coccal cells yielded so much precipitate that the total mass remained far above the graduated level in Hopkins tubes. The difference between this and the normal serum control was indeed striking, and could have been interpreted as capsular swelling had not the experiment with washed cells been conducted.

Although there is no capsular swelling, the data presented here suggest that there is some degree of capsular shrinkage. This is particularly apparent in cells harvested from mice because these cells have extremely large capsules. Further experimentation will be necessary to determine whether this apparent shrinkage has biological significance although from a statistical standpoint the differences in mean diameters presented in Table III are significant. It is possible that the apparent shrinkage seen under the microscope does not represent any volume change in the capsule but merely the change from an ellipsoid to a sphere during the antigen-antibody reaction. A shortcoming of the direct microscopic measurement is the fact that capsular boundaries in India ink are not as sharp as in antiserum, particularly in the counting chamber where the fluid depth is 0.1 mm. This accounts for the somewhat larger standard deviations obtained in counting chamber experiments. This criticism cannot be directed at centrifugation experiments; however, it is possible that the shrinkage observed here represents better packing in the presence of antibody.

Summary. 1. In studying the mechanism of the "capsular reaction" one of the first questions to be answered is whether or not the capsule swells when antibody is added. Data presented here indicate that it does not, using *C. neoformans* as a test organism. Exposure of washed, encapsulated *C. neoformans* to antibody results in a prominent clarification of capsular outline, but no increase in capsular size as determined by packed cell volume after centrifugation and by direct microscopic measurement. When cells with large capsules are employed, there is an apparent *decrease* in capsular size during the reaction with antibody. 2. If capsulated cells are not washed free of soluble capsular polysaccharide present in the cultural supernate, the soluble antigen coprecipitates when antibody is added. This may lead to the erroneous conclusion that there is an increase in capsular volume.

1. Neufeld, F., *Z. Hyg.*, 1902, v40, 54; Neufeld, F., and Etinger-Tulczynska, *ibid.*, 1931, v112, 492.
2. Klienberger, Nobel E., *J. Hyg.*, 1948, v46, 345.
3. Duguid, J. P., *J. Path. and Bact.*, 1951, v63, 673.
4. Edwards, P. R., and Fife, M. A., *J. Inf. Dis.*, 1952, v91, 92.
5. Tomcsik, J., *Ann. Rev. Biochem.*, 1953, v22, 351.
6. Johnson, F. H., and Dennison, W. L., *J. Immunol.*, 1944, v48, 317.
7. Neill, J. M., Castillo, C. G., Smith, R. H., and Kapros, C. E., *J. Exp. Med.*, 1949, v89, 93.
8. Evans, E. D., *J. Immunol.*, 1950, v64, 423; Banish, G., and Evans, E. E., unpublished data.
9. Evans, E. E., Sorensen, L. J., and Walls, K. W., *J. Bact.*, 1953, v66, 287.
10. Wood, W. B., and Smith, M. R., *J. Exp. Med.*, 1949, v90, 85; ———, *ibid.*, 1950, v92, 85.

Received August 30, 1956. P.S.E.B.M., 1956, v93.

Effect of Biotin Deficiency on Follicular Melanocytes of Mice. (22726)

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It has been known for some time that pigmented mice and rats produce grey hairs when made biotin deficient(1,2,3). Unlike greying induced by X-rays, which is permanent(4), greying resulting from biotin deficiency is reversible(3). It would appear that melanocytes persist within the hair follicles of biotin deficient mice whereas they are destroyed in X-irradiated mice. Thus far,

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no attempt has been made either to trace the fate of melanocytes within the greyed hair follicles of biotin deficient mice or to determine the extent to which such depigmented melanocytes are morphologically and physiologically similar to the "pigmentless" melanocytes (clear cells) of albino mice. In addition, whereas it is well established in mice that the synthesis of the black-brown melanins (eumelanins) is inhibited by biotin deficiency, it is not known whether yellow melanin (phaeomelanin) synthesis is similarly affected.

It is the purpose of this paper to describe the effects of biotin deficiency on the follicular melanocytes of both yellow (A^y) and black (C57BL) mice.

Materials and methods. To produce biotin deficiency, 25 black (C57BL) and 5 yellow mice, 16 days of age, were weaned to diet #9A described by Wilson *et al.*(2). The effectiveness of this diet in causing greying of hair in C57Black mice was demonstrated by these investigators and subsequently by Rauch(3). Biopsies of skin were taken approximately 20 days post-weaning, shortly after the hairs of the second coat grew visibly above the skin. The skin samples were placed in Bouin's fixative and routine histological procedures were followed. Paraffin sections were cut at 7 μ and stained with Delafield's hematoxylin. Sections of depigmented follicles of the biotin deficient mice were compared with the follicles of albino mice in a comparable stage of hair growth. The activity of the dopa oxidase enzyme system within the depigmented melanocytes was determined by testing their response to exogenously supplied d,l-3-4-dihydroxyphenylalanine (dopa). Fresh frozen sections of skin from biotin deficient C57Black mice were cut on the freezing microtome at 30 μ and incubated in a buffered 0.76M solution of dopa according to the method of Laidlaw and Blackberg(5). Control sections were incubated in buffer alone. In addition, fresh frozen sections of skin from normal C57Black mice were treated in identical fashion, some sections being incubated in dopa solution, others in buffer alone.

