Effect of Strychnine on the Cat's Electrocerebellogram. (17589)

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In the course of our experiments on the response of the cerebellum to various epileptogenic agents, we have observed an interesting change in the electrocerebellogram of the cat following the use of strychnine. Dow(1) has previously reported no effect on the electrical activity of the cerebellum following the application of 1% strychnine to the cerebellum except for some lowering of the amplitude and frequency of the action potentials. In one of his experiments, however, the application of 1/1000 strychnine caused the appearance of groups of large rhythmic waves at a frequency of about 70 per second. Miller(2) applied 1% strychnine nitrate to the surface of the cerebellum of cats and observed rhythmical tremors.

Methods. 75 cats were used in these studies. The animals were prepared under a brief period of ether anesthesia followed by local anesthesia with 1% procaine. The trachea was cannulated, the animal curarized, and respirations maintained artificially. Recordings were made from the cerebral cortex by removing the calvarium and applying silver-tipped electrodes to the surface of the cortex, and from the cerebellum by means of electrodes applied directly to the surface of the cerebellum, or by phonograph needle electrodes inserted through the suboccipital bone and bony tentorium. A Grass, eight-channel, ink-writing electroencephalograph was used. Following a period of control recording, 1% strychnine was injected in varying amounts intravenously, into the cerebellar cortex or into the dentate nucleus. It was also placed on the surface of the cerebellum by means of small pledgets of cottonoid.

Results. When strychnine is applied topically to the cerebellum, injected into the cerebellar cortex or dentate nucleus, or given intravenously, the electrocerebellogram develops rhythmical waves with a frequency of 10-30 per second, and a voltage of 100 to 400 microvolts occurring in periodic discharges. The time and type of onset, duration of each discharge, length of time between discharges, and persistence of the discharges depend to a great extent on the dose and mode of administration, although there is considerable variation from animal to animal (Fig. 1). The dose of strychnine required to produce an effect varied with the mode of administration. Intravenous administration or injection into the dentate nucleus of strychnine in doses of .018 to .022 mg per kilo produced in most instances rhythmical discharges. At times even smaller amounts (.011 mg per kilo) given intravenously were effective. When injected into the cerebellar cortex, approximately 0.3 mg was the necessary dose. Topical application by means of a small pledget of cottonoid soaked in 1% strychnine caused no changes, whereas the use of 2½% strychnine was effective only after 1½ to 2 hours. The time and type of onset varied. In some instances, within a few seconds after administration of the strychnine, a synchronous, rhythmical discharge appeared from the cerebellum with a frequency of 10-30 per second. The voltage increased rapidly to about 200 to 400 microvolts. In other cases changes occurred in the electrocerebellogram in from 2-30 minutes after strychnine was given. At times, the onset of a long rhythmical discharge was preceded by many minutes of intermittent bursts of 5-6 high voltage waves. This type of burst was also frequently seen between the periodic rhythmical discharges. The frequency of the discharge would also change, being perhaps 10 per second initially, gradually increasing to 15-20 per second and then gradually decreasing again. These rhythmical

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1. Dow, R. S., *J. Physiol.*, 1938, v94, 67.
2. Miller, F. R., *Science*, 1920, v51, 413.

