I. INTRODUCTION

Wavelengths below 300 nm form a unique region of the photobiological spectrum, the final effects on living cells being almost always deleterious. In this respect, their action resembles that of ionizing radiation. Chief among these effects, and the only one we consider here, is cell killing.

Unlike our less fortunate fellows in the ionizing radiation field, we have known for over 30 years the nature of the primary chromophore for killing. The pioneering work of Gates (1930) showed that this was nucleic acid. One might have thought that such knowledge would rapidly lead to elucidation of the mechanism of u.v. killing, but little has been learned of the mechanism until quite recent times. In 1960, Beukers and Berends showed that u.v. could dimerize thymine in frozen solution, and, further, that the same wavelength that produced the dimers could then split them upon melting of the solution. This finding inaugurated a resurgence of interest in the action of u.v. on biological materials and marks the beginning of the present phase of research, in which information on the molecular modes of action of u.v. is being accumulated at a remarkable rate.

Why did it take 30 years to reach the point where we began to understand the mechanism of u.v. action? I think there are at least two important contributing factors. One is that u.v. radiation, like ionizing radiation, is extremely efficient in killing cells. The bacterium Escherichia coli B, shows a 37 per cent survival dose \( D_{37} \) of about 100 erg mm\(^{-2} \), and some bacteria have a \( D_{37} \) as low as 1 erg mm\(^{-2} \) (Hill and Simson, 1961). In contrast, doses of the order of \( 10^6 \) erg mm\(^{-2} \) are needed to alter measurably the physical properties of large nucleic acids in vitro, and even tiny chemical changes cannot usually be detected with doses of less than about \( 10^4 \) erg mm\(^{-2} \). Apparently, it was only through luck, and the fact that clever people kept probing the matter, that the induction of thymine dimers was discovered.
This induction is sensitive, drastic in its biological consequences and, though ordinarily yielding a stable product, can nevertheless be reversed in at least two ways. The point, to which we shall have occasion to refer later in this discussion, is that the so-called 'biological doses' of u.v. (below about 1000 erg mm\(^{-2}\)) are far below the doses required for most observable effects \textit{in vitro}, and the products that we seek are therefore difficult to find.

The other factor which contributed to the slow development of knowledge of mechanisms is that a considerable amount of biological information had to be accumulated. During the 30-year period I mentioned, many protection and recovery phenomena, including photoreactivation (for review, see RUPERT, 1964) and dark recovery, were discovered and characterized. 'Dark recovery' includes phenomena like host-cell reactivation of bacteriophage (for discussion, see HARM, 1963) and liquid holding recovery of bacteria (HOLLAENDER and CLAUS, 1937; ROBERTS and ALDOUS, 1949). The point I wish to make is that, while we are all, in the last analysis, seeking to understand biological phenomena in molecular terms, this understanding cannot be obtained before we recognize the biological phenomena themselves. As a plant depends on the sun for both energy and control, so molecular biology must always turn to biology for (1) the source of all problems it seeks to solve, (2) much of the information that contributes to molecular solutions, and (3) determination of the biological importance of effects that are discovered in the test tube.

The series of papers that we are about to discuss is primarily concerned not, as the first part of the session title indicates, with photochemistry of nucleic acids, but, as the second part indicates, with the biological implications of this photochemistry. We know that u.v. acts on nucleic acids in cells, and we know a good deal now about the photochemistry of this interaction \textit{in vitro}. The question we ask here is, which test-tube reactions are of importance to the survival of the cell?

We shall use certain abbreviations. DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) are familiar ones. Radiation of wavelengths 230–300 nm, which is highly lethal to small cells, we call 'far ultraviolet' (far u.v.), and radiation of 300–380 nm, which is relatively non-lethal but highly effective for photoreactivation and induction of growth-division delay, we call 'near ultraviolet' (near u.v.). If the term 'u.v.' is not modified, 'far u.v.' is implied, and usually the wavelength is 254 nm. 'Photoreactivation' is abbreviated as 'PR'.

