Effect of Biotin Deficiency Upon the Skin of Mice. (17600)

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Wilson *et al.*(1) have shown that nutritional biotin deficiency in the mouse, is expressed in severe dermatotic symptoms which are superficially similar to those described by Sullivan and Nicholls(2) in the rat. This paper reports the histological changes in the skin of biotin-deficient mice. Several of these details, and particularly the changes in the sebaceous glands, and the behavior of connective tissue cells in the dermis have not been reported hitherto either in the rat or the mouse.

Methods. The animals used in this study are those described by Wilson *et al.*(1) and Wilson and Leduc(3) in a study of biotin deficiency in the mouse. The procedures for rendering the animals deficient in biotin are described in detail by these authors. The 50 animals used here were chosen to represent several degrees of symptomatic deficiency. Those animals which will be referred to as very deficient showed extreme symptoms and were nearly moribund. Skin was removed from the mid-dorsum and from the perineal region, including the whole anal canal. The perineal skin was particularly useful in the study of sebaceous glands which are large and numerous in this region. Tissues fixed in Zenker-formol were stained in Delafield's hematoxylin and eosin, and in Heidenhain's hematoxylin. Other sections were stained in eosin-methylene blue to reveal cytoplasmic basophilia, and with toluidin blue for the study of metachromasia (see Lison,(4) for evaluation and manipulation of this technic).

The lipids were demonstrated in frozen sections of tissues fixed in formol-calcium and then chromated in dichromate-calcium of Baker(5). Sections were treated with Sudan black and with oil-blue N. It has become increasingly evident that the lipids are much more clearly discerned in tissues thus treated than in tissues simply fixed in formol-calcium. For the demonstration of delicate lipid granules, Sudan black is superior to oil-blue N. Unstained frozen sections were studied under the phase-contrast microscope for the fine morphology, and under polarized light for birefringence. Representative sections were treated with the Romieu test for cholesterol and its esters (from Lison)(6), after being oxidized for 3 days in 2.5% ferric alum at 37°C. In all of these studies, the skin of experimental mice is compared with that of normal control animals.*

Results. The slightly thicker than normal epidermis of moderately deficient mice is usually keratotic, and contains numerous keratohyalin granules. Mitotic figures in the lower layers of the stratum spinosum and in the basal layer appear slightly more numerous than in normal skin. Hair follicles are normal, but in the sebaceous glands some of the peripheral cells are fragmented. The non-sebaceous epithelial elements associated with the sebaceous glands are thicker than in normal glands. There is some mitotic activity in the non-sebaceous elements and in the cells at the periphery of the glands. Histiocytes, mast-cells, eosinophiles and other leucocytes, in the dermis appear to have increased in number.

In very deficient mice, all of the changes mentioned above are accentuated. The stratum corneum and the whole epidermis are thicker and the keratohyalin granules in the upper cells of the stratum spinosum are more numerous. In some animals conspicuous epi-

