sa follicle, which is similar in morphology to the tylotrich follicle (*7*). Forward or upward movement of the vibrissa produces a slowly adapting response, whereas pulling or pushing of this hair often fails to produce a discharge (*12*). Furthermore, Iggo (*2*) reports that an occasional discharge of impulses can be elicited if the hair nearest the touch spot (tylotrich pad) of the cat is moved in a particular direction. Possibly an effective directional movement is one that depresses the larger area of the pad. In the cat the tylotrich must be moved cephalad if it is to press against the pad. In other mammals, such as the rabbit, most of the pad is caudal to the orifice of the tylotrich follicle (*7*), and thus a caudad movement of the hair should be more effective.

The growing tylotrich follicle of the cat differs markedly from the resting follicle. For example, when the hair is growing, the annular complex appears compressed and is difficult to observe. This suggests that changes in neurophysiology may occur during the growth cycle of the tylotrich follicle. Unfortunately, such changes would be difficult to study in the cat because the pelage follicles grow asynchronously (*13*). In the rabbit, hair cycle staging can be assessed accurately by timing the waves of synchronous growth or by use of the plucking stimulus (*14*). The rabbit tylotrich follicle should provide better material for neurophysiological studies because the components of the annular complex are large and show extreme physiological and morphological specialization (*7*).

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Ionizing Radiation: Effect of Irradiated Medium on Synthetic Processes

Abstract. *The incorporation of uracil-C^{14} into macromolecules in* Escherichia coli *cells is decreased by doses of ionizing radiation when the cells are in very dilute suspension. The decrease results from an action of irradiated medium on the cells, and a similar reaction is observed during the incorporation of thymine (indication of DNA synthesis) and of proline and valine (indicative of protein synthesis). Irradiated medium reduces the formation of β-galactosidase but does not cause the degradation of DNA.*

Studies on the effect of ionizing radiation on the incorporation of labeled metabolites into macromolecules of bacterial cells show that the processes of synthesis are sensitive to ionizing radiation. However, the conditions of irradiation seem to produce striking differences in sensitivity. The incorporation of a radioactive label either as uracil-C^{14} or phosphate-P^{32} into cells of *Escherichia coli* is reduced by ionizing radiation, but only at relatively high doses, 50,000 roentgens or higher (*1*, *2*). On the other hand, at much lower doses there is a striking effect on *E. coli* DNA; it is synthesized at a reduced rate after irradiation and also degraded to very small fragments (*3*, *4*).

We now report two seemingly separate effects of radiation: first, an effect which is produced primarily by a radiochemical action on the medium in which the cells are subsequently grown, and second, an effect which requires radiation of the cells themselves. Both effects are greater in the presence of oxygen.

In a representative uptake experiment (Fig. 1) the culture was oxygenated before irradiation, and the time of exposure to a Co^{60} source is indicated by the shaded region. For 12,000 roentgens there is clearly a cessation of uptake, this period lasting for about 20 minutes. The results of the same experiment carried out after nitrogenation of the medium indicates that a dose ten times larger produces, if anything, less effect than is produced in the presence of oxygen. In experiments similar to this for doses of 6,000 r, 12,000 r, and 30,000 r in the presence of oxygen, incorporation was only observed for 6,000 r, and in that case there was a 50 percent decrease.

The foregoing experiments were performed at cell concentrations of less than 10^8 cells per milliliter. At concentrations of 5×10^8 cells per milliliter and upward the sensitivity of incorporation was much less. In this range our results agree with those of Frampton (*2*). However, two experiments imply that radiation affects the medium as well as the cells. The effects of exposing cells which had not been irradiated to medium which had been irradiated (Fig. 2) is almost as great as would be produced by irradiation of a dilute culture of cells in the presence of oxygen. The medium is Roberts C minimal medium (NH_4Cl, 2 g; Na_2HPO_4, 6 g; KH_2PO_4, 3 g; NaCl, 3 g; $MgCl_2 \cdot 6H_2O$, 62 mg; Na_2SO_4, 80 mg; glucose, 5 g per liter), and separate irradiation of distilled water indicated that the significant component is water. When bovine serum