\textit{Photochemistry of Nucleic Acids}
Discussion of the papers is rather arbitrarily divided into three parts. The first two papers concern effects of u.v. on RNA. Although it is believed that the primary effects of u.v. in small cells are on DNA, this is a first-order approximation, and we are reaching a level now where it behoves us to consider other possible effects. The next three papers deal with u.v. effects on protein–DNA linkage. Action spectra and other information have implicated nucleic acids as both chromophores and sites of primary lethal damage, but usually with varying degrees of protein involvement. The role of protein in lethal damage is not at all understood. The last three papers deal with dark recovery from u.v. damage to DNA, a subject that has implications reaching far beyond the relatively narrow field of u.v. photobiology. Such repair ability may be crucial in the maintenance of species integrity over long periods of time. Without this stability, evolution, presumably, would be difficult if not impossible.

II. EFFECTS ON RNA

It seems likely that the nucleic acids are the only molecules that suffer photoreactivable damage (Jagger, 1958). There is no question that such damage is produced in DNA. Is it produced in RNA?

The evidence is largely affirmative. All of the plant viruses so far tested, except the rigid rods, are photoreactivable. Furthermore, the only rigid rods that have been examined in this respect, tobacco mosaic virus and tobacco rattle virus, do not show PR if the intact virus is irradiated with u.v., but do show PR if their extracted RNA is irradiated. Thus, the plant viruses without exception can be made to exhibit PR. Clover yellow mosaic virus is of particular interest since it is a flexible rod and therefore might be expected to show a behaviour intermediate between that of the spheres and the rigid rods. However, this virus, like two other flexible rods, potato virus X and cabbage black ringspot virus, has now been shown by Chessin to exhibit PR after irradiation of the intact virus. Thus, the flexible rods behave like the spheres.

In spite of all this evidence for the photoreactivability of RNA, no one has yet demonstrated a PR enzyme that works on RNA. Consequently, one cannot help wondering if plant virus PR may not be a basically different phenomenon, caused by some other response of the plant to photoreactivating light, which is a normal component of sunlight. An example of the sort of effect that is disturbing is shown by
the data of Chessin for clover yellow mosaic virus, shown in Fig. 1. Though at some u.v. doses the irradiated virus has a higher survival when the plant is in the light, and the slope of the curve in the light is much less steep than in the dark, still there is a very large lethal effect of the light alone (zero u.v. dose). Chessin thought this might be caused by a photodynamic action of chlorophyll, which is present both

Fig. 1. Survival of local lesions in Gomphrena globosa induced within 13 days by clover yellow mosaic virus. Circles refer to plants kept dark for 24 h after u.v. irradiation, triangles to plants left in sunlight. Experiments started at noon (from data of Chessin).
in the plant and in the virus inoculum. However, he found that virus in a colourless inoculum, containing no chlorophyll, showed the same sensitivity to photoreactivating light after inoculation, which suggests, but does not prove, that plant chlorophyll is not inactivating the virus. Thus the cause of the lethality remains unknown.

We now consider a study that suggests that RNA in cells can be photoreactivated. PITTMAN has studied photoreactivation of killing and mutation in haploid yeast. The mutation is the famous one leading to respiratory deficiency, resulting in the so-called 'petite' colonies. A variety of indirect evidence, mostly genetic, has indicated that the mutation is non-genic. PITTMAN studied both zero-point mutants, which give rise to entirely petite colonies, and delayed mutants, which yield one-half or one-quarter mutant sectors. He also examined cultures in two states, stationary phase, consisting of 72-h broth cultures, and a phase just at the inception of budding, obtained by growing stationary cells in fresh medium for 1 h, at which time the DNA content has roughly doubled and the RNA content roughly quadrupled. Figure 2 illustrates his findings. Stationary-phase cells show PR of killing and of zero-point mutation, but no PR of delayed mutation. Cells at the inception of budding are much more resistant to killing and to the induction of zero-point mutation, but are more sensitive to delayed mutation. The PR picture here is the same as for the stationary cells, namely, that PR of killing and of zero-point mutation occurs, while PR of delayed mutation does not.

