ground" synthesis was high owing to the secretion of endogenous insulin, or the effect of insulin was in part counterbalanced by the secretion of adrenal medullary and adrenal cortical hormones in response to hypoglycemia; epinephrine (*4*) and adrenal cortical steroids (*5*) are inhibitory of protein synthesis in muscle.

The assay was carried out in circumstances in which incorporation was proportional to the concentration of ribosomes; that is, where all other factors necessary for protein synthesis were added in optimal or excess amounts. In those circumstances, the insulin effect was present even when polyuridylic acid was added in concentrations from 25 to 150 μg/ml; moreover, the stimulation due to insulin treatment was observed at each of several concentrations of ribosomes (50 to 250 μg/ml of ribosomal RNA).

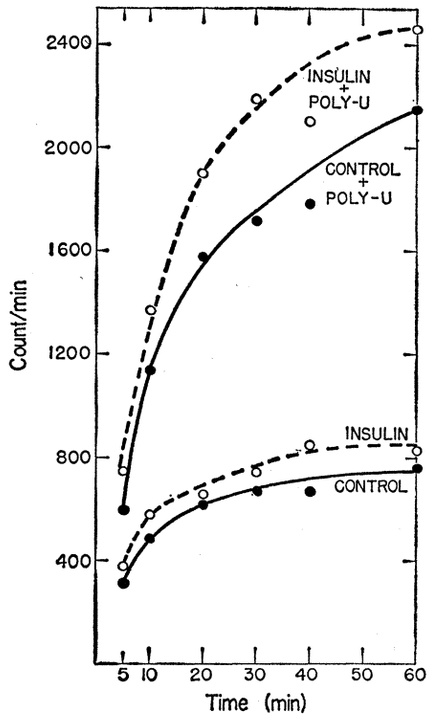

Fig. 2. Incorporation into protein of radioactivity from sRNA charged with C^{14}-phenylalanine by ribosomes from heart muscle of insulin-treated animals. The treated animals received 1 unit of insulin intraperitoneally 1 hour before they were killed; at that time the average blood glucose concentration was 43 mg per 100 ml of blood (range 31 to 77). The assay was carried out as described in Fig. 1, except that 150 μg of sRNA from *Escherichia coli* charged with C^{14}-phenylalanine (8.47×10^3 count/min) and 100 μg of ribosomal RNA were added. The amount of polyuridylic acid, when present, was 100 μg. The ribosomes from control animals had an absorbancy ratio (260:280 mμ) of 1.73; those from insulin-treated animals, an absorbancy ratio of 1.74.

The results have relevance for several of the theories of insulin action. The accelerated rate of protein synthesis due to insulin (or the decreased rate in its absence) is not likely to have resulted from an alteration of the rate of transport of amino acids (*6*), because synthesis was from sRNA "charged" with a full complement of amino acids, and the sRNA was added in excess to ribosomes from both normal and insulin-treated animals; for the same reason, the increase in protein synthesis is not likely to have resulted from an increase in the transport of any other substrate. The possibility that insulin affected the ribosomes as the result of an increase in the transport of some critical substrate during the hour before the ribosomes were isolated cannot, of course, be excluded. However, that alternative requires that the change, once initiated, persists even after the ribosomes are isolated and the concentration of substrates in the assay system are made equal for the control and experimental groups. The results also make it less likely that the stimulation of protein synthesis by insulin is the secondary result of a stimulation of the generation of adenosine triphosphate (ATP), of the distribution of ATP in the cell (*7*), or of the turnover of high-energy phosphate compounds (*8*), for all were, once again, added to the assay system in optimal or excess amounts. Nor do the results support the idea that the crucial action of insulin is on the cell's cytostructure (*9*). Finally, the defect that occurs in the absence of insulin would not appear to be due to a deficiency of template RNA (*10*), for the defect persists in the presence of even large amounts of polyuridylic acid just as does the increased incorporation of amino acid into protein by ribosomes from insulin-treated animals. The results do support the suggestion that the locus of action of insulin in stimulating protein synthesis is the ribosome. Conceivably, the hormone produces an alteration in the ribosome of a type that leads to a modification in the translation of messenger RNA.

