the damage due to enzymatic dissolution of parts of cell wall is not repaired; bacteria swell and burst in a sort of sterile lysis, as evidenced by drop in optical density and concomitant loss of infective centers. As the kinetics of lysis of frozen and thawed bacteria are similar with different phages, one might assume with Weidel(6) that the substrate for them is common. Comparison of phage lysis with lysozyme lysis of frozen and thawed cells(11) indicates that phage enzyme is isodynamic with lysozyme. All this is consistent with an assumption that the shock of freezing and thawing causes tears or holes in the outer lipoproteid layer of the cell wall(6), thus admitting the enzyme or phage's tail to act on the inner, lipopolysaccharide layer. Integrity of enzyme substrate in this rigid layer is essential for maintenance of bacterial, rod-like, structure. When the substrate is lysed, and the repair is blocked by freezing shock, the inner wall layer disintegrates(2) and the bacteria either lyse, or form spheroplasts(12) if suspended in proper hypertonic medium. The formation of such spheroplasts by lysis from without (1000 T2 phage particles/bacterial cell) had been previously observed by Carey *et al.*(13). In the present study, spheroplasts were formed from frozen and thawed *E. coli* under the influence of only few phages.

Summary. Frozen and thawed cells of *E. coli* B and B/r lyse immediately upon addition of various T-bacteriophages (T2r$^+$, T4D, T4Dr47, T3 and T7), at multiplicities of 1-15. Lysis is complete before the end of latent period and is more rapid as higher multiplicities of infection. When phage is added to thawed bacteria in 0.3 M sucrose, spheroplasts are formed.

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Influence of Hair Growth Cycle on Cytochrome Oxidase and DPNH-Cytochrome C Reductase in Mouse Epidermis.* (24946)

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Although normal epidermis has been demonstrated to undergo pronounced morphological changes in relation to hair growth cycles, relatively little is known about corresponding alterations in chemical composition. Recently, histochemical studies have clearly demonstrated certain chemical changes in epidermal cells paralleling hair growth activity (1). It is well established that the chemical constitution of epidermis is significantly altered when malignant transformation occurs by chemical carcinogens(2). Incidence of skin tumors induced by chemical carcinogens also has been shown to vary considerably with hair growth activity(3). A proper interpretation of such findings depends on a complete knowledge of normal quantitative and qualitative alterations in epidermal chemical composition which accompany hair growth cycles. In the present study the activity of 2 enzymes, namely cytochrome oxidase

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and DPNH†-cytochrome c reductase, was determined in the epidermis at the 4th (anagen III), 12th (anagen VI) and 22nd (telogen) days of hair growth cycle(4). These stages represent maximal alterations in epidermal hypoplasia and hyperplasia or growth. Activity of cytochrome oxidase in actively proliferating hair follicles was also determined for comparison with normal hypoplastic and hyperplastic epidermis obtained at known stages of the hair growth cycle(5).

Methods. The hair was plucked by hand from adult Swiss albino mice with quiescent (telogen) hair. Skin samples were obtained from mice killed by cervical dislocation 4, 12 and 22 days following plucking. Epidermis was separated from the dermis at 50°C by procedure of Baumberger *et al.*(6) and dropped into several ml of water in 7 ml Ten Broeck homogenizer, immersed in crushed ice. The hair follicle preparation (hair bulbs, hair shafts and some hair) was obtained from skin of A and C_3H mice 10 to 14 days of age, by first dissecting the layer of skeletal muscle (panniculus carnosus) and a considerable amount of adipose layer from the skin (epidermis down). Hair follicles and associated material were scraped off with iris knife and placed into 2 ml distilled H_2O in 25 ml lusteroid centrifuge tube immersed in crushed ice. Hair follicle preparations were centrifuged at 13,000 rpm (International multispeed attachment and rotor No. 269) for 10 minutes at 0°C. The sedimented material was then homogenized by hand in several ml distilled H_2O. Epidermal preparations were also homogenized in similar fashion and aliquots of both homogenates were retained for nitrogen determinations by Kjeldahl procedure. Nitrogen appears to be as good a basis of reference as any other tissue constituent(2). The remainder of the homogenates were centrifuged at 1500 rpm (International horizontal rotor No. 241 for Model V, size 2 centrifuge) for 10 minutes at room temperature. Supernatant fractions thus obtained were kept cold and used immediately for enzyme assay. Epidermis removed from skin at various phases of hair growth cycle and follicular material were disrupted to about the same extent by homogenization. The reaction mixture for determination of cytochrome oxidase activity was as follows: A total volume of 3 ml contained 0.2 ml 0.17 M sodium phosphate buffer, pH 7.4; 0.4 ml 1.7×10^{-4} M reduced cytochrome c and 0.02 to 0.1 ml of sample. The volume was adjusted to 3 ml with H_2O. Cytochrome oxidase activity was determined according to the macro procedure of Cooperstein and Lazarow(7) by measuring change in optical density at 550 mμ every 15 seconds in a Beckman model DU spectrophotometer. For assay of DPNH-cytochrome c reductase activity, 3 ml of reaction mixture containing the following materials in concentrations indicated were used: 0.027 M nicotinamide; 2×10^{-4} M NaCN; 0.033 M phosphate buffer, pH 7.4; 1 mg cytochrome c; 300 μg DPNH; 0.03 to 0.1 ml sample and H_2O to make a final volume of 3 ml. Activity of DPNH-cytochrome c reductase was determined from rate of reduction of cytochrome c at 550 mμ every 15 seconds according to procedure of DeDuve *et al.*(8).