The interpretation of these findings is not clear. PITTMAN assumes that both types of mutation are caused by u.v. damage to RNA, thus reflecting a different class of damage from that leading to killing, which is presumably in DNA. However, it is tempting to imagine that both zero-point mutations and killing are caused by damage to DNA. There are several reasons why this might be so: (1) roughly the same photoreactivable sector is found for both killing and zero-point mutation in both growth phases studied, (2) the enzyme in yeast extracts that photoreactivates DNA has been shown by RUPERT (1964) not to combine with u.v.-damaged RNA, and (3) the mutation of *E. coli* B/r to resistance to phage T1 was shown by KELNER (1949) to show PR of zero-point mutation but no PR of delayed mutation, and there is no reason to expect that the mutation in this system occurs in RNA. Finally, although the evidence seems clear that the 'petite' mutation of yeast is non-genic, this does not say that it does not occur in DNA. Indeed, a variety of recent papers report the normal presence
Fig. 2. Survival and mutation to respiration deficiency in yeast after u.v. irradiation of cells in the stationary phase (parts 1 and 2) and at inception of budding (parts 3 and 4). In parts 1 and 3, solid circles represent survival to u.v., open circles survival to u.v. plus maximum photoreactivation. In parts 2 and 4, circles refer to zero-point mutations, squares to delayed mutations, solid symbols to treatment with u.v., open symbols to u.v. plus maximum photoreactivation. Source of photoreactivating light was a 500-W projection bulb with a 1-in filter of 0.2 N CuSO₄ (PITTMAN).
of DNA in cytoplasm, so that the mutation could even have a cytoplasmic locus and still reside in DNA. Thus the intriguing results of Pittman remain unexplained at this time.

III. PROTEIN INVOLVEMENT

Many action spectra for u.v. damage to cells are not identical to the absorption spectrum of nucleic acid, but suggest possible involvement of protein. It is also known that the chromosomes of many cells contain protein. These facts have suggested to many workers that part of the lethal effect of u.v. may involve protein, either as a chromophore or as a substance that might bind to sites of u.v. damage in DNA. Some experimental evidence for such DNA-protein binding is now appearing.

Moroson, Gallego and Alexander have irradiated log-phase E. coli 15 T− and then removed and studied the DNA, using detergent lysis. As the dose increases, the amount of DNA that can be extracted from the cells decreases. Furthermore, at relatively low doses (1600–6600 erg mm−2), the extracted and purified DNA has a higher viscosity than DNA from unirradiated cells. They suggest that both phenomena reflect DNA-protein linkage, but offer no further evidence bearing on this matter. It would seem that their suggestion could be easily tested by seeing whether proteolytic enzymes eliminate the increased viscosity.

At doses roughly ten-fold higher, the viscosity falls to much lower levels than that of the control, and they attribute this to main-chain scission of the DNA. Such doses also decrease the hypochromic effect, but, contrary to expectation, they do not alter the melting temperature.

Finally, all of the effects they observe with DNA extracted from irradiated cells occur at doses as much as fifty times lower than those required to produce the same effects by in vitro irradiation of DNA. This, incidentally, should remind us of the fact that test-tube reactions cannot always be extrapolated to the biological situation.

The interpretations given of these results, however, suffer from the possibility that the actual damage to DNA within the cell may be relatively moderate, becoming serious only as a result of the unnatural events that occur during extraction and purification of the DNA. It would be interesting to see if PR of the cells causes any reduction in the observed effects. If not, then they probably are involved in a minor fraction of the u.v. biological effect.

These effects are of great interest, since they occur at doses close to
the biological range. However, it is probable that much broader studies will be required before we can say with assurance that they are biologically important.

A similar, but more detailed, study has been made by Smith. He finds, in agreement with Moroson et al, that when E. coli is irradiated with increasing doses of u.v., a proportional decrease is observed in
the amount of DNA that can be extracted from the cells with detergent. In a CsCl gradient, about 80 per cent of the unextractable DNA floats on top of the gradient with protein, and about 50 per cent of this DNA can be freed with trypsin. These results indicate that the unextractable DNA is bound to protein.