1. Wilson, J. W., Leduc, E. H., and Winston, D. H., *J. Nutr.*, 1949, v38, 73.

2. Sullivan M., and Nicholls, J., *Arch. Derm. and Syph.*, 1942, v45, 295.

3. Wilson, J. W., and Leduc, E. H., *Growth*, in press.

4. Lison, L., *Arch. Biol.*, 1935, v46, 559.

5. Baker, J. R., *Quart. J. Micro. Sci.*, 1946, v87, 441.

6. Lison, L., 1936, Gauthier-Villars, Paris.

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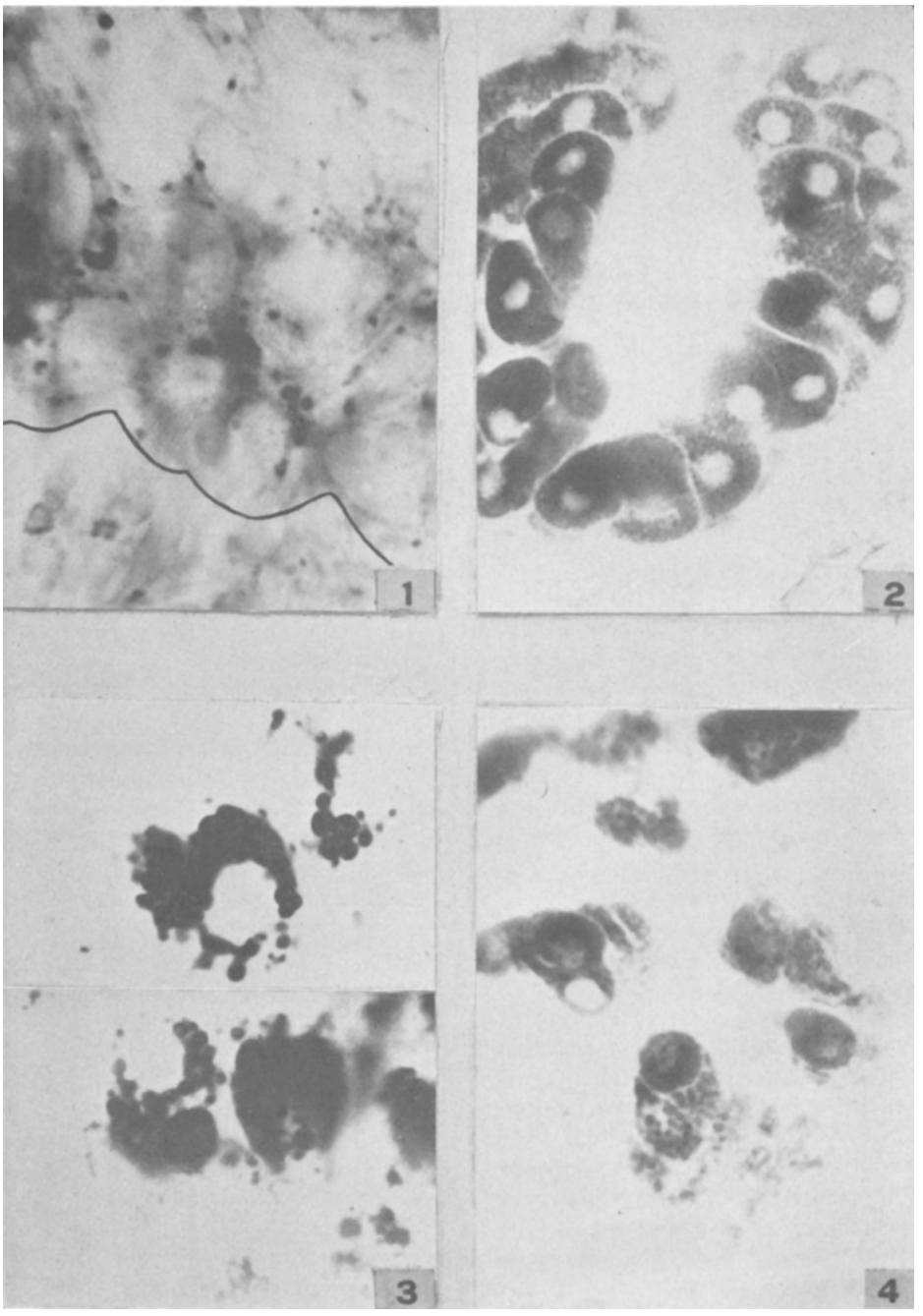

PLATE 1.

All of the frozen sections figured are cut at 5 μ and colored with Sudan black.

FIG. 1. Skin of a biotin-deficient mouse. Inked line separates epidermis from dermis. The coarser lipid bodies in the epithelial cells resemble Golgi elements. The finer lipid bodies, not clearly shown, surround the nuclei. 1125 ×.

