sectomized animal. Accordingly it is proposed that endotoxin acts directly on the central nervous system to result in ACTH release. Experiments have been undertaken to determine site of action of endotoxin in the central nervous system for both adrenal cortical stimulating and fever-promoting properties.

Summary. Dogs given large doses of bacterial endotoxin do not become tolerant to either fever producing or adrenal cortical stimulating effects. Dogs receiving small doses of endotoxin readily become tolerant to the fever-producing effect, but either do not become tolerant at all or achieve only partial tolerance to adrenal cortical stimulating properties. The partial adrenal cortical tolerance observed following endotoxin administration probably represents decreased pituitary release of the ACTH rather than tolerance of adrenal cortex itself.

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A New Type of Lysis from Without. (24945)

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According to the enzyme hypothesis of phage action on susceptible bacterial cells(1-6), holes are made in bacteria cell wall, by phage enzyme following adsorption, and nucleic acids of the phage are injected through these holes into the cell. Within a few minutes after adsorption these holes are sealed (3), provided the damage to cell walls is not too great, as in lysis from without(1). One might therefore expect that if the phage enzyme acted on bacteria which were blocked in their ability to reseal even the minor "tears" in their walls, initial lysis would not be halted and the enzymatic action would disintegrate the wall completely. Such immediate lysis caused by T2r phage was observed by Heagy(7) in partially starved *Escherichia coli*. The present study shows that freezing and thawing of exponentially growing bacteria provides a shock which blocks repair of damage to bacterial wall, and that such thawed bacteria lyse immediately upon addition of the phage.

Methods. E. coli, strain B or B/r, was grown in broth or in a synthetic medium(8) until an optical density of 0.4-0.6 (at 540 mμ) was reached. This corresponded to a viable count of 2 to 4 x 10^8 cells/ml. The suspension was either directly frozen in the growth medium, using a CO_2-acetone bath, or it was washed by centrifugation and frozen in fresh medium. The bacterial suspensions were infected with various bacteriophages (T2r$^+$, T4D, T4Dr47, T3, or T7), either before freezing or after thawing. In the first case, phage was added to cells at a desired multiplicity of infection, the suspension incubated 3 min. at 37°, and frozen. In the second type of experiment, phage was added immediately upon thawing, *i.e.,* at moment of final disappearance of ice. The volume of added phage was 1/30 to 1/50 of the bacterial suspension, while multiplicity of infection varied between 1 and 15. For determination of onestep growth curves, unadsorbed phage was removed by centrifugation.

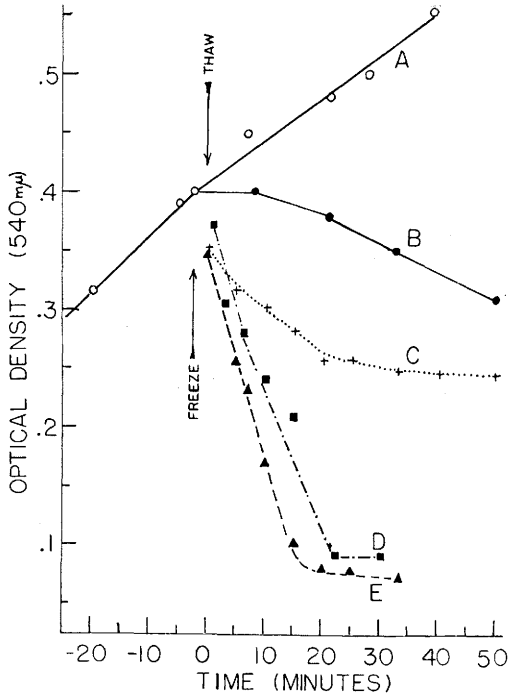

FIG. 1. Effect of T2r+ bacteriophage on frozen and thawed cells of *E. coli* B/r. Multiplicity of infection = .4. Curve (A) represents exponentially growing culture (at 37°) from which, at −3 min., 4 separate samples were derived. At this time phage was added to samples (B) and (E). Sample (B) was incubated undisturbed at 37°. Samples C, D, and E were immediately frozen at −80° and 2 min. later thawed. Phage was added to sample (D) immediately after thawing. (C) is the control lysis curve due to freezing and thawing alone.

Results. The fate of infected or non-infected frozen and thawed bacteria was followed by viable counts of bacteria (or of infective centers), as well as by measuring changes in optical density of suspension. One-step growth curves and single cell bursts were also determined in bacteria infected before freezing(8).

Freezing and thawing of a control suspension caused a loss of viability of 30 to 60% of bacteria. This loss was accompanied by 10 to 25% drop in optical density during first 10 to 20 min. after thawing at 37° (Fig. 1-C). Thawed bacteria did not resume growth for 1 to 2 hr. Freezing and thawing of infected bacteria caused a loss of about 80% of infective centers and an approximately equal drop in optical density (Fig. 1-E). Upon addition of phage after thawing of frozen bacterial suspension, lysis occurred in a manner and at rate similar to that in bacteria infected before freezing (Fig. 1-D). The concomitant lysis, as observed in photometer, reached 60 to 70% in nutrient broth at multiplicities of infection below 10, and within 20 min after thawing (*i.e.*, during latent period when no lysis should occur). In synthetic medium the lysis was less pronounced. Higher multiplicities of infection caused a more rapid and extensive lysis. In presence of 0.3 M sucrose and phage, the thawed bacteria form spheroplasts.

The effects of freezing of exponentially growing cells of *E. coli* are evident not only in decrease of viability of thawed suspension, partial lysis, and interruption of exponential growth of survivors for 1 to 2 hr, but also in their striking response to lytic phages. Frozen and thawed bacteria infected before freezing exhibit a longer than normal latent period at 37° and their burst size is smaller. The fact that such cells produce phage at all indicates that their synthetic mechanism is not damaged by freezing and thawing, although the ability of frozen, non-infected cells to divide is seriously impaired. As mentioned before, frozen bacteria to which T2r+, T4D and T7 phages were added after thawing lysed considerably, although multiplicity of infection was below that causing lysis from without. It has been shown by Puck and Lee (3) that in the second, enzymatic stage of adsorption of phages to bacteria, a local leakage of bacterial contents occurred and that this leakage was soon repaired. Similarly, Doermann(9) showed that after attachment of T2 phages to *E. coli,* a transient drop in optical density occurred. This was interpreted by him as a permeability change in bacterial cell walls infected by phage.

Discussion. In cell-wall preparations, lysis by phage enzymes (whole phages(5) or frozen and thawed phages(4)), as evidenced by appearance of labeled split products from the wall, was demonstrated by Barrington and Kozloff(5), Weidel and Primosigh(4), and Koch and Weidel(10). All these findings give strong support to the original Delbrück (1) enzyme hypothesis of phage action.

Our experiments fit this hypothesis. Because of dormancy induced by freezing shock,

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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