Figure 3 shows how the per cent recovery of DNA decreases with u.v. dose, the behaviour being similar for strains B/r, B, and B,T-. Thirty per cent of the DNA in these strains is quite sensitive to the cross-linking, a dose of 1800 erg mm\(^{-2}\), which leaves 1 per cent survival of B/r, causing a 10 per cent drop in recoverable DNA. The top line in Fig. 3 shows the loss of thymine due to thymine-dimer formation; the cross-linking reaction appears to be much more sensitive than thymine-dimer formation.

Various experiments with thymine-requiring strains indicate that cross-linking is related to the growth cycle. That portion of the genome that is being actively copied is the most sensitive to cross-linking. These variations correlate well with the variations in sensitivity to u.v. during the growth cycle, and thus suggest that cross-linking may play a role in cell killing.

*In vitro* experiments also show cross-linking. DNA irradiated in the presence of bovine serum albumin shows extensive cross-linking, and some is obtained if either the DNA or the bovine serum albumin is irradiated separately before mixing, suggesting a remarkable stability of the molecular configuration that leads to cross-linking.

These experiments show clearly that u.v. produces lesions *in vivo* that result in cross-linking of DNA with protein upon extraction by detergent. They also show that the same phenomenon occurs if irradiation of DNA and/or protein is carried out *in vitro*. Questions arise, however, regarding the biological significance of the reaction. For one thing, the cross-linking has only been shown to occur with extraction by detergent; it does not occur if the cells are first ground with alumina and then treated with detergent (Smith, 1964), although Smith (personal communication) believes this to be due to the drastic shearing caused by this treatment. It must be pointed out also that the response *in vitro* does not require detergent. Secondly, the phenomenon is not photoreactivable. U.v. biological damage in these strains is roughly 70 per cent photoreactivable, suggesting that the cross-linking can involve not more than 30 per cent of the biological damage.

As with the experiments of Moroson et al, these results of Smith
could possibly be artifacts caused by the extraction procedures. There is no doubt that u.v. increases the susceptibility of the DNA to cross-linking and that this susceptibility correlates with cell sensitivity to u.v. The question that arises is whether or not the cross-linking actually occurs in the intact cell.

If it does occur in intact cells, it is conceivable that the cross-linking reflects repair by dark enzymes that are still attached to the DNA at the time of extraction. SMITH finds (personal communication) the same effect for the same u.v. dose with strains B/r, which has dark recovery ability, and B_{s-1}, which shows no evidence of dark recovery (HARM, 1963). This would seem to eliminate the possibility of attachment of dark repair enzymes. However, it also shows that cross-linking has nothing to do with the unusual sensitivity of B_{s-1}, and that in this case the correlation of cross-linking with cell sensitivity to u.v. is zero.

The next paper we shall discuss concerns effects of u.v. on echinoderm cells. This work has a bearing on the question of protein involvement because it concerns cells in which protein is intimately associated with the chromosomes.

It has been shown in bacteria (GREER, 1960) and in phage (SAUERBIER, 1961) that incorporation of 5-bromouracil or 5'-'bromodeoxyuridine (BUDR) in place of thymine or thymidine (TdR) in the DNA leads to a greatly increased sensitivity to killing by u.v. There is also a concomitant sharp drop in photoreactivability. COOK has extended this work to echinoderm zygotes (the sand dollar, Echinarachnius parma).

Figure 4 shows, at the top, the normal time course of the first and second cleavages in a population fertilized at time zero. The middle part of the figure shows that addition of TdR and BUDR simultaneously at 30 min, followed by u.v. at 70 min, causes no delay in the first cleavage but a large delay in the second cleavage. While no photoreactivation is seen in the first cleavage, an almost complete PR of the delay in the second cleavage is observed. This behaviour is similar to that found if no TdR or BUDR is added, suggesting that in the presence of both precursors the cell uses only TdR. (The reason for using TdR at all is that the cells do not survive if exposed indefinitely to BUDR alone.) If the BUDR is presented to the cells 20 min before TdR, it becomes incorporated, and the bottom of the figure shows that the second cleavage is even further delayed and PR is eliminated. The additional delay in second cleavage is much greater than appears from this figure; in terms of dose modification, it is usually about a factor of
treatments are given early enough, uptake of BUDr does not affect either of these phenomena. Since the uptake of BUDr can occur during DNA replication in preparation for the first division, it is clear that BUDr, which affects only the second division, shows its effects only if it is present in parental DNA at the time of replication. A final point of great interest is an implication from the data that PR can
IV. DARK RECOVERY

