M. K., *Nature*, 697–699 (1956).
[98] Veis, A., and Cohen, J., *J. Amer. Chem. Soc.*, **78**, 6238–6244 (1956).
[99] Witkop, B., *Chem. Soc. (London) Spec. Publ. No.* **3**, 60–82 (1955).
[100] Witkop, B., *Science*, **124**, 941 (1956).
[101] Orekhovich, V. N., *Congr. Intern. Biochim., Resumes Communs.* 3e *Congr., Brussels*, 1955, p. 50.
[102] Zaides, A. L., Tustanovskii, A. A., and Orlovskaya, G. V., *Doklady Akad. Nauk. S.S.S.R.*, **104**, 563–566 (1955).
[103] Zaides, A. L., *Biofizika*, **1**, 279–283 (1956).
[104] Marsh, C. L., Olson, C., and Blore, I. C., *Am. J. Vet. Research*, **17**, 410–414 (1956).
[105] Damodaran, M., Sivaraman, C., and Dhavalikar, R. S., *Biochem. J.*, **62**, 621–625 (1956).
[106] Burton, D., *et al.*, *Nature*, **176**, 966–969 (1955).
[107] Keech, M. K., Reed, R., and Wood, M. J., *J. Path. Bacteriol.*, **71**, 477–493 (1956).
[108] Walker, D. G., and Wirtschafter, Z. T., *Arch. Pathol.*, **61**, 125–135 (1956).
[109] Gaebler, O. H., Editor, *Enzymes: Units of Biological Structure and Function*, Academic Press, N.Y., 1956.
[110] Spiegelman, S., *Proc. 3rd intern. Congr. Biochem. Brussels* 1955, 185–195.
[111] Monty, K. J., *et al.*, *J. Biophys. Biochem. Cytol.*, **2**, 127–145 (1956).
[112] Tuppy, H., *et al.*, *Acta Chem. Scand.*, **9**, 353–64; 365–74 (1955).
[113] Voevodskii, V. V., Volkinshtein, F. F., and Semenov, N. N., *Akad. Nauk. S.S.S.R.* 1955, 423–440.
[114] Sheehan, J. C., and Beck, C. W., *J. Amer. Chem. Soc.*, **77**, 4875–4877 (1955).
[115] Ballard, D. G. H., and Bamford, C. H., *Nature*, **177**, 477–478 (1956).
[116] Riehl, N., *Zhur. Fiz. Khim.*, **29**, 1537–1548 (1955).
[117] Green, D. E., *Proc. 3rd Intern. Congr. Biochem., Brussels*, 1955, 281–284.
[118] Theorell, H., *Discus. Faraday Soc. No.* **20**, 224–31 (1955).
[119] Ishibashi, M., *Osaka Daigaku Igaku Zassi*, **8**, 7–11 (1956).
[120] Turpaev, T. M., *Biokhimiya*, **20**, 456–462 (1955).
[121] Frieden, E. H., *J. Amer. Chem. Soc.*, **78**, 961–965 (1956).
[122] Friess, S. L., Patchett, A. A., and Witkop, B., *J. Amer. Chem. Soc.*, **79**, 459–462 (1957).
[123] Koshland, D. E., *J. Cellular Comp. Physiol.*, **47**, Suppl. 1, 217–234 (1956).
[124] Theorell, T., *Science*, **124**, 467–472 (1956).
[125] Wilson, I. B., Kewitz, H., and Ginsburg, S., through *Drug Trade News*, **31**, No. 7, 43 and 61 (1956).
[126] Popov, I. D., Karabashev, N., and Karabasheva, T., *Contes rendues Acad. Bulgare Sci.*, **8**, No. 1, 65–68 (1955).
[127] Larson, P. S., Finnegan, J. K., and Haag, H. B., *J. Pharmacol. Exptl. Therap.*, **116**, 119–122 (1956).
[128] Gero, A., and Reese, V. J., *Science*, **123**, 100 (1956).
[129] Bruice, T. C., Kharasch, N., and Winzler, R. J., *Arch. Biochem. and Biophys.*, **62**, 305–317 (1956).
[130] Russell, G. A., and Brown, H. C., *J. Amer. Chem. Soc.*, **77**, 4578–4582 (1955).
[131] Schlenk, W., *Svensk Kem. Tidskr.*, **67**, 435–462 (1955).
[132] Szwarc, M., and Leavitt, F., *J. Amer. Chem. Soc.*, **78**, 3590–3593 (1956).