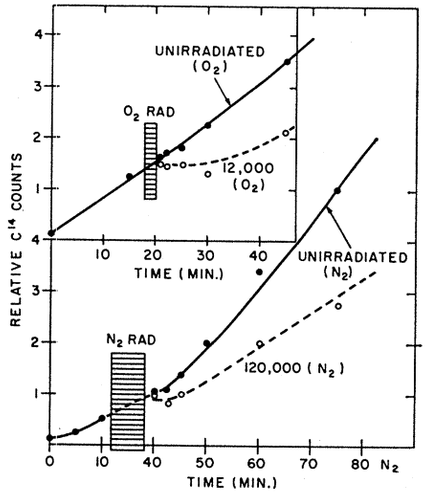

Fig. 1. Uracil-C^{14} in the macromolecular fraction (insoluble in trichloroacetic acid) for unirradiated cells and cells irradiated in oxygen and nitrogen (count/min). Upper curve, effect of 12,000 r in oxygen and the lower for ten times that dose in nitrogen. Irradiation in oxygenated medium halts the incorporation of uracil.

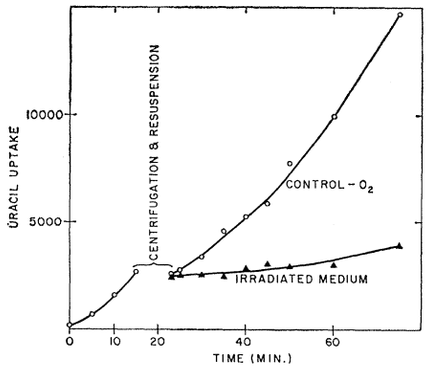

Fig. 2. Addition of irradiated medium to unirradiated cells. The cells were centrifuged and resuspended in irradiated (28,000 r) oxygenated medium, and the incorporation of uracil-C^{14} into the macromolecular fraction was observed. The effect of irradiating the medium is comparable to the effect of irradiating the cells.

albumin was added to a dilute culture the effect of radiation was drastically reduced.

Since the bovine serum albumin is much too large a molecule to penetrate the cell, it must act on the medium and thus provides a natural explanation of the effect of concentration. At high concentrations this action on the medium is distributed among many cells and the effect per cell is less, whereas at low concentrations the effect per cell is much greater.

We also studied the effect of radiation of the medium on DNA degradation, DNA synthesis after irradiation, protein synthesis, and the formation of β-galactosidase. To study DNA degradation, a phenomenon observed by Stuy (*3*), we grew cells (*E. coli* 15T⁻L⁻, which requires thymine and leucine for growth) on thymine-C^{14} for several hours and centrifuged and resuspended them in nonradioactive medium which had been irradiated. The control received unirradiated medium. The amount of radioactivity in the fraction insoluble in trichloroacetic acid remained constant for 90 minutes in both control and exposed cells. We also irradiated such previously labeled cells with and without the addition of bovine serum albumin to the medium. The degree of degradation was the same in each case, an indication that the bovine serum albumin had no effect on this process even though in the case of uracil uptake there had been a striking effect. We conclude that the degradation of DNA is not produced by irradiation of the medium but requires the irradiation of the cells.

To study the effect of exposing cells to irradiated medium on the synthesis of DNA, the labeled thymine was kept in the medium after exposure (Fig. 3). The incorporation of label into macromolecules is clearly much less. Direct irradiation of the cells (*5*) has a somewhat greater effect, although the difference lies more in a prolongation of the depression for direct irradiation than in a greater initial effect.

To observe the effect of exposure to irradiated medium on protein synthesis, we conducted a series of experiments in which labeled valine and proline were introduced into the medium (Fig. 4). The effect of the irradiated medium is not very different from that which occurs when the cells alone are irradiated. Similar results were observed for proline-C^{14}.

