PERSORPTION*

By Amos E. Light

The Wellcome Research Laboratories, Tuckahoe 7, N. Y.

As defined in the dictionary, skin is "the membranous external investment of an animal." Actually it has turned out to be an "investment" of inestimable value for the human animal and the study of ways and means of keeping the skin in good condition has become a very profitable investment for the drug and cosmetic industries.

A human being weighing 70 kg. has approximately 1.83 square meters of skin. This average value is obtained by raising one-tenth of the value of the weight to the two-thirds power (1). The skin weighs about 16 per cent of the body weight. In burns during mass casualties, the area of skin damage is most important in planning for the care of the patient especially in regard to fluid administration. Normally the skin prevents a large loss of moisture except in times of increased body heat during fever or activity at which times the sweat glands are called into action. It may be of value to remember the Rule of Nine where the area of each arm and head is 9 per cent of the total and the surface of each leg and each side of the trunk is 18 per cent of the total (2). The thickness of the skin usually measures between 2.5 mm. and 8 mm. and the different layers also vary in a similar manner (3). It has been recorded that an average strip of skin one inch square contains some 3,000,000 tissue cells, one yard of blood vessels, four yards of nerves with 25 sensory ends, some 400 or more sweat glands, and from 15 to 30 hair follicles with like numbers of sebaceous glands (4). The skin is an organ and uses all of the above structures to protect the body. The production of keratin is considered one of the skin's greatest achievements (5).

Usually it is extremely unwise to introduce a new term to describe old events; however, after careful perusal of the literature it became apparent that no single word yet used could describe the act of substances passing through various layers of the skin. After considering many combinations of prefixes and suffixes the word "Persorption" evolved to amply describe this action. The cosmetic chemist is probably more interested in absorption, an example of which would be the stratum corneum swelling following

* Presented at the September 26, 1956, Meeting, New York Chapter, New York City.

the application of water, whereas the pharmaceutical chemist is concerned with persorption which indicates passage of material into the deeper sections of the dermis and possibly into the lymph or blood systems. In fact the cosmetic chemist may not desire to have the lower layers of the skin affected. Penetration of the stratum corneum by detergents to the area of living cells may be a major factor in the severity of some eczemas. Protective creams have been devised to prevent such penetration (6).

Penetration of the skin may be by solid matter causing trauma, or by energy such as heat and x-radiation. With the administration of heat the temperature of the skin rises, the rapidity of this rise depending on the thickness of the tissue; it is less rapid when the superficial tissue is thinner allowing deeper penetration and faster dissipation of the heat by the blood (7). The study of the passage of chemicals through the skin, persorption, offers a great challenge to research workers in the field of dermatology. Much information has been gained in the past few years but much more is needed to treat the various skin disorders prevalent today (8).

It would be out of place here to present all published data on skin structure and physiology (9) and the literature on percutaneous absorption (10). These subjects have been reviewed before as listed in the bibliography. In general it has been found that a substance with water and lipoid solubility has a much better chance of passing through the skin than other types. The rate of penetration is related to the ether-water partition coefficient, and the barrier to be passed appears to be an aqueous phase separating two lipoidal layers. There are many exceptions to this so that each individual compound should be tested for persorption. Water itself has been studied for its persorption properties, but it has been found that the most important property of this substance is to keep the stratum corneum hydrated with resultant improvement in dry skin (11). The surface layer of epidermis usually contains only 10 per cent of moisture in the keratin as compared with 40 per cent in the deeper layers and 70 per cent below the stratum granulosum (12). The skin as a whole becomes a depot of water for the rest of the body in certain cases of electrolyte imbalance (13). It is possible that a trace of water may go through the membrane at the stratum lucidum boundary but the eccrine sweat glands and hair follicles are probably the main pathways for any slight passage of moisture through the skin. It is interesting to observe that the diameter of the eccrine sweat gland opening is only about 30 microns or less. This small opening will undoubtedly prevent particles having a large diameter from entering this gland. The much narrower tube spirals down into the skin with only a double layer of cells along its lining; this would facilitate persorption (14). The much larger hair follicle has a lining whose cells become more and more undifferentiated as it penetrates into the skin allowing easier passage of material. The sebaceous gland openings into the

hair follicle likewise are points of entry especially for fat soluble substances. In certain regions such as in the armpits the apocrine glands also open into the hair follicles and offer additional pathways (15).