Results. The pattern of greying is the same in C57Black mice and in yellow mice weaned to the experimental diet. The first hair growth cycle is completed approximately 2 days post-weaning when the mice are 18-19 days old. The hairs of the first generation are normal in pigmentation. The hairs of the second generation, appearing above the skin at approximately 20 days post-weaning, show a marked reduction in pigment content. Longitudinal sections of the hair follicles reveal a similar reduction in the amount of pigment within the hair bulbs. Except for the presence of small granules of pigment within the melanocytes and recipient epithelial cells, the greyed hair follicles are strikingly similar to those of the albino (Figs. 1, 2). The depigmented melanocytes contain a large hyalinated cytoplasm as do the clear cells or "pigmentless" melanocytes of the albino. Depigmented melanocytes are found in the hair bulbs of both biotin deficient black and yellow mice and in all instances they are restricted to the upper halves of the hair bulbs. This is also the position of clear cells within the hair bulbs of the albino mouse.

Depigmented melanocytes resulting from biotin deficiency react positively to exogenously supplied dopa. Abundant dopa melanin is observed in the hair follicles which are incubated in dopa solution (Fig. 3). The amount of dopa melanin formed indicates clearly that with regard to the activity of the dopa oxidase system, the melanocytes are capable of greater melanin synthesis than is expressed in the biotin deficient individual. In this way depigmented melanocytes differ from clear cells which have been shown to be dopa negative(6).

Discussion. The present experiments demonstrate that melanocytes persist within the depigmented hair follicles of biotin deficient pigmented mice and that they are similar in morphology to the follicular clear cells of albino mice. This observation lends support to the view held by previous investigators (7,8) that the clear cells of the albino are melanocytes which are incapable of melanin synthesis due to a gene controlled enzymatic deficiency. Unlike clear cells, however, de-

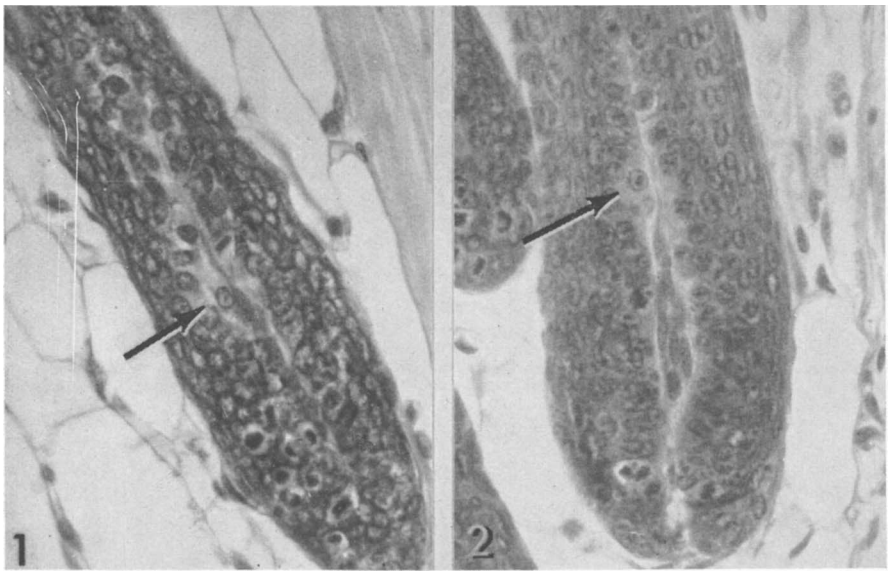

FIG. 1. Vertical section through a hair follicle of the albino mouse. Note the large clear cells (arrow) in the upper bulb. ×430.

FIG. 2. Longitudinal section of a hair follicle from a biotin deficient C57 Black mouse. Depigmented melanocytes (arrow) are visible in the upper bulb. ×430.

pigmented melanocytes retain the ability to respond *in vitro* to dopa. It is probable that the histological appearance of greyed hair follicles observed in biotin deficiency is also typical for the reversibly greyed hair follicles resulting from other nutritional deficiencies, *e.g.*, copper, zinc, and pantothenic acid deficiency(9). However, it would not be surprising if the response of depigmented melanocytes to exogenous dopa is subsequently found to vary considerably with the type of nutritional deficiency.