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17 June 1965

Ultraviolet Damage to Bacteria and Bacteriophage at Low Temperatures

Abstract. *The survival of* Escherichia coli *B/r WP2 (tryptophan-requiring) from ultraviolet irradiation when suspended in 0.067*M *phosphate buffer (pH 7) has been studied over the temperature range 22° to −269°C. In unfrozen suspensions there was no appreciable change in sensitivity between 22° and −10°C. The sensitivity in the presence of ice progressively increased by a factor of 7 when the temperature was lowered to −79°C. Between −79° and −196°C the sensitivity decreased to less than four times the sensitivity at 22°C and was not appreciably different at −269°C. Evidence from experiments with bacteriophage T1 and* E. coli *WP2 HCR− (a strain unable to excise thymine dimers) indicates that a new, qualitatively different lesion, less amenable to repair, may replace the thymine dimer in* E. coli *irradiated at −79°C.*

Many physicochemical properties of liquids and solutions alter abruptly in the process of cooling to the frozen state. From present knowledge of the interaction of ultraviolet light with organic molecules in solution (*1*) one would anticipate that the efficiency of the resulting photochemical changes would also change abruptly with change of state (*2*). One example of such a change is the enhancement of the dimerization of nucleotide bases when

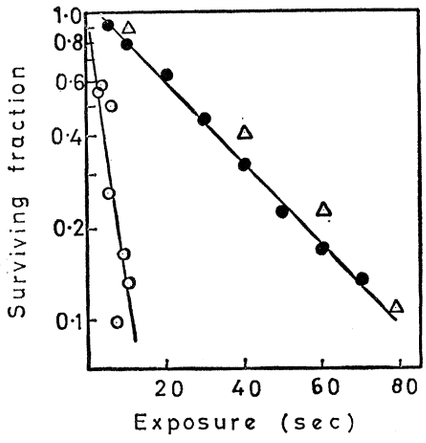

Fig. 1. Survival from ultraviolet irradiation (at a distance of 58.5 cm) of *Escherichia coli* B/r WP2; closed circles, irradiated at 22°C; open circles, irradiated at −79°C; triangles, irradiated at 22°C immediately after freezing to −79°C and then thawing.

irradiated with ultraviolet light in the frozen state (*3–6*). Since it seemed possible that similar effects might occur with living systems, the action of ultraviolet light on suspensions of *Escherichia coli* was studied over a wide range of temperatures. Both inactivation and induction of mutations were enhanced in passing from the liquid to the frozen state. This report deals with observations concerning the lethal effects of ultraviolet light.

The organisms used in these studies were *Escherichia coli* B/r WP2 (tryptophan-requiring) and a strain derived from it, *E. coli* WP2 HCR⁻ (Hill), which has a reduced ability to excise thymine dimers from irradiated DNA and consequently is more sensitive to ultraviolet light and less able to perform host-cell reactivation of irradiated bacteriophage T1 (*7*). Bacteria were grown at 37°C in a minimal medium (*8*) containing 6 μg of tryptophan per milliliter, centrifuged and resuspended in 0.067*M* phosphate buffer (*p*H 7) during irradiation, and plated on the surface of agar containing minimal medium and 0.75 μg of tryptophan per milliliter. Colonies were counted after having been incubated 24 hours at 37°C. The plating medium was that used in our mutation studies. Identical results were obtained in our experiments when a minimal medium with a larger tryptophan supplement (20 μg/ml) was used. Some experiments were carried out with bateriophage T1 assayed in the above bacterial strains by means of the layer-plate technique and minimal agar supplemented with 20 μg of tryptophan per milliliter. The irradiations were performed with a low-pressure mercury lamp emitting predominantly at 2537 Å. The approximate dose rate at a distance of 58.5 cm was 11 ergs mm^{-2} sec^{-1} as judged by bacterial-survival curves previously determined with a calibrated lamp.

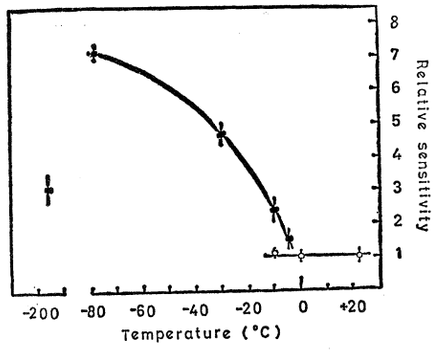

Fig. 2. Ultraviolet sensitivity of *Escherichia coli* B/r WP2 at different temperatures (ratios of survival curve regressions at different temperatures to those at 22°C); open circles, unfrozen; closed circles, frozen. Vertical lines represent standard errors of regressions at each temperature.