It is essential for good persorption to assure prolonged contact with the substance in an easily spread ointment base or lotion (16). Rubbing and pressure would certainly force more material into the skin openings. Washing the skin to remove sebum and other matter, including the "acid mantle" (17) that might interfere with the application, usually affords more rapid persorption. For deep fungal infection it is useful at times to remove the upper layers by abrasion (18) or with scotch tape (19) before administering the topical drug; or it may be necessary to soften the keratin layer with alkaline solutions, or with organic solvents. Breaking the sulfide linkages in the keratin layer with reducing agents may also prove useful (8). Low concentration of salicylic acid softens the outer structures while higher strengths damage the tissue (20). Damaged epidermal tissue increases passage by permitting material to enter the blood and lymph vessels of the dermis directly (21). During persorption tests on intact skin, therefore, the investigator must exercise utmost care in preventing skin damage during cleaning or shaving the skin. Damage also changes the negative charge on the outside of the skin which usually repels anions.

The methods of determining persorption merit consideration for by these means it is possible to compare drugs and drug vehicles, and obtain other pertinent data necessary to establish good therapeutic action. It is well to remember that experiments on laboratory animals do not always give the same results as are obtained with humans (22). Known amounts of ointments or solutions in enclosed containers or compresses (23) have been applied to the skin for various periods of time; the difference between the original and final amounts represents the amount going into the skin. However, this quantity may remain in the epidermis without exhibiting persorption, or a part will go into the skin orifices with immediate persorption, or produce a depot effect by liberating material gradually into the dermis. This difference may also be determined by extraction, but again this need not indicate persorption. Excretion of inuncted material or its derivatives in feces, urine, or breath or identification in tissues or organs proves persorption. The depth of percutaneous penetration has recently been investigated by means of radioactive (24) and fluorescent (25) materials. By the former method autoradiograms give pictures of the tissues containing the test material at different depths, at various time intervals. Hydrocortisone appears to pass generally through the epidermis, and not preferentially through the hair follicles. However, radioactive chemicals with short wave emission may indicate a deeper penetration than actually obtained. The fluorescent method shows that oleic acid is very easily persorbed; it indicates that linoleates and oleates may be especially valu-

able for persorptive purposes. *In vitro* tests with isolated skin tissue may prove useful in certain instances (26).

The prevention of cutaneous disturbances has also been used to estimate percutaneous penetration. Thus, histamine solutions applied to the skin either directly or by iontophoresis, produce the usual response; however, antihistamine preparations when applied to the skin may interfere with or prevent this action (27). Incidentally, iontophoresis is an excellent method for enabling certain ions to pass the epidermal barrier. Usually it is more applicable with cations applied at the anode; it was used over fifty years ago for introducing the iodide ion into the neck for the successful treatment of deficiency goiter.

Histamine, because of its biological importance, has been widely tested for percutaneous absorption. The base will penetrate from most solutions and ointments, but it is such a potent drug that only very small quantities are needed to elicit vascular responses. The salts of histamine are less easily persorbed. An attempt was made to inunct "48–80" (28), a potent histamine liberator, into the skin of several species of animals. A water washable ointment containing 1 per cent of "48–80" was rubbed vigorously into the skin of the backs of a rabbit, rat, guinea pig and dog from which the hair had been removed with an electric clipper. No cutaneous effects or temperature changes were observed in any of the animals, and no drop in blood pressure was found in the dog. Later a 1 per cent aqueous solution was applied on the same regions with still no effects except for a slight blister on the dog, possibly due only to the rubbing. Administered intravenously, 25 to 50 micrograms of "48–80" will produce a 50 per cent reduction in the dog's blood pressure. Thus the above data indicate that a polymer (formaldehyde with N-methylhomoanisylamine) of large molecular weight (about 471 or more), will not rapidly pass the epidermal membrane barrier and reach the mast cells where much of the skin histamine is located.

In order to determine the persorption of chlorocyclizine hydrochloride from a water washable ointment* the amount of drug actually found in the blood was biologically measured (29). This was accomplished by taking blood samples from the inuncted dog at various time intervals and determining the inhibition of histamine induced contractions of the isolated guinea pig ileum following the addition of plasma samples to the tissue bath. During this procedure it was discovered that an appreciable amount of chlorocyclizine became bound in the plasma and did not exhibit full activity in this type of assay. Control samples of standards in plasma were thus made necessary in order to evaluate the actual amount passing through the skin and found in the blood.