Since ionizing radiation (*6*) interferes with the formation of an induced enzyme, we studied the effect of irradiated medium on the formation of induced β-galactosidase in a culture of *E. coli*. There is a definite reduction in the rate of formation of enzyme followed by a later recovery (Fig. 5). Medium irradiated after nitrogen gas had been passed through it had a much smaller effect. In order to see whether this reduction is related to messenger RNA (mRNA), we performed pulsed-induction experiments of the type described by Swenson and Setlow (*7*), who showed that ultraviolet light causes inactivation of the RNA. This technique, in which the inducer is given and removed, causes a burst of mRNA production which then ceases. The amount of enzyme formed is proportional to the amount of active mRNA produced during the burst. These experiments showed that irradiated medium does inactivate mRNA. Medium which had been oxygenated and irradiated with 33,000 r reduced the amount of enzyme formed to 50 percent.

Probably the only well-known product of the irradiation of water which is of long life, namely hydrogen peroxide, takes part in these actions of the irradiated medium. Adler (*8*) showed that the cells surviving radiation treatment, which were also catalase-negative mutants, were sensitive to irradiated medium, and he concluded that the action was due to hydrogen peroxide. Our experiments agree with his findings and,

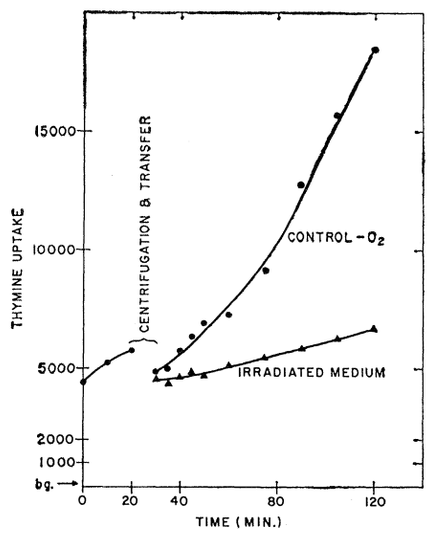

Fig. 3. Effect of irradiated medium on thymine incorporation. Oxygenated unirradiated medium was used for the control; similar medium irradiated with 28,000 r was added to the irradiated fraction.

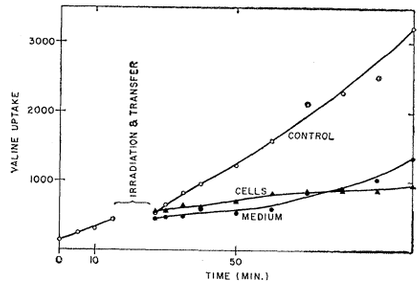

Fig. 4. Effect of irradiated medium on valine incorporation for control cells, irradiated cells, and cells exposed to irradiated medium.

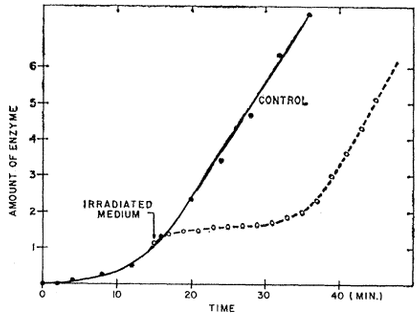

Fig. 5. Cells (*E. coli* B) grown on maltose are induced with thiomethylgalactoside at zero time. At 15 minutes, an equal volume of oxygenated unirradiated medium is added to the control; at the same time similar medium, irradiated for 58,000 r, is added to the other cells. The amount of β-galactosidase is measured at intervals. The irradiated medium decreases the amount of β-galactosidase formed, but there is recovery to the normal rate.

in addition, suggest that the effect of radiation on the medium, while closely related to synthetic processes and to mRNA, is not related to the degradation of DNA. Thus two aspects of the action of ionizing radiation on simple cells can be studied separately.