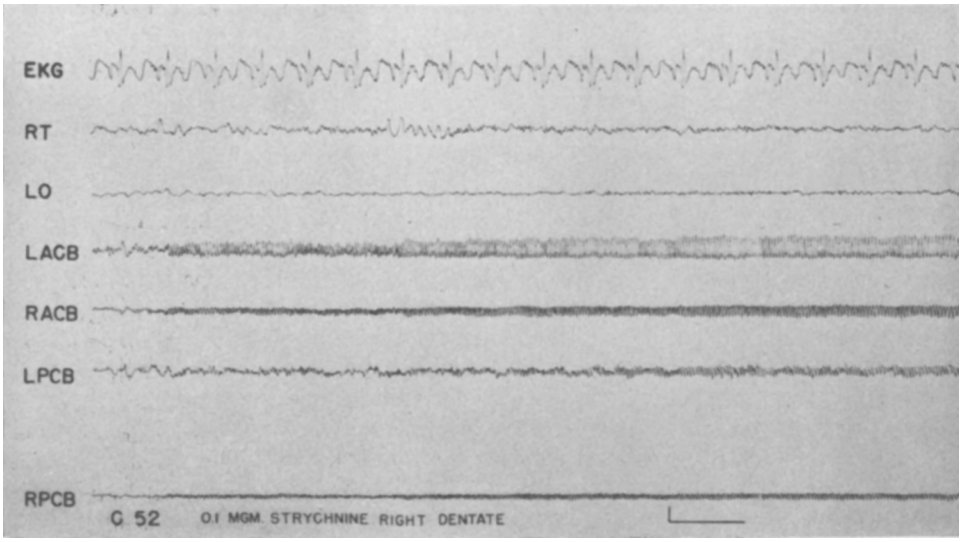

FIG. 1.

This record shows the electrocorticogram and electrocerebellogram of a cat after the injection of 0.1 mg of strychnine into the right dentate nucleus. The change in the electrocerebellogram is shown with the development of high voltage synchronous rhythmical waves. The abbreviations are: EKG, electrocardiogram; RT, right temporal region; LO, left occipital; LACB, left anterior cerebellum; RACB, right anterior cerebellum; LPCB, left posterior cerebellum; RPCB, right posterior cerebellum; LD, left dentate nucleus. The records are bipolar recordings made on a Grass electroencephalograph. The horizontal line at the base represents one second and the vertical line a calibration of 200 microvolts.

discharges lasted for varying lengths of time, from 10 seconds to as long as 2½ hours. Usually, however, the individual discharge had a duration of 30 seconds to 3 minutes, recurring at intervals of 1 to 3 minutes for 30 minutes to an hour. In one animal the rhythmical discharges recurred for a period of 4 hours.

In general, when the strychnine was given in doses just sufficient to produce a response, changes occurred in less than a minute after the intravenous injection, in a slightly longer interval after injection into the dentate nucleus, and in 10 to 15 minutes after injection into the cerebellar cortex. The changes, however, appeared to be more persistent after the intracortical injection, lasting for 1 to 2½ hours, whereas after the intravenous injection, the changes usually lasted for only about 30 minutes. Individual discharges of the highest voltage and longest duration were obtained following intravenous injection. Recordings were generally taken from the anterior and posterior surfaces of the two cerebellar hemispheres, the dentate nuclei, and from the temporal, occipital, and frontal cortex of the cerebral hemispheres. In most animals the discharge appeared from all areas of the cerebellum simultaneously. Frequently, however, the voltage was considerably higher from the dentate nucleus and the anterior surface of the cerebellum. In 2 animals the changes appeared first from these regions. These changes in the electrocerebellogram appear in the decerebrate animal as well as from the intact brain. Also, if decerebration is performed after the discharges have appeared, they continue unchanged (Fig. 2). With the amounts of strychnine used, usually an increase in frequency and a decrease in amplitude of the electrocorticogram was observed.

Discussion. Strychnine results in a different type of electroencephalographic response from the cerebellum than it does from the cerebrum. The so-called "strychnine spikes" have not been observed from the cerebellum. The site of origin of this discharge is not definitely known at this time. It appears to be an unusual type of response of the central