* Supplied as "Perazil" brand Chlorcyclizine Hydrochloride Cream, Burroughs Wellcome & Co. (U.S.A.), Inc., Tuckahoe, N. Y.

Boric acid powders, solutions and ointments have been investigated but no persorption could be detected through normal skin, as determined by an increase of boron content in blood by chemical assay (30). The passage of cortisone through the skin of adrenalectomized mice has been measured by the eosinopenic response (31). Most vitamins have been found to penetrate the skin enough to relieve the various deficiency symptoms in rats when vigorous application and special ointments are used (32). With human skin, similar activities (33) have been reported and in one instance actual recovery of vitamin B-6 in the urine has been recorded (34). Nicotinic acid derivatives, used for peripheral vasodilation when applied locally, have been found to raise the blood level of the vitamin (35). Tetraethylpyrophosphate certainly passed through the skin when it almost caused the death of a child by its blocking action on the heart (36). In a high concentration, DDT exhibits toxicity when placed in contact with the skin especially when chlorinated solvents are present (37). Of the war gases, the mustard type at low concentration appears to penetrate uniformly through the epidermal barrier, whereas lewisite has been found more in the cellular lining of the hair follicles. Both have much less effect in cold weather (38). Local anesthetic action is also a unique but valuable way of observing the passage of compounds through the skin (39). A polyethylene glycol ointment base permits the dye, phenolsulfonphthalein, to pass through the skin much better than a petrolatum base (40).

Radioactive tracers are now being used to determine the amounts and the pathways of persorption. Mercuric chloride with radioactive mercury has been found to have no penetrative ability (41). On the other hand C^{14} hydrocortisone was easily followed through the skin and into the blood system by autoradiography (42). Sodium retention has also been measured following the application of fluorohydrocortisone lotion (43). Hair growth has been inhibited in rats by application of both cortisone and estrogens (44) but this does not necessarily indicate that persorption has taken place. However, in other tests, it was found that the sex hormones exhibited their respective actions on the rat, estradiol being catabolic and methyl testosterone anabolic (45). With humans, it has been reported that an estrogen lotion applied to male scalps has reduced the amount of falling hair (46). These examples could possibly involve persorption.

When the applied material has passed through the epidermal barrier its continued movement largely depends on the vascularity and circulation in the underlying tissues (47). This has been increased by the use of peripheral vascular dilators such as salicylic acid, nicotinic acid, benzedrine and their derivatives (48). Some of these may be administered orally or by inunction. Benzyl nicotinate has been shown to increase the rapidity with which S^{35} may be excreted in the urine following inunction (49). Besides blood flow, the size of the molecule may affect the passage of the material.

The pore size of the membranes in the blood vessel walls as well as in the epidermis (50) may be smaller than the molecular volume of the substances. For instance, a pore diameter of 55 Å. will retain egg albumin (51). Hydrostatic pressure may also affect the passage (52).

In other aspects of persorption it has been found recently that the skin color may be controlled by topical application of certain chemicals. The monobenzyl ether of hydroquinone inhibits the oxidation of tyrosine so that melanin formation may be prevented (53). On the other hand, white skin patches of vitiligo may be made darker by using 8-methoxypsoralen along with sunlight. This chemical stimulates tyrosinase activity in the cells of the lower layers of the epidermis (54). This suggests the study of enzymes in the skin which may possibly influence persorption. Acid phosphatase has been found in regions of keratin transition (55). Esterases have been reported in high concentration near the surface (56). Succinic dehydrogenase (57) and cytochrome oxidase have also been observed (58). Monamine oxidase may be responsible for the ammonia from sweat glands (59). Various aspects of this phase offer a wide field for future research work.

Age offers many problems in persorption (60). For instance, estrogenic ointments have been recorded to have a beneficial effect (61) on senile skin though a visible improvement has been questioned in one instance (62). There is some question as to whether the collagenous and elastic tissues change markedly with older age thus affecting persorption (63). Aging of protein, ground substance, hyaluronic acid, hyaluronidase, glycogen and other constituents must be further investigated (64). Observation of circulatory changes of both blood and lymph in the dermis along with oxidation studies will be of value in considering persorptive measures (65). The scalp (66) should be considered specifically, for it has peculiarities not found in other parts of the skin.