We now move on to discussion of papers that concern dark recovery processes. One of the most important concepts that is now emerging from this type of work is that a very great fraction indeed of u.v. damage is probably rendered harmless by the cell itself. Thus, it seems likely that the one hundred-fold difference in sensitivity of *E. coli* strains B and B<sub>s-1</sub> (HILL) reflects merely a difference in ability of the cells to recover from the initial damage, which is probably identical in both strains. This means, of course, that when we observe the survival of typical cells, we see a population that, even under our 'control' conditions, has already undergone a great deal of repair. The additional repair that we induce by various treatments may in some instances be little more than minor enhancements of recovery processes that are already very active. This complicates our study of recovery phenomena. However, it also means that these phenomena are much more important than was previously thought, and they may have a very general application to genetic damage, whether induced by u.v. or by other agents.

Hanawalt and Pettijohn have made extensive studies on the physical nature of the DNA synthesized by *E. coli* during growth after u.v. irradiation. Cells of a thymine-requiring mutant were grown with radioactivity labelled 5-bromouracil (a thymine analogue) after u.v. The DNA was then extracted and its density distribution was examined in a CsCl gradient. All their experiments involved a u.v. dose of 500 erg mm<sup>-2</sup>, which totally inhibited the normal semi-conservative replication of DNA. Concurrent with 5-bromouracil incorporation into DNA, there was a steady loss with time of some of the thymine that was originally present in the DNA. This non-conservative mode of replication eventually resulted in density heterogeneity among the isolated DNA fragments. This brings to mind the thymine dimer 'excision' after u.v., reported by Setlow and Carrier (1964) and by Boyce and Howard-Flanders (1964).

Further experiments of Hanawalt and Pettijohn showed that, in cells incubated in nutrient medium for several hours after u.v., DNA synthesis is indeed quite abnormal. Normal incorporation of 5-bromouracil should lead to DNA of hybrid density, in which one
strand is light and one heavy. After u.v., however, the new DNA has a
density intermediate between normal and hybrid, suggesting that if
one strand is light, the other is heavy only in certain regions. This again
fits with the notion of 'excision' followed by new synthesis of certain
parts of one or the other strand of a two-stranded DNA. Furthermore,
they showed that this 'cut-and-patch' phenomenon occurs at random
positions throughout the genome.

They then designed experiments to test the four models shown in
Fig. 5. In this figure, new DNA is indicated by the jagged line. The
first model (A) illustrates normal synthesis, involving a single 'growing
point'. Model B illustrates intermolecular cross-linking that has
occurred after considerable synthesis within one of the molecules.
Now, with either of these models, the DNA extracted from a cell,
which is usually broken down into several hundred fragments in the
process, should show some fragments of normal and some of hybrid
density. This is not found, even after further breakage by moderate
sonication. These two models are therefore rejected.

Models C and D involve localized replication, either occurring in
parallel on both strands, as in C, or of the 'cut-and-patch' type, as in D.
Now, sonication plus thermal denaturation (separation of strands)
should yield some heavy fragments if model C is correct. Such heavy
fragments are not found, but fragments of hybrid density or of density
between hybrid and normal are found. It is concluded that model C is
wrong and model D is right. Finally, thermal denaturation alone,
without sonication, causes little change in molecular weight, indicating
that the phosphodiester backbone is not broken, and again contra-
dicting model C and supporting model D.

These findings provide support for the earlier observations of
thymine dimer 'excision' after u.v. It was supposed in this earlier
work that, if thymine dimers were excised and if this led to viable cells,
then the cut-out sections must in some way have been patched up with
the proper bases. The experiments of Hanawalt and Pettijohn
provide the first clear evidence that patching does indeed take place in
cells. They do not prove, however, that the patched regions are truly
back to normal.