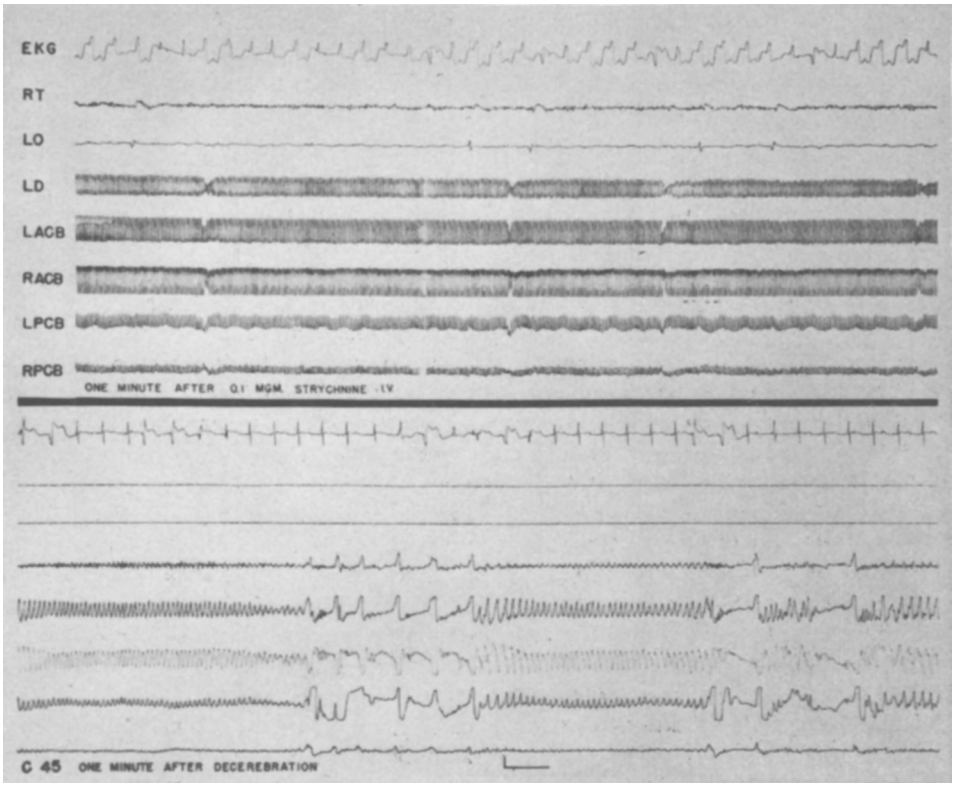

FIG. 2.

After the intravenous injection of 0.1 mg of strychnine, the electrocerebellogram of this cat shows rhythmical waves of 200 to 400 microvolts at a frequency of 24 per second. The same type of waves, although at a slower frequency, are seen to persist after decerebration. The abbreviations are the same as in Fig. 1.

nervous system to stimulation. A few preliminary experiments have indicated that the same response can be obtained from the spinal cord, medulla, and pons in the absence of the cerebellum, so that it would seem that this effect of strychnine is not peculiar to the cerebellum. It is interesting to speculate as to why the response of the cerebellum to strychnine is so different from the response of the cerebrum. The synchronous rhythmical discharge may be related to the uniform histologic structure of the cerebellum.

Summary. When strychnine is applied topically to the cat's cerebellum, injected intravenously or into the dentate nucleus in doses of .018 to 022 mg per kilo, or injected into the cerebellar cortex in doses of 0.3 mg, the electrocerebellogram develops rhythmical waves with a frequency of 10-30 per second and a voltage of 100 to 400 microvolts occurring in periodic discharges of 30 seconds to 3 minutes duration and recurring at intervals of 1 to 3 minutes for 30 minutes to an hour.