FIG. 2. Sebaceous gland of skin of a normal mouse. Note especially the fine lipid granules in sebaceous cells. 562.5 ×.

FIG. 3. Details of sebaceous glands of a very deficient animal. Compare with Fig. 2. 675 ×.

FIG. 4. Fragmenting sebaceous gland of a very deficient mouse. 562.5 ×.

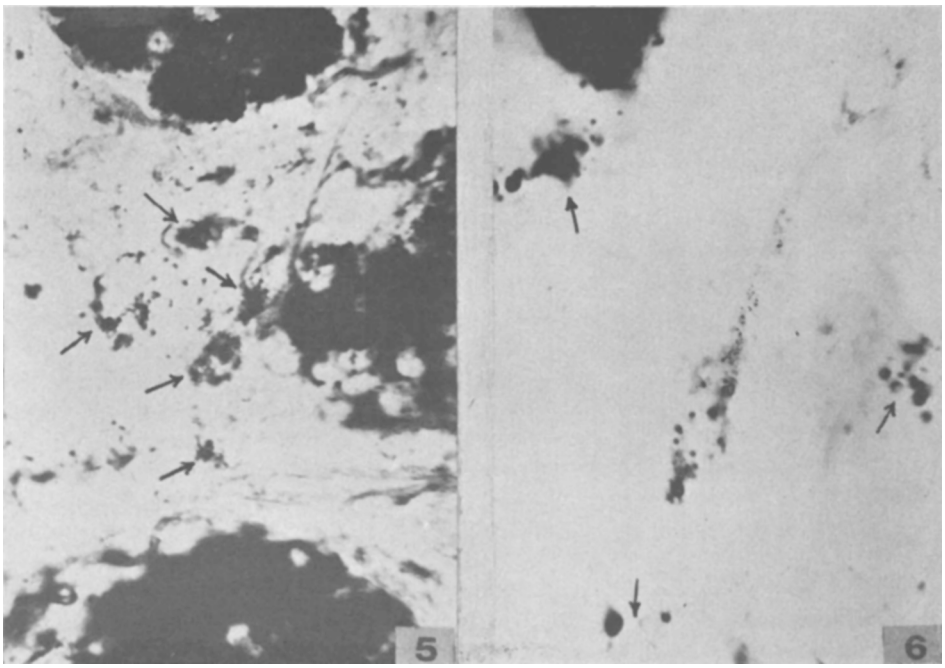

PLATE 2.

FIG. 5. Macrophages, laden with lipids, surrounding fragmenting sebaceous cells of a very deficient mouse. Arrows indicate the larger macrophages. 450 ×.

FIG. 6. Pigment-bearing cell with one pole laden with lipid spherules. Arrows indicate surrounding macrophages. 900 ×.

dermal papillae grow down into the dermis as described in the rat by Sullivan and Nicholls.(2) Many sebaceous glands show cellular fragmentation at the periphery. Some mitotic figures can be seen at the periphery of the sebaceous glands of very deficient animals. The cell population of the dermis is increased. Some of the cells appear mesenchymatous, with a dense nucleus and basophilic cytoplasm. Numerous macrophages surround the fundus of sebaceous glands (in normal skin it is almost impossible to recognize macrophages). Mast cells are more numerous than in normal skin. Although some of the mast cells are normal, many possess long filamentous processes. In these cells metachromatic granules are sparsely distributed in the cytoplasm; occasionally they are found only in the cytoplasmic processes. While in normal cells the metachromatic granules are fairly uniform in size, their size is variable in cells where they are scantily disseminated in the cytoplasm. The differences in the state of these cells recall those described by Paff *et al.*(7) in mast cells cultivated *in vitro*. Since Oliver *et al.*(8) have presented evidence that mast cells elaborate heparin, there is a suggestion here of an increase in the amount of heparin released in the dermis of biotin deficient mice. Numerous heterophylic leucocytes and lymphocytes, and a variable number of eosinophiles, also invade the dermis.