[133] Arnold, J. W., and Meakins, R. J., *Trans. Faraday Soc.*, **51**, 1667–1673 (1955).
[134] Kapustinskii, A. F., *Izvest. Akad. Nauk. S.S.S.R. Otdel. Khim. Nauk.* 1956, No. **4**, 427–434.
[135] Arshid, F. M., Giles, C. H., and Jain, S. K., *J. Chem. Soc.*, 1956, 559–569.
[136] Anon., *Aminco Lab. News*, Vol. **13**, No. 6, P. 1 (Nov. 1956).
[137] Anon., *Chem. and Eng. News*, **34**, 4759 (1956).
[138] Peiss, C. N., Randall, W. C., and Hertzman, A. B., *J. Inves. Dermatol.*, **26**, 459–470 (1956).
[139] Mom, A. M., and Clerc, N. A., *J. Inves. Dermatol.*, **26**, 427–429 (1956).

[140] Fretzdorff, A. M., and Weitzel, G., *Hoppe-Seyler's Z. Physiol. Chem.*, **301**, 17–25 (1955).
[141] Weitzel, G., Fretzdorff, A. M., and Heller, S., *Hoppe-Seyler's Z. Physiol. Chem.*, **301**, 26–45 (1955).
[142] Kato, H. P., Zwolinski, B. J., and Eyring, H., *J. Phys. Chem.*, **60**, 404–410 (1956).
[143] Christensen, H., and Riggs, T. R., *J. Biol. Chem.*, **220**, 265–278 (1956).
[144] Davis, B. D., *Intern. Sympos. on Enzymes. Units of Biol. Structure and Function, Detroit*, 1955, 509–522.
[145] Puck, T. T., and Lee, H. H., *J. Exptl. Med.*, **101**, 151–175 (1955).
[146] Hughes, T. R., and Klotz, I. M., *J. Amer. Chem. Soc.*, **78**, 2109–2116 (1956).
[147] Klotz, I. M., Klotz, T. A., and Fiess, H. A., *Abstracts Biol. Chem. Division, A.C.S. Meeting*, Sept. 1956, No. 48.
[148] Bhargava, P. M., and Heidelberger, C., *J. Amer. Chem. Soc.*, **78**, 3671–3677 (1956).
[149] Peticolas, W. L., and Klotz, I. M., *Abstracts Biol. Chem. Division, A.C.S. Meeting*, Sept. 1956, No. 20.
[150] Blei, I., and Carroll, B., *Abstracts Biol. Chem. Division, A.C.S. Meeting*, Sept. 1956, No. 47.
[151] Schellman, J. A., *Contes Rendues Lab. Carlsberg, Ser. Chim.*, **29**, 223–9 (1955).
[152] Cann., J. R., and Clark, E. W., *J. Amer. Chem. Soc.*, **78**, 3627–3631 (1956).
[153] Singer, S. J., *Proc. Nat. Acad. Sci. U.S.*, **41**, 1041–1045 (1955).
[154] Singer, S. J., *Abstracts Biol. Chem. Division, A.C.S. Meeting*, Sept. 1956, No. 80.
[155] Epstein, S. I., and Singer, S. J., *Abstracts Biol. Chem. Division, A.C.S. Meeting*, Sept. 1956, No. 79.
[156] Menkin, V., *Science*, **123**, 527–534 (1956).
[157] Scott, A., and Kalz, F., *J. Inves. Dermatol.*, **26**, 361–378 (1956).
[158] Haurowitz, F., Reller, H. H., and Walter, H., *J. Immunol.*, **75**, 417–422 (1955).
[159] Rostenberg, A., *et al.*, *J. Inves. Dermatol.*, **26**, 209–216 (1956).
[160] Saunders, J. W., *et al.*, *J. Nat. Cancer Inst.*, **16**, 475–485 (1955).
[161] Kelly, E. W., and Pinkus, H., *J. Inves. Dermatol.*, **25**, 453–456 (1955).
[162] Marcus, A. D., Wetstein, E., and Ruderman, M., *J. Amer. Pharm. Assn., Pract. Pharm. Ed.*, **17**, 453, 472–473 (1956).
[163] Davies, J. T., Haydon, D. A., and Rideal, E., *Proc. Roy. Soc.*, B **145**, 375–383 (1956).
[164] Boyd, J. S. K., *Biol. Revs. Cambridge Phil. Soc.*, **31**, 71–107 (1956).
[165] Loewenthal, L. A., *J. Morphol.*, **98**, 275–303 (1956).