Since aging of skin does not necessarily correspond to chronological aging other factors such as skin exposure, nutrition (67), sensitivity and possibly stress (68) should be investigated. Poison ivy (69) and other similar skin reactions are only partly understood with respect to skin penetration and present many research problems. The action of lysergic acid diethylamide (70) on capillary resistance introduces serotonin inhibition for study. Cortisone and its derivatives may suppress vascularization through action on the ground substance and hyaluronic acid (71). Temperature, humidity, season and sex also probably contribute to differences in persorption (72). Skin-fold thickness measurements (73) may prove useful to predict persorption values.

Summary

Due to considerable discussion of terminology for the passage of materials through the skin, a new term "Persorption" has been proposed. Different

degrees of persorption have been discussed, and various methods of determining persorption have been reviewed. Many factors such as fat and water solubility, contact with the skin, environmental conditions, intactness, thickness and age of skin, and circulation of blood and lymph all need much additional research work to evaluate their effect on "Persorption."

References

(1) Dawson, W. T., *Ann. Internal Med.*, **13,** 1594 (1940).
(2) Armstrong, G. E., Shaeffer, J. R., and Artz, C. P., *U. S. Armed Forces Med. J.*, **7,** 320 (1956).
(3) Leider, M., and Bunche, C. M., *Arch. Dermatol. and Syphilol.*, **69,** 563 (1954).
(4) *Drug and Cosmetic Ind.*, **77,** 259 (1955).
(5) Giroud, A., and Leblond, C. P., *Ann. N. Y. Acad. Sci.*, **53,** 613 (1951). Leblond, C. P., and Walker, B. E., *Physiol. Revs.*, **36,** 255 (1956).
(6) Suskind, R. R., *Ind. Med. and Surg.*, **24,** 413 (1955). Lubowe, I. I., J. Soc. Cosmetic Chem., **6,** 19 (1955). Bateman, F. J. A., *Brit. Med. J.*, 554 (March 10, 1956). Schwartz, L., "The Prevention of Occupational Skin Diseases," New York, McGraw-Hill Book Co., Inc. (1955).
(7) Benjamin, F. B., *J. Invest. Dermatol.*, **26,** 471 (1956).
(8) Rothman, S., *Trans. N. Y. Acad. Sci., Ser. II,* **12,** 27 (1949). Seeberg, V. P., Hildago, J., Wilken, W., Beniams, H. N., and Lundblad, J., *J. Am. Pharm. Assoc., Sci. Ed.*, **45,** 342 (1956). Oster, K. A., and Golden, M. J., *Trans. N. Y. Acad. Sci., Ser. II,* **12,** 132 (1950). Barlow, A. J. E., and Chattaway, F. W., *Lancet*, **269,** 1269 (1955). Stoughton, R. B., and Novak, N., *J. Invest. Dermatol.*, **26,** 127 (1956).
(9) Montagna, W., "The Structure and Function of Skin," New York, Academic Press (1956). Behrman, H. T., "The Scalp in Health and Disease," St. Louis, C. V. Mosby Co. (1952). Kalish, J., "Skin Research." In each monthly issue of *Drug and Cosmetic Ind.*, starting with Vol. **76,** 393 (1955). Carruthers, C., and Suntzeff, V., *Physiol. Revs.*, **33,** 229 (1953). Kooij, R., *Dermatologica*, **112,** 62 (1956). Pillsbury, D. M., Shelley, W. B., and Kligman, A. M., "Dermatology," Philadelphia, W. B. Saunders Company (1956). Pillsbury, D. M., *Ann. Rev. Physiol.*, **2,** 151 (1940). Kalish, J., *Drug and Cosmetic Ind.*, **76,** 622 (1955). Flesch, P., J. Soc. Cosmetic Chem., **6,** 14 (1955).
(10) Rothman, S., *J. Lab. and Clin. Med.*, 28, 1305 (1943). Rothman, S., J. Soc. Cosmetic Chem., **6,** 193 (1955). Rothman, S., "Physiology and Biochemistry of the Skin," Chicago, The Univ. of Chicago Press (1954), Calvery, H. O., Draize, J. H., and Laug, E. P., *Physiol. Revs.*, **26,** 495 (1946). Treherne, J. E., *J. Physiol.*, **133,** 171 (1956). Peck, S. M., *Drug and Cosmetic Ind.*, **72,** 46 (1953). Hadgraft, J. W., and Somers, G. F., *J. Pharm. and Pharmacol.*, **8,** 625 (1956). Guillot, C. F., and Valette, G., *Technique Pharmaceutique*, **1,** No. 9 (1954).
(11) Newburgh, L. H., and Johnston, M. W., *Physiol. Revs.*, **22,** 1 (1942). Blank, I. H., *Drug and Cosmetic Ind.*, **76,** 758 (1955).
(12) Peiss, C. N., Randall, W. C., and Hertzman, A. B., *J. Invest. Dermatol.*, **26,** 459 (1956).
(13) Woodbury, D. M., *Am. J. Physiol.*, **185,** 281 (1956).
(14) O'Brien, J. P., *J. Invest. Dermatol.*, **15,** 95 (1950). Montagna, W., Chase, H. B., and Lobitz, W. C., *Ibid.*, **20,** 415 (1953).
(15) Shelley, W. B., and Levy, E. J., *Ibid.*, **25,** 249 (1956).
(16) McConnell, W. E., *Chem. Products*, **11,** 391 (1955). Florestano, H. J., Bahler, M. E., and Jeffries, S. F., *J. Am. Pharm. Assoc., Sci. Ed.*, **45,** 538 (1956). Lesser, M. A., *Drug and Cosmetic Ind.*, **73,** 764 (1953). Hilfer, H., *Ibid.*, **77,** 41 (1955). Kalz, F., and Scott, A., *Arch. Dermatol.*, **73,** 355 (1956). Collins, A. P., and Zopf, L. C., *Am. Prof. Pharmacist*, **22,** 691 (1956). Mutimer, M. N., Riffkin, C., Hill, J. A., Glickman, M. E., and Cyr, G. N., *J. Am. Pharm. Assoc., Sci. Ed.*, **45,** 212 (1956). Perlman, H. H., and Leuallen, E. E., *J. Pediat.*, **43,** 578 (1953).
(17) Jacobi, O., and Heinrich, H., *Drug and Cosmetic Ind.*, **75,** 34 (1954). Lubowe, I. I., *Ibid.*, **77,** 43 (1955). Kvorning, S. A., *Acta Pharmacol. et. Toxicol.*, **6,** 13 (1950). Kvorning, S. A., and Svendsen, I. B., *J. Invest. Dermatol.*, **26,** 421 (1956). Frazier, C. N., and Blank, I. H., "A Formulary for External Therapy of the Skin," Springfield, Ill., Charles C Thomas (1954). Martin-Scott, I., and Ramsay, A. G., *Brit. Med. J.*, 1525 (June 30, 1956). Brain, R. T., *Ibid.*, 299 (Aug. 4, 1956).
(18) Burks, J. W., Jr., *J. Louisiana Med. Soc.*, **107,** 29 (1955). Grady, E. D., *U. S. Armed Forces Med. J.*, **7,** 1471 (1956).