Results. Cytochrome oxidase activity of epidermis at day 4 of hair growth cycle was considerably greater than that found for epidermis removed at 12th and 22nd days of hair growth cycle, whether the results were expressed on the homogenate or supernatant nitro basis (Table I). The epidermis is hyperplastic at 4th day of hair growth cycle apparently due to increased mitotic activity(5,9, 10). The elevated level of cytochrome oxidase activity observed at this time thus may be associated with increase in cell division.

TABLE 1. Cytochrome Oxidase Activity of Epidermis of Skin in Various Stages of the Hair Growth Cycle.*

Stage of hair growth	Day of cycle	Specific cytochrome oxidase activity† of Homogenate	Supernatant
Anagen III	4	2.2 ± .60	4.4 ± .80
"	12	.8 ± .25	1.7 ± .25
Telogen	22	1.1 ± .18	2.5 ± .22

* Results are avg with dev. from mean of 3 determinations using epidermis of 3 to 5 mice/analysis.

† △ log ferrocytochrome c/min./mg nitrogen.

† The following abbreviations are used: DPNH, reduced diphosphopyridine nucleotide; DNA, desoxyribonucleic acid; RNA, ribosenucleic acid.

TABLE II. DPNH-Cytochrome c Reductase Activity of Epidermis of Skin in Various Stages in the Hair Growth Cycle.*

Stage of hair growth	Day of cycle	Specific DPNH-cytochrome c reductase activity† of Homogenate	Supernatant
Anagen III	4	1.2 ± .18	2.2 ± .50
"	12	.3 ± .05	.9 ± .20
Telogen	22	1.0 ± .15	2.1 ± .27

* Results are avg with dev. from mean of 3 determinations using epidermis of 3 to 5 mice/analysis.

† △ log ferrocytochrome c/min./mg nitrogen.

This assumption appears to be strengthened by the fact that cytochrome oxidase activity of growing hair follicles, which contain actively dividing epidermal cells(9) was much higher than that of epidermis. Average value of follicular material was 10.1 ± 3.87; even the lowest values were twice that of the epidermis removed on 4th day of hair growth cycle when cytochrome oxidase activity was a maximum (2.2 ± 0.6). Histological examination of follicular preparations showed no evidence of skeletal muscle which has a high cytochrome oxidase activity(11,12).

DPNH-cytochrome c reductase activity of epidermis on 12th day of hair growth cycle was significantly less than the activity of epidermis on 4th or 22nd days (Table II). At 12th day of hair growth cycle the epidermis is thinner than at any other stage of the cycle (5). No values were obtained for hair follicle preparation. Thus, both cytochrome oxidase and DPNH-cytochrome c reductase activities within epidermis appear correlated with the mitotic status of its constituent cells.

Griesemer has shown that the succinic dehydrogenase DNA† and cytochrome oxidase DNA† ratios of 4th day rat epidermis was about 30% higher than that of the epidermis of 0 day of hair growth cycle(13). However, no significant difference was found in activity of these enzymes on 0 and 4th day of cycle when enzyme activities were expressed on dry weight or RNA† basis.

Summary. 1. Cytochrome oxidase activity of epidermis of mice on 4th day of hair growth cycle was appreciably higher than of epidermis of 12th and 22nd days of cycle. 2. Hair follicle preparations from very young mice had much greater cytochrome oxidase activity than did epidermis of 12th and 22nd days of cycle. 2. Hair follicle preparations from very young mice had much greater cytochrome oxidase activity than did epidermis regardless of stage of hair growth. 3. DPNH-cytochrome c reductase activity of epidermis of 12th day of hair growth cycle was significantly less than that of epidermis removed on 4th and 22nd days of cycle. 4. Activity of cytochrome oxidase appears associated to some extent with mitotic activity of tissues examined.

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