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Itch and Vibration

Abstract. *Itch produced by application of cowage to the wrist was reduced in intensity by vibration of the stimulated area. Application of vibration to the opposite wrist also reduced intensity. The results may be attributed to physiological activities occurring at the early stages of information transmission.*

Itch is relieved by scratching, but when the scratching has ceased it often returns with increasing intensity, sometimes changing to frank pain (*1*). Two theories have been proposed to account for these facts. According to traditional specificity theory (*2*) itch is the result of weak stimulation of pain receptors; and interactions between inputs from scratching and the itch-producing stimulus occur at the thalamus or cortex. These propositions are based on the assumption that each modality is carried by direct-line pathways from receptors to a brain center that registers a specific sensation. According to the pattern theory (*3, 4*), itch perceptions are subserved by unique spatial and temporal patterns of nerve impulses, and these afferent patterns can be modified by interaction with tactile inputs beginning at the earliest stages of information transmission.

Physiological and behavioral evidence (*4*) lends strong support to pattern theory. Wall and Cronly-Dillon (*5*) showed that the first central cell of the spinal cord responded with characteristic firing patterns to various skin stimuli. They observed that the high-frequency bursts of impulses recorded when itch powder was applied to the skin were abolished by simultaneous vibration of the surrounding skin. Their data suggest that the vibratory input exerts an inhibitory effect on the afferent pattern produced by pruritogenic substances. Although vibration raises (*5*) thresholds for tactile, thermal, and noxious stimuli, there are no comparable psychological data for itch. We have therefore examined the effects of vibration applied to different parts of the body on the intensity of perceived itch.

Our subjects were 34 male and 16 female university students assigned at random to one of five groups containing ten subjects each. Itch was produced by applying small amounts of cowage (*Mucuna pruriens* spicules) to the flexor surface of one of the wrists until the subjects reported a desire to scratch. A few subjects who failed to experience itch were not tested further. After the subjects reported the desire to scratch, a small patch of adhesive tape was placed over the wrist (to hold the cowage spicules in place), and one of four skin areas was vibrated by lowering onto the subject's skin a 60-cy/sec vibrator fitted with a rubber disc 5.7 mm in diameter and mounted on a retort stand. Vibration was applied to the itch-stimulated wrist (group I), to the flexor surface of the same arm half-way between the elbow and the wrist (group II), to the opposite wrist (group III), and to the flexor surface of the opposite arm (group IV). A control group (group V) received no vibration, although the inactivated vibrator was placed on the itch-stimulated skin.

All five groups received identical instruction. The subjects were told to report *0* when they felt no itch, *1* when itch was just perceptible and without annoying effect, *3* when itch was bothersome or annoying, *5* when the itch was at its worst and produced an intense desire to scratch, *2* and *4* being appropriate intermediates. The subjects were also instructed to report the scale numbers (which were presented on a card) continuously throughout the experiment, starting a few seconds after cowage was applied, until they were told to stop. When each subject reported a constant itch intensity at *3* or higher, the vibrator was placed on the skin for 100 seconds and then removed.

A decrease in itch intensity of two scale units or more was the criterion for effective reduction of itch. Itch was reduced significantly ($p = .05$ or better, Fisher tables, *6*) by application of vibration to the same wrist (group (I) beginning at 10 seconds, to the opposite wrist (group III) at 20 and 30 seconds, to the same lower arm (group II) at 30 seconds, and to the opposite lower arm (group IV) at 50, 90, and 100 seconds.

The reduction of itch intensity and its subsequent return toward initial intensity during vibration is shown in Fig. 1. Since the control group exhibited a slow, rhythmic, spontaneous decrease in itch intensity in the absence of vibration, the values on the curves for the experimental groups in Fig. 1 have been corrected by subtracting the corresponding values of the control group.

Except for an occasional increase, vibration generally decreased itch intensity. The occasional increase appeared to depend, in part, on the original intensity of the itch. Three subjects who, at first, felt severe, almost painful itch reported that the vibration immediately transformed the itch into frank pain. Others reported a decrease in intensity and a subsequent "overshoot" to greater intensity. Most subjects in groups I and III reported that after cessation of vibration itching returned, and that it was sometimes more intense than before vibration.

The results show that vibration effectively reduces the intensity of moderate degrees of itching. The long delays of the effect of vibration experienced in groups II, III, and IV, compared with the rapid and more marked effect in group I, indicate that the reduction in intensity cannot be attributed simply to distraction or to implicit suggestion. Rather, it appears to be due to an interaction of the two