(19) Pinkus, H., *J. Invest. Dermatol.*, **19,** 431 (1952).
(20) Kvorning, S. A., *Acta Pharmacol. et. Toxicol.*, **12,** 222 (1956).
(21) Michelfelder, T. J., and Peck, S. M., *J. Invest. Dermatol.*, **19,** 237 (1952).
(22) Draize, J. H., and Alvarez, E., *Proc. Sci. Sect. Toilet Goods Assoc.*, **12,** 12 (1949). Traub, E. F., and Spoor, H. J., *J. Soc. Cosmetic Chem.*, **6,** 200 (1955).
(23) Linoli, O., *Scientia Med. Ital.*, **3,** 173 (1954).
(24) Witten, V. H., Brauer, E. W., Loevinger, R., and Holmstrom, V., *J. Invest. Dermatol.*, **26,** 437 (1956). Scott, A., and Kalz, F., *Ibid.*, **26,** 149 (1956).
(25) Butcher, E. O., *J. Invest. Dermatol.*, **21,** 43 (1953).
(26) Flesch, P., *Proc. Sci. Sect. Toilet Goods Assoc.*, **23,** 24 (1955).
(27) Shelley, W. B., and Melton, F. M., *J. Invest. Dermatol.*, **13,** 61 (1949). Matoltsy, A. G., and Matoltsy, M., *J. Pharmacol. and Ex. Therap.*, **102,** 237 (1951). Perry, D. J., Falk, M. S., and Pillsbury, D. M., *J. Invest. Dermatol.*, **11,** 461 (1948). Peck, S. M., Finkler, B., Mayer, G. G., and Michelfelder, T., *Ibid.*, **14,** 177 (1950). Hearin, D. L., and Mori, P. P., *Ibid.*, **14,** 391 (1950). Inman, P., and Cowan, I. C., *Brit. Med. J.*, 1064 (May 17, 1952).
(28) Dews, P. B., Wnuck, A. L., Fanelli, R. V., Light, A. E., Tornaben, J. A., Norton, S. Ellis, C. H., and deBeer, E. J., *J. Pharmacol. and Exp. Therap.*, **107,** 1 (1953).
(29) Light, A. E., and Tornaben, J. A., *Ann. Allergy*, **9,** 607 (1951).
(30) Fisher, R. S., *Arch. Dermatol.*, **73,** 336 (1956). Fisher, R. S., Freimuth, H. C., O'Connor, K. A., and Johns, V., *J. Am. Med. Assoc.*, **157,** 503 (1955).
(31) Speirs, R. S., *Science*, **113,** 621 (1951).
(32) Schaefer, A. E., Sassaman, H. L., Slocum, A., and Greene, R. D., *J. Nutrition*, **59,** 171 (1956).
(33) Siemers, G. F., and Sleezer, P. E., *Drug and Cosmetic Ind.*, **74,** 38 (1954). Sobel, A. E., *Arch. Dermatol.*, **73,** 388 (1956).
(34) Villela, G. G., *Rev. brasil. biol.*, **14,** 443 (1954).
(35) Weiss, W., *Am. J. Med. Sci.*, **231,** 13 (1956).
(36) *Drug and Cosmetic Ind.*, **74,** 641 (1954).
(37) Council on Pharmacy and Chemistry, *J. Am. Med. Assoc.*, **145,** 728 (1951).
(38) Thomson, J. F., Savit, J., and Goldwasser, E., *J. Pharmacol. and Exp. Therap.*, **89,** 1 (1947). Sinclair, D. C., *Brit. Med. J.*, 290 (Aug. 7, 1948).