Bacterial and phage suspensions containing not more than 10^4 organisms per milliliter were irradiated without agitation in flag-bottomed glass vessels, the depth of the liquid (3 mm) being insufficient to cause appreciable absorption of ultraviolet light by the buffer alone. Solutions were cooled to the desired temperature by placing the glass vessels on copper platforms immersed either in alcohol cooled with solid carbon dioxide or in liquid nitrogen or liquid helium. Preliminary experiments did not indicate any appreciable effect of the freezing and thawing rate on bacterial survival. Bacteria were irradiated 20 minutes after they were placed in the cooling bath, and measurements with thermocouples indicated that the desired temperatures were reached within 7 minutes. Immediately after irradiation, samples were thawed in a water bath at 37°C and then plated. The experiments were performed in a darkened laboratory as a precaution against photoreactivation.

The change from the liquid to the frozen state had a large effect on the ultraviolet sensitivity of *E. coli* B/r WP2 (Figs. 1 and 2). Bacteria that were irradiated while in a suspension supercooled at −10°C showed the same sensitivity as bacteria irradiated at either 22° or 0°C, but there was an approximately twofold increase in sensitivity when frozen, as compared with unfrozen, suspensions were irradiated at −10°C. The sensitivity in the frozen state at −79°C was seven times that at 22°C. At the temperature of liquid nitrogen (−196°C) sensitivity to ultraviolet irradiation was only between three and four times that at 22°C, and a few experiments with liquid helium (−268°C) gave essentially similar results to those obtained at −196°C. When bacteria were frozen to −79°C and then thawed before ultraviolet irradiation their sensitivity was not altered. This evidence, together with results obtained in experiments with supercooled bacteria, demonstrated that increased sensitivity is observed only when samples are irradiated in the frozen state.

It is generally believed that one of the chief modes of ultraviolet inactivation of *E. coli* in the absence of photoreactivation is the production of lesions in DNA, such as thymine dimers, a large proportion of which may be repaired (by excision) in bacteria possessing the necessary enzymes, such as *E. coli* B/r WP2 (*9, 10*). If the effect of irradiation at low temperatures were to increase the amount of this thymine dimer–type damage, bacteria lacking repair enzymes (and therefore killed almost exclusively by this type of damage) should exhibit a temperature-dependence at least as great as those which possess the enzymes. In addition, the effect on bacteria deficient in repair systems should be dose-modifying, that is, the shapes of the curves at both temperatures should be identical.

Some results with the very sensitive strain *E. coli* WP2 HCR⁻ are shown in Fig. 3. That some repair is possible in this strain after low doses of radiation at 22°C is suggested by the shoulder on the survival curve, which is what one would expect of a repair enzyme present at a very low level and therefore readily saturated by its substrate. Experiments carried out in total darkness, except for a red safety light, showed that this shoulder was not caused by a small amount of photoreactivation nor was it abolished by freezing and thawing immediately before irradiation. At −79°C there was no shoulder to the curve and the slope was not significantly different from the ultimate slope of the curve at 22°C (that is, the slope where the repair sys-

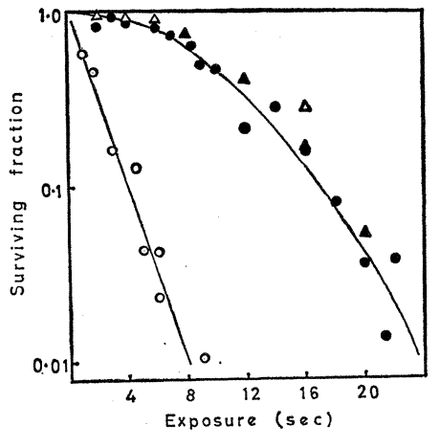

Fig. 3. Survival from ultraviolet irradiation of (at a distance of 90 cm) of *Escherichia coli* WP2 HCR⁻ (Hill); closed circles, irradiated at 22°C; open circles, irradiated at −79°C; open triangles, irradiated at 22°C in complete absence of photoreactivating light; closed triangles, irradiated at 22°C immediately after freezing to −79°C and then thawing.