The thickened stratum corneum of deficient mice is moderately sudanophilic. The cells of the epidermis possess discrete sudanophilic spherules and rodlets around the nucleus (Fig. 1). The spherules, which contain a sudanophobic center, are usually clustered at one pole of the nucleus. Sudanophilic spherules and perinuclear rodlets are also present in the cells of the bulb and root-sheath of hair follicles and in the ducts of sebaceous glands. In the sebaceous ducts the sudanophilic bodies

7. Paff, G. H., Bloom, F., and Reilly, C., *J. Exp. Med.*, 1947, v86, 117.

8. Oliver, J., Bloom, F., and Mangieri, C., *J. Exp. Med.*, 1947, v86, 107.

described are more conspicuous. The distribution of the lipid bodies just described is identical with that in the skin of normal mice.

The sebaceous cells of biotin deficient mice, regardless of their position in the gland, contain very large lipid globules (Fig. 3). In nearly all of the glands some peripheral cells show degenerative fragmentation (Fig. 4). The central sebaceous cells seldom show normal sebaceous maturation.

Numerous macrophages, whose cytoplasm is laden with lipid globules or granules, surround the sebaceous glands of deficient animals (Fig. 5). In colored mice, the pigment-bearing cells which surround sebaceous glands also contain variable numbers of lipid droplets (Fig. 6). The numerous mast cells in the dermis contain delicate lipid granules scantily distributed in the cytoplasm.

Although there is a mild reduction in the amount of adipose tissue in the subcutaneous tela, the fatty depots are never depleted as they are in the rat (Sullivan and Nicholls) (2). Furthermore, in some animals there is a fatty infiltration in the dermis.

Sebum is practically absent from the sebaceous glands of very deficient mice. When the pilosebaceous exits have become cystic, the contents of the cysts are meagerly sudanophilic. In deficient animals the sebaceous lipids are usually isotropic. The application of the Liebermann-Burchard test to frozen sections of skin of deficient mice seldom gives a positive reaction in the sebaceous glands.

Discussion. Although the dermatotic symptoms of biotin deficient mice resemble superficially those described by Sullivan and Nicholls(2) in the rat, there are some differences between the rat and the mouse. In deficient rats the skin is covered by a brownish encrustation (Sullivan and Nicholls),(2) but in the mouse it is dry. Keratosis is well developed in the rat but only moderate in the mouse. The pilosebaceous exits are dilated in deficient rats but only rarely in the mouse. The stratum corneum is sudanophilic in deficient rats but it is only mildly so in the mouse. The subcutaneous fatty depots are resorbed in deficient rats, but they remain virtually unchanged in the mouse.

The orifices of the pilosebaceous units in deficient mice are usually plugged by keratinized debris, yet the glands are seldom cystic. This suggests that there is practically no excretion of sebum upon the surface of the skin, and explains the dryness of the skin and the small amount of demonstrable lipid in the stratum corneum of deficient animals.

The sudanophilic inclusions described in the epidermal cells, and in the cells of the pilosebaceous units resemble the lipid part of the Golgi element such as described by Baker(9) and Cain,(10) or the myelin figures of Palade and Claude(11,12) (Fig. 1). Since these and other lipid bodies are more numerous in the cells of the ducts of sebaceous glands of normal and biotin deficient mice, it seems that the early stages in sebaceous accumulation are normal in the skin of biotin deficient mice.