(39) Bhatia, V. N., and Barber, R. H., *J. Am. Pharm. Assoc., Sci. Ed.*, **44,** 342 (1955). Brockemeyer, E. W., and Guth, E. P., *Ibid.*, **44,** 706 (1955).
(40) Bhatia, V. N., and Zopf, L. C., *J. Am. Pharm. Assoc., Sci. Ed.*, **41,** 543 (1952).
(41) Wernsdorfer, R., *Klin. Wochsch.*, **33,** 626 (1955).
(42) Scott, A., and Kalz, F., *J. Invest. Dermatol.*, **26,** 361 (1956). Malkinson, F. D., and Ferguson, E. H., *Ibid.*, **25,** 281 (1955).
(43) Livingood, C. S., Hildebrand, J. F., Kay, J. S., and Smith, R. W., Jr., *Arch. Dermatol.*, **72,** 313 (1955). Haxthausen, H., *J. Invest. Dermatol.*, **26,** 111 (1956). Fitzpatrick, J. B., Griswold, H. C., and Hicks, J. H., *J. Am. Med. Assoc.*, **158,** 1149 (1955).
(44) Whitaker, W. L., and Baker, B. L., *J. Invest. Dermatol.*, **17,** 69 (1951).
(45) Light, A. E., *Proc. Sci. Sect. Toilet Goods Assoc.*, **22,** 10 (1954). Dunaif, C. B., and Finerty, J. C., *J. Invest. Dermatol.*, **15,** 363 (1950).
(46) Shapiro, I., *J. Med. Soc. N. J.*, **50,** 17 (1953).
(47) Horwitz, O., Montgomery, H., Longaker, E. D., and Sayer, A., *Am. J. Med. Sci.*, **218,** 669 (1949). Haley, T. J., and Andern, M. R., *J. Pharmacol. and Exp. Therap.*, **100,** 393 (1950). Scott, M. G., *Nature*, **175,** 395 (1955).
(48) Lange, K., and Weiner, D., *J. Invest. Dermatol.*, **12,** 263 (1949). Freedman, I., *Angiology*, **6,** 52 (1955). Friend, D., and Edwards, E., *Arch. Int. Med.*, **93,** 929 (1954). MacArthur, J. G., and Alstead, S., *Lancet*, **265,** 1060 (1953). A Useful Vasodilator, *Ibid.*, **260,** 1263 (1951).
(49) Stuttgen, G., and Wurst, H., *Hautarzt.*, **6,** 172 (1955).
(50) Selby, C. C., J. Soc. Cosmetic Chem., **7,** 584 (1956).
(51) Pappenheimer, J. R., *Physiol. Revs.*, **33,** 387 (1953). Osterhout, W. J. V., *J. Gen. Physiol.*, **39,** 963 (1956).
(52) Farber, E. M., and Batts, E. E., *Arch. Dermatol. and Syphilol.*, **70,** 653 (1954).
(53) Kanof, N. B., *N. Y. State J. Med.*, **55,** 3103 (1955). Lerner, A. B., *Am. J. Med.*, **19,** 902 (1955).
(54) Fitzpatrick, T. B., Hopkins, C. E., Blickenstaff, D. D., and Swift, S., *J. Invest. Dermatol.*, **25,** 187 (1955). Hilfer, H., *Drug and Cosmetic Ind.*, **78,** 462 (1956).
(55) Moretti, G., and Mescon, H., *J. Invest. Dermatol.*, **26,** 347 (1956).
(56) Findlay, G. H., *Brit. J. Dermatol.*, **67,** 83 (1955). Montagna, W., *J. Biophysics, Biochem. Cytol.*, **1,** 13 (1955). Berliner, R. W., and Orloff, J., *Pharmacol. Revs.*, **8,** 137 (1956).