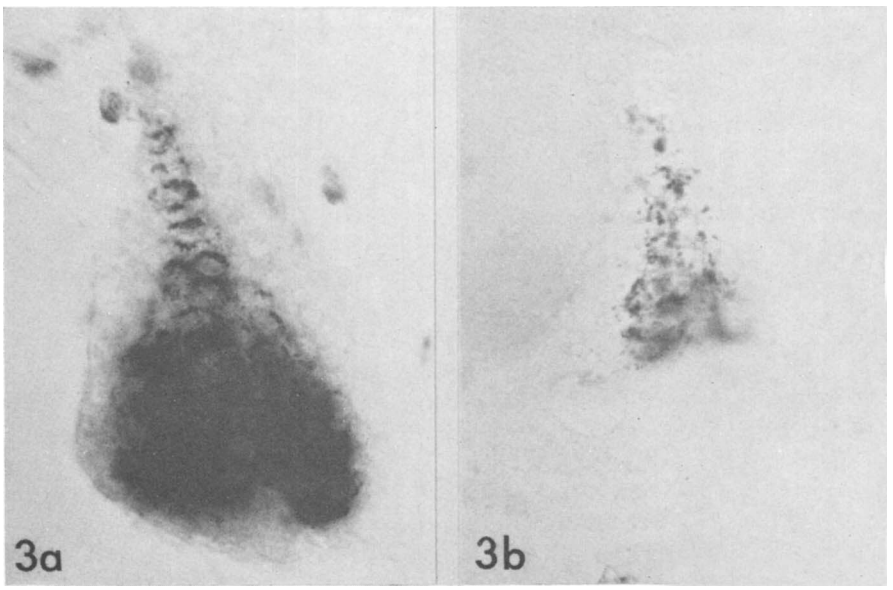

FIG. 3. a. Frozen section of a hair follicle from a biotin deficient C57 Black mouse, following incubation of the section in dopa. ×430. b. Control section to that shown in a., incubated in phosphate buffer only. ×430.

It would appear that both phaeomelanin and eumelanin synthesis are equally sensitive to biotin deficiency. In both yellow mice and black mice deficient in biotin a marked reduction in pigmentation is found in the hairs of the second hair generation. At present, with the role of biotin in the pigmentary process undetermined, no statement can be made as to the nature of this inhibition.

Summary. Both yellow (A^y) and black (C57BL) mice produce grey hairs when weaned to a diet known to cause biotin deficiency. Histologically, the greyed hair follicles of biotin deficient mice bear a striking resemblance to the hair follicles of albino mice. The depigmented melanocytes of biotin deficient mice are similar in morphology to the follicular clear cells of the albino. Unlike clear cells, however, depigmented melanocytes react positively to tests for the presence of dopa oxidase.

1. Emerson, G. A., and Keresztesy, J. C., PROC. SOC. EXP. BIOL. AND MED., 1942, v51, 358.
2. Wilson, J. W., Leduc, E. H., and Winston, D. H., *J. Nutrition,* 1949, v38, 73.
3. Rauch, H., *Physiol. Zool.,* 1952, v25, 145.
4. Chase, H. B., *J. Morphol.,* 1949, v84, 57.
5. Laidlaw, G. F., and Blackberg, S. N., *Am. J. Path.,* 1932, v8, 491.
6. Russell, L. B., and Russell, W. L., *Genetics,* 1948, v33, 237.
7. Taylor, A. C., *J. Exp. Zool.,* 1949, v110, 77.
8. Silvers, W. K., Thesis, University of Chicago, 1954.
9. Frost, D. V., *Physiol. Revs.,* 1948, v28, 362.

Received August 31, 1956. P.S.E.B.M., 1956, v93.

Measurement of Total Body Water in Sheep Using I^{131} Labeled 4-Iodo-Antipyrine. (22727)

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Antipyrine has been employed extensively as an indicator for the *in vivo* estimation of total body water in man(1,2) and animals (3-6). The method is based upon the dilution principle and is an adaptable and economical procedure for studies with the intact animals. Despite certain theoretical shortcomings(7,8) this method has been found to compare favorably with parallel procedures for body water measurement employing whole body analysis(3-5), specific gravity(6,9,10, 11) or the isotope dilution technic using deuterium(7), or tritium oxide(12). However, these methods require tedious procedures of sample preparation and time consuming analyses that discourage their use in routine experimentation. The recent introduction of I^{131}-labeled 4-iodo-antipyrine as a simple and rapid method for estimating total body water in man(13) made feasible the adaptation of this procedure for use with farm animals(14). This study is concerned with comparative behavior and volume of distribution of antipyrine and I^{131} labeled 4-iodoantipyrine for total body water estimation in sheep.

Materials and methods. The animals used in these studies were normal, healthy individual wether lambs and mature sheep maintained under typical summer and fall farm management conditions. The procedures employed for handling animals and performing quantitative injections of test substances have been described(15). Animals were taken off

* This article is published with approval of Director of the University of Tenn. Agric. Exp. Station.

The technical assistance of T. G. Clark, Henry Pace, Gladys Tapp and H. M. Crowder is gratefully acknowledged. The iodine-131 labeled 4-iodo-antipyrine was procured from Abbott Laboratories, North Chicago, Ill., on allocation from U. S. Atomic Energy Com.

This work was completed under Contract No. AT-40-1-GEN-242 between University of Tennessee, College of Agriculture, and the Atomic Energy Commission.