tem is almost completely saturated) (*11*). This result does not appear to be compatible with an increased production of thymine dimer–type damage. If the assumption is correct that the shoulder on the curve at 22°C is due to a small capacity for repair of thymine dimer–type damage, then the abolition of the shoulder could mean that the lesion at −79°C is different from that at 22°C and is not subject to the repair system present in the HCR⁻ strain. Further, the fact that the ultimate slopes at both temperatures are similar would indicate that the new lesion has an efficiency for killing similar to that of thymine dimer–type lesions. It would also be necessary to assume that *E. coli* B/r

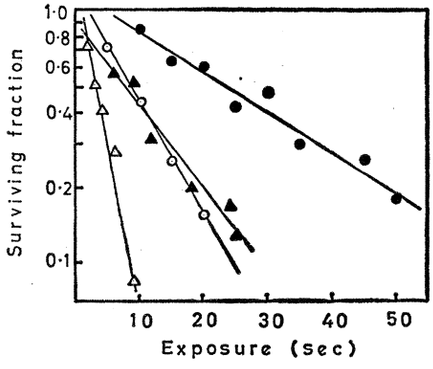

Fig. 4. Survival from ultraviolet irradiation (at a distance of 58.5 cm) of phage T1 irradiated at 22°C (circles) or −79°C (triangles) and assayed in *E. coli* B/r WP2 (solid symbols) or *E. coli* WP2 HCR⁻ (Hill) (open symbols).

WP2 can effect some repair of the different lesion produced at −79°C, otherwise its sensitivity at that temperature would not differ from that of the HCR⁻ strain. This is not unreasonable since any repair system in the HCR⁻ strain need not be identical with the excision-repair system known to be present in *E. coli* B/r WP2.

These results may also be explained by postulating that the repair system itself is very sensitive to ultraviolet light at −79°C, more so in the case of *E. coli* WP2 HCR⁻ than in the case of strain B/r WP2. Besides being rather unlikely at these low doses (from the known sensitivity of other enzymes), this interpretation cannot apply to the 2½-fold increase in sensitivity (relative to 22°C) observed when bacteriophage T1 is irradiated in vitro at −79°C and assayed in unirradiated bacteria (*12, 13*). The increase in damage observed with T1 differs from that with bacteria in that it has the same magnitude when either *E. coli* B/r WP2 or WP2 HCR⁻ is used as the assay organism (Fig. 4) and would therefore appear to consist largely of thymine dimer–type damage. A certain caution is indicated before correlating results with phage and bacteria in these strains, since the introduction of the HCR⁻ gene into WP2 affects its own ultraviolet sensitivity by a factor of about 20, but the sensitivity of irradiated T1 phage assayed in it by a factor of only 2½. This difference cannot be explained entirely by the different sizes of the phage and the bacterial chromosomes. We have, however, performed some experiments to determine whether the ability of *E. coli* B/r WP2 to reactivate irradiated phage T1 is inactivated by doses of ultraviolet light given at −79°C comparable with doses used in the bacterial survival curves; no appreciable inactivation was detected. While the evidence is not conclusive it seems unlikely that the results obtained with *E. coli* are due to a large increase in the ultraviolet sensitivity at −79°C of the thymine-dimer excision system.

We favor the hypothesis that during ultraviolet irradiation at −79°C thymine dimers (and other excisable lesions) are replaced by lesions which are less readily repaired. A similar situation may exist in the (perhaps) analogous solid-state environment inside spores of *Bacillus megaterium*. Donnellan and Setlow (*14*) found no thymine dimers after ultraviolet irradiation, but rather a relatively high yield of a new photoproduct involving thymine.

The greater sensitivity of reactions involving photodimerization of nucleotide bases in the frozen (or solid) state as compared with those in the liquid state has been explained in terms of concentration effects concomitant with phase changes during freezing (*6*). It is possible that ultraviolet-induced changes in precursors of DNA with low molecular weights could lead to the production of defective DNA, in which case one might expect a large increase in lethal effect because of localized concentration of precursors during freezing. It is difficult to envisage how concentration effects of the type which occur during freezing could affect chromosomal DNA. Indeed, Beukers (*5*) has found DNA in solution to be no more sensitive in the frozen than in the liquid state as measured by change in extinction at 259 mμ.

The possibility that damage to some nongenetic system might be involved cannot, of course, be entirely excluded. The interesting reversal of temperature-dependence which occurs below −79°C, and which is probably associated with physical processes such as the migration of excitation energy and charge transfer, deserves further study.

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