Finally, Hanawalt and Pettijohn have shown that photoreactiva-
tion prevents all this unusual synthesis, presumably caused primarily
by the presence of thymine dimers, which are known to be split by PR.
It is of interest that the 'dark repair' ('cut-and-patch'), even after
several hours of post-u.v. growth, does not bring the cells back to the
point of producing normal DNA, as does PR. This implies that dark repair and PR do not overlap completely, which is contrary to indications in the literature (Metzger, 1963; Castellani, Jagger and Setlow, 1964). A possible explanation is that much of the abnormal synthesis observed by Hanawalt and Pettijohn may be unrelated to the dark repair processes that lead to higher survival. Their u.v. dose leaves only $4 \times 10^{-4}$ surviving fraction, loss of thymine after u.v. is drastic, and repair processes are expected to be complete after 90 min.
in nutrient medium, whereas their observed synthesis becomes progressively aberrant for hours after this time. It would seem that studies at lower u.v. doses and under conditions where biological recovery could be studied in parallel, would provide more information on the biological significance of these processes. Such experiments, however, are not easy, for at lower u.v. doses normal synthesis obscures the aberrant synthesis.

It has been found by Jagger and Stafford that there are two distinct types of photoreactivation in *E. coli* B. The relation of the work to dark recovery lies in the mechanism of photoprotection. Before we can discuss their findings we must first review what is known of the phenomenon called 'photoprotection'.

It is generally known that photoreactivation involves a treatment with near u.v. or visible radiation after u.v., and that this treatment results in higher survival of the cells. In some systems, however, a higher survival may be obtained by such a treatment before u.v., and this effect has been called 'photoprotection'. Jagger and co-workers have shown that the rate of photoprotection does not saturate at high dose rates of the protecting radiation, nor does the reaction show much dependence upon temperature. This behaviour is quite different from that of the usual photoreactivation and suggests that the initial reaction in photoprotection is purely photochemical and does not involve enzymes, as does the usual PR. Furthermore, the action spectrum for photoprotection, which is not nearly as broad as that for PR (see Fig. 6) is identical to that for the induction of growth delay in the near u.v. These and other data suggest that photoprotection operates by inactivating components of the electron transport system, thereby inducing both a growth delay and a division delay. This permits more time for dark repair systems within the cell to act upon the u.v. damage in nucleic acid, and this then leads to higher survival. Thus, photoprotection acts in a very indirect way to repair u.v. damage, while photoenzymatic PR acts in a very direct way.

Now, if this hypothesis of the mechanism of photoprotection is correct, then there seems to be no reason why one should not be able to induce the required growth-division delay by near-u.v. treatment after u.v. as well as before u.v. In most cells, such an 'indirect photoreactivation' would be difficult to detect, because it would be masked by the usual 'direct photoreactivation', caused by photoenzymes. However, a mutant of *E. coli* B, called 'phr−', was isolated a few years ago by Dr W. Harm. This mutant does not contain the
photoreactivating enzyme. However, Jagger and Stafford have demonstrated PR in this mutant. This observation alone shows that there are two different mechanisms of PR in E. coli B.

![Graph showing action spectra for photoprotection and photoreactivation](image)

**Fig. 6.** Action spectra for photoprotection from killing in *E. coli* B (solid line) and for photoreactivation of killing in *E. coli* B/r (broken line). Curves are normalized to 100 at wavelength of peak efficiency (from Jagger and Stafford, 1962, and Jagger and Latarjet, 1956).

They have also shown that PR in this mutant is, in every respect examined, similar to photoprotection and dissimilar to the usual enzymatic PR. For example, it shows no saturation at high dose rate and only a slight temperature dependence. It occurs at 334 nm, but not at 405 nm (see Fig. 6). Finally, 334 nm radiation induces a growth delay, but 405 nm does not. Therefore, they conclude that they have observed in this organism an 'indirect PR' that is similar in mechanism to photoprotection, and that therefore does not utilize a 'photoreactivating enzyme'.
The question arises whether or not this indirect PR may be peculiar to this one mutant