(57) Foraker, A. G., and Wingo, W. J., *Arch. Dermatol.*, **72,** 1 (1955).
(58) Griesemer, R. D., and Gould, E., *J. Invest. Dermatol.*, **25,** 383 (1955).
(59) Shelley, W. B., Cohen, S. B., and Koelle, G. B., *J. Invest. Dermatol.*, **24,** 561 (1955).
(60) Lesser, M. A., *Drug and Cosmetic Ind.*, **73,** 178 (1953). Rattner, H., and Sutton, C., *Med. Clin. N. A.*, **40,** 33 (1956). Andrew, W., *J. Soc. Cosmetic Chem.*, **6,** 299 (1955). Strauss, J., Jr., and Necheles, H., *J. Lab. and Clin. Med.*, **33,** 612 (1948). Canizares, O., *N. Y. State J. Med.*, **56,** 2967 (1956). Cooper, Z. K., "Aging of the Skin," in Cowdry's "Problems of Aging," Edited by A. I. Lansing, 3rd Ed., Baltimore, The Williams & Wilkins Co. (1952), p. 764. Chieffi, M., "Cosmetological Aspects of Aging," *Ibid.*, p. 909.
(61) Goldzieher, J. W., Roberts, I. S., Rawls, W. B., and Goldzieher, M. A., *Arch. Dermatol. and Syphilol.*, **66,** 304 (1952). Eller, J. J., and Eller, W. D., *Ibid.*, **59,** 449 (1949).
(62) Behrman, H. T., *J. Am. Med. Assoc.*, **155,** 119 (1954).
(63) Still, W. J. S., and Boult, E. H., *Lancet*, **271,** 117 (1956). Hill, W. R., and Montgomery, H., *J. Invest. Dermatol.*, **3,** 231 (1940). Ejiri, I., *Jap. J. Dermatol. and Urol.*, **41,** 95 (1937). Bourne, G. H., *Nature*, **177,** 467 (1956). Kirk, E., and Kvorning, S. A., *J. Gerontol.*, **4,** 273 (1949). Calkins, E., and Bauer, W., *Med. Clin. N. Am.*, **39,** 325 (1955). Reed, R., Wood, M. J., and Keech, M. K., *Nature*, **177,** 697 (1956).
(64) Flesch, P., *J. Soc. Cosmetic Chem.*, **6,** 377 (1955). Carruthers, C., Woernley, D. L., Baumler, A., and Shorts, H., *Ibid.*, **6,** 324 (1955). Schmidli, B., and Paschoud, J. M., Dermatologica, **110,** 315 (1955). Steincke, K., *Acta Pharmacol. et Toxicol.*, **12,** 126 (1956). Matoltsy, A. G., and Herbst, F. S. M., *J. Invest. Dermatol.*, **26,** 339 (1956). Washburn, W. W., *Ibid.*, **23,** 97 (1954). Montagna, W., Chase, H. B., and Hamilton, J. B., *Ibid.*, **17,** 147 (1951). Montagna, W., Eisen, A. Z., Rademacher, A. H., and Chase, H. B., *Ibid.*, **23,** 23 (1954). Kvorning, S. A., and Kirk, E., *J. Gerontol.*, **4,** 113 (1949). Sheinaus, H., Christian, J. E., and Sperandio, G. J., *J. Am. Pharmaceut. Assoc., Sci. Ed.*, **44,** 483 (1955).