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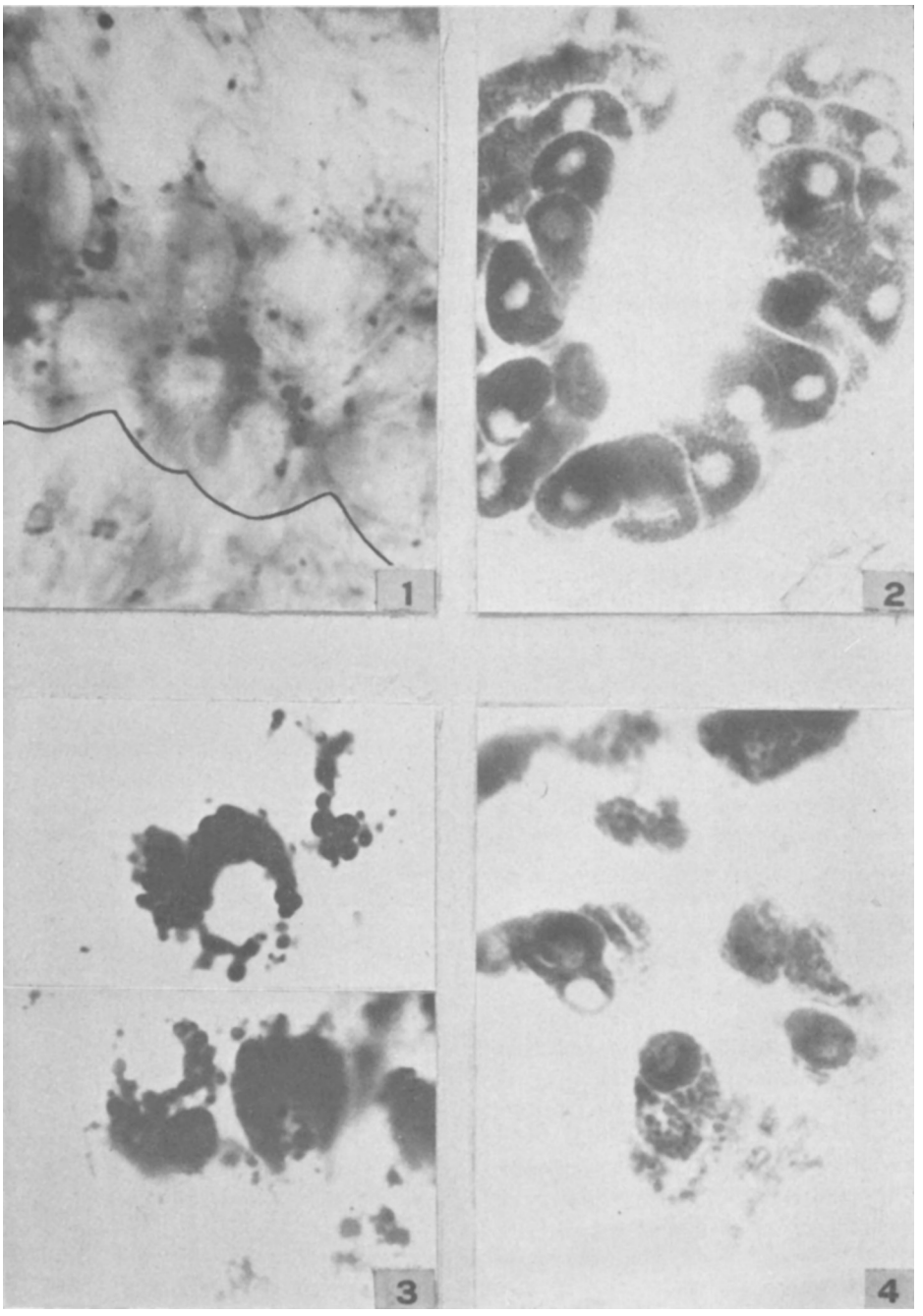

PLATE 1.

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 ×.

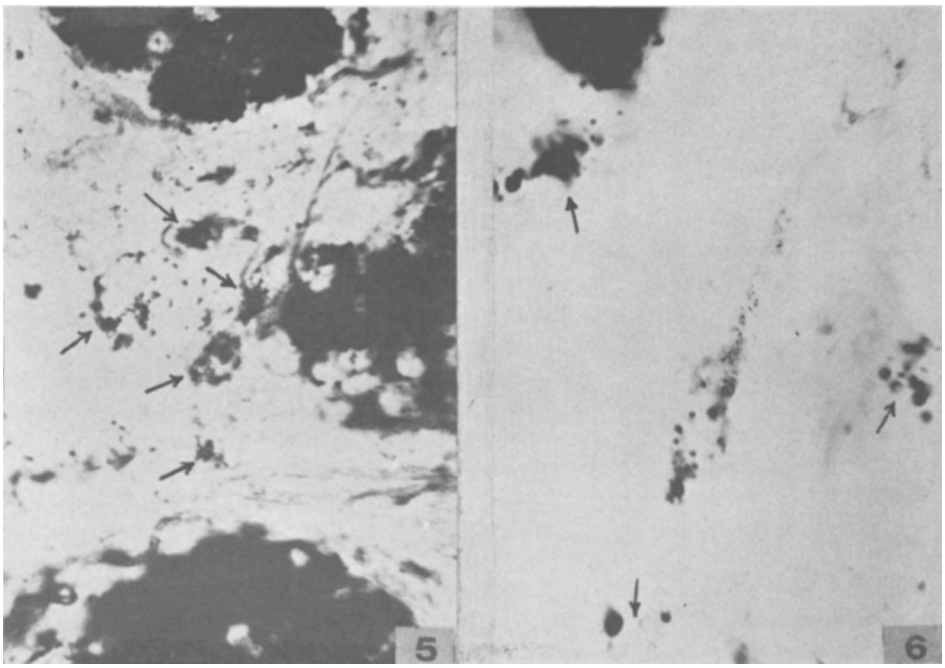

PLATE 2.

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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(Introduced by W. E. Ehrich)

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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.