The differences between the sebaceous glands in normal skin and in the skin of biotin deficient animals have been described and are illustrated in Fig. 2, 3, and 4. In biotin deficient skin, the fragmenting sebaceous glands are surrounded by baskets of histiocytes whose cytoplasm is engorged with lipid granules of varying sizes (Fig. 5) whereas in normal skin histiocytes seldom contain lipids. In colored mice, many of the pigment-bearing cells also contain lipid granules and spherules. Lipid inclusions are more abundant in the small pigment-bearing cells than in the larger dermal chromatophores, although some of these also contain demonstrable lipids (Fig. 6). It is not established if these pigment-bearing cells are melanophores which have phagocytized lipids, or if they are dying cells, or if they are macrophages which have engulfed the pigment of fragmented melanophores as well as the lipids released by the breakdown of sebaceous cells. Unlike the situation in man (Masson),(13) lipids are not normally found in the melanoblasts of the mouse. Becker(14)

9. Baker, J. R., *Quart. J. Micro. Sci.*, 1944, v85, 1.

10. Cain, A. J., *Oxford Sci.*, 1949, v2, 30.

11. Palade, G. E., and Claude, A., *J. Morph.*, 1949, v85, 35.

12. Palade, G. E., and Claude, A., *J. Morph.*, 1949, v85, 71.

13. Masson, P., from The Biology of Melanomas, *Spec. Publ. N. Y. Acad. Sci.*, 1948, v4, 15.

has observed that in human skin the perivascular histiocytes in the dermal papillae phagocytize lipids as well as pigment.

Growth of sebaceous glands and sebaceous accumulation is not impeded by biotin deficiency in mice, but normal storage of lipids in the sebaceous cells and the release of sebum is impaired. Growth of the glands is indicated by the presence of intermediate sebaceous cells and by mitotic activity in the peripheral cells (cf. Montagna and Kenyon(15) for the growth of sebaceous glands in the rabbit). This is in accord with the reports of Bosse and Axelrod(16) who find that wound healing in the skin of biotin deficient rats occurs as rapidly as in normal skin. There is an agreement here also with the conclusions of Wilson and Leduc(3) that in animals deficient in biotin "the capacity of tissue cells for mitosis is not impaired", despite a decline in the mitotic activity in the liver during the development of the deficiency. Abnormal functions of the sebaceous glands, however, is indicated by the absence of brown seborrheal incrustation, the scantiness of sudanophilia in the stratum corneum, the absence of birefringent lipids, the failure of the lipids to give a positive Liebermann-Burchard test, and by the fragmentation of the peripheral sebaceous cells.

Summary and Conclusions. In the mouse the growth potentials of the skin and its appendages are not appreciably affected by biotin deficiency, but normal differentiation and function are impaired. There is a slight increase in mitotic activity in the epidermis and in the sebaceous glands. The pilosebaceous units are blocked by keratinized debris and little or no sebum is released upon the surface of the skin. In the sebaceous cells, the stored lipid droplets are much larger than in those of normal animals. Unlike the sebaceous lipids of normal glands, those of biotin deficient mice are isotropic and negative to the Liebermann-Burchard test. There is abundant cellular fragmentation at the periphery of sebaceous glands and the lipids are apparently phagocytized by histiocytes in the dermis. The melanin-bearing cells around the sebaceous cells, when present, also contain lipid droplets. As a rule, neither histiocytes nor melanin-bearing cells contain demonstrable lipids in the skin of normal mice. There is an increase in the number of mesenchymatous cells, mast cells and leukocytic cells in the dermis of biotin deficient mice.

14. Becker, S. W., from The Biology of Melanomas, *Spec. Publ. N. Y. Acad. Sci.*, 1948, v4, 82.

15. Montagna, W., and Kenyon, P., *Anat. Rec.*, 1949, v103, 665.

16. Bosse, M. D., and Axelrod, A. E., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 418.

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Preliminary Observations on the Antiarthritic Effect of 21-Acetoxypregnenolone.* (17601)

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It has been shown by Seifter, Baeder, and Begany(1) that the permeability of the synovial membrane in rabbits is influenced by enzymes and by steroids. Hyaluronidase and desoxycorticosterone (DOCA) were found to markedly increase permeability, while corti-

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1. Seifter, J., Baeder, D. H., and Begany, A. J., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 277.