(65) Edholm, O. G., Fox, R. H., and Macpherson, R. K., *J. Physiol.*, **132,** 15P (1956). Montgomery, H., and Horwitz, O., *J. Clin. Invest.*, **29,** 1120 (1950). Wayne, E. J., *Brit. Med. J.*, 718 (Sept. 25, 1954). Zweifach, B. W., and Metz, D. B., *Am. J. Physiol.*, **182,** 155 (1955). Burton, A. C., *Physiol. Rev.*, **34,** 619 (1954). Reynolds, S. R. M., Hamilton, J. B., di Palma, J. R., Hubert, G. R., and Foster, F. I., *J. Clin. Endocrinol.*, **2,** 228 (1942). Drinker, C. K., and Field, M. E., "Lymphatics, Lymph and Tissue Fluid," Baltimore, The Williams & Wilkins Co. (1933). Wolstenholme, G. E. W., and Freeman, J. S., "Peripheral Circulation in Man," Boston, Little, Brown and Co. (1954). "Vascular Patterns as Related to Function," Second Conference on Microcirculatory Physiology and Pathology, April 5, 1955, Philadelphia, Pa., The Williams & Wilkins Co., Baltimore (1955). Zweifach, B. W., *Ann. N. Y. Acad. Sci.*, **61,** 670 (1955). McMaster, P. D., *Ibid.*, **46,** 743 (1946). Andrus, E. C., *Circulation*, **7,** 437 (1953). Akers, R. P., and Zweifach, B. W., *Am. J. Physiol.*, **182,** 529 (1955). Blum, L., *J. Mt. Sinai Hosp.*, **21,** 87 (1954).
(66) Light, A. E., *J. Invest. Dermatol.*, **13,** 53 (1949).
(67) Carruthers, C., and Suntzeff, V., *J. Invest. Dermatol.*, **23,** 77 (1954). Wolbach, S. B., and Bessey, O. A., *Physiol. Rev.*, **22,** 233 (1942).
(68) Jambor, J. J. and Suskind, R. R., *J. Invest. Dermatol.*, **24,** 379 (1955). Wolf, S., *Am. J. Med.*, **20,** 919 (1956). Kramár, J., Meyers, W. V., and Wilhelmj, C. M., Jr., *Proc. Soc. Exp. Biol. and Med.*, **89,** 528 (1955). Hartman, M. M., *Am. J. Med.*, **21,** 85 (1956). Tuft, L., Heck, M., and Gregory, D. C., *J. Allergy*, **26,** 359 (1955). Germuth, F. G., Jr., *Pharmacol. Revs.*, **8,** 1 (1956). Ginsberg, J., and Duff, R. S., *Brit. J. Pharmacol.*, **11,** 180 (1956).
(69) Dawson, C. R., *Trans. N. Y. Acad. Sci., Ser. II*, **18,** 427 (1956).
(70) Blair, E. L., Wakefield, M., and Ingram, G. I. C., *Nature*, **176,** 563 (1955). Asboe-Hansen, G., and Wegelius, O., *Ibid.*, **178,** 262 (1956).
(71) Jones, I. S., and Mayer, K., *Proc. Soc. Exp. Biol. and Med.*, **74,** 102 (1950). Gillman, T., Penn, J., Brooks, D., and Roux, M., *Nature*, **176,** 932 (1955).
(72) Peters, J. P., *Physiol. Revs.*, **24,** 491 (1944). Hellon, R. F., Lind, A. R., and Weiner, J. S., *J. Physiol.*, **133,** 118 (1956). Hardy, J. D., Milhorat, A. T., and Dubois, E. F., *J. Nutrition*, **21,** 383 (1941). Farber, E. M., and Lobitz, W. C., *Ann. Rev. Physiol.*, **14,** 519 (1952). Magnin, P., Fournie, G., and Gaté, A., *Bull. fédération soc. gynécol. et obstét. langue franc.*, **5,** 190 (1953).
(73) Crowley, L. V., Ryder, R. R., III, and Pollack, H., *Metabolism*, **5,** 272 (1956).