[166] Rademacher, A. H., and Montagna, W., *J. Inves. Dermatol.*, **26**, 69–75 (1956).
[167] Kohler, H., *Arch. Tierernahr.*, Beihefte No. **5**, 283–289 (1954).
[168] T. Yokozeki, *Shikoku Acta Med.*, **7**, 80–90 (1955).
[169] Engle, E. T., and Pincus, G., Editors, *Hormones and the Aging Process*, Academic Press, New York, 1956.
[170] Comfort, A., *The Biology of Senescence*, Routledge and Paul, London, 1956.
[171] Schulze, W., *Excerpta Med., Sect. II*, **8**, 188 (1955).
[172] Hemingway, A., Pocock, D., and Short, J. J., *J. Chronic Diseases*, **3**, 301–310 (1956).
[173] Martinelli, M., and Montanari, L., *Bol. Soc. Ital. Biol. Sper.*, **31**, 1316–1317 (1955).
[174] Isler, O., *et al.*, *Helv. Chim. Acta*, **39**, 897–904 (1956).
[175] Tchen, T. T., and Bloch, K., *Abstracts Biol. Chem. Section, A.C.S. Meeting*, Sept. 1956, No. 126.
[176] Anon., *Chem. and Eng. News*, **34**, 6180 (1956).
[177] Schroeder, H. A., *J. Chronic Diseases*, **2**, 28–41 (1955).
[178] Shetlar, M. R., and Masters, Y. E., *Proc. Soc. Exptl. Biol. Med.*, **90**, 31–33 (1955).
[179] Cruise, A. J., *J. Soc. Leather Trades Chemists*, **40**, 321–329 (1956).
[180] Verzar, F., *Helv. Physiol. et Pharm. Acta*, **14**, 207–221 (1956).
[181] Anon., *Chem. and Eng. News*, **33**, 3390 (1955).
[182] Boucek, R. J., Noble, N. L., and Kao, K. Y. T., *Circulation Research*, **3**, 519–524 (1955).
[183] Serri, F., and Rabbiosi, G., *Boll. Soc. Med. Chir. Pavia*, **69**, 373–380 (1955).
[184] Bruening, C. F., *J. Assoc. Offic. Agr. Chemists*, **39**, 391–396 (1956).
[185] Turner, W. R., Brown, D. S., and Harrison, D. V., *Ind. and Eng. Chem.*, **47**, 1219 (1955).
[186] Kapitel, W., *Fette, Seifen, Anstrichmittel*, **58**, 91–94 (1956).
[187] James, A. T., and Martin, A. J. P., *Biochem., J.*, **50**, 679–690 (1952).
[188] McInnes, A. G., Hansen, R. P., and Jessop, A. S., *Biochem. J.*, **63**, 702–705 (1956).
[189] Brenner, N., *Proc. Sci. Section, T.G.A.*, No. **26**, 3–8 (Dec. 1956).
[190] Root, M., *J. Soc. Cosm. Chemists*, in press.

[191] Anon., *Chem. and Eng. News*, **35**, No. 1, 112 (Jan. 7, 1957).
[192] Sacks, J., *Biochim. et Biophys. Acta*, **16**, 436 (1955).
[193] Anon., *Chem. and Eng. News*, **33**, 4652 (1955).
[194] Szent-Gyorgyi, A., *Science*, **124**, 873–875 (1956).
[195] Buswell, A. M., and Rodenbush, W. H., *Scientific American*, **194**, No. 4, 77 (Apr. 1956).
[196] Jacobson, B., *J. Am. Chem. Soc.*, **77**, 2919 (1955).
[197] Anon., *Chem. and Eng. News*, **34**, 4616–4617 (1956).
[198] Anon., *Time Magazine*, July 9, 1956.
[199] *Federal Grants and Contracts for Unclassified Research in the Life Sciences, Fiscal Year* 1954. National Science Foundation, Washington, D.C.
[200] Lauffer, P. G. I., *Proc. Sci. Section, T.G.A.*, No. **25**, 20–23 (May 1956).
[201] *Federal Grants and Contracts for Unclassified Research in the Life Sciences, Fiscal Year* 1955. National Science Foundation, Washington, D.C.
[202] Anon., *Chemical Week.*, **80**, No. 1, p. 27 (Jan. 5, 1957).
[203] Anon., *Chem. and Eng. News*, **35**, No. 3, p. 7 (Jan. 21, 1957).
[204] Anon., *Chem. and Eng. News*, **34**, 459 (1956).
[205] Anon., *Chem. and Eng. News*, **34**, 5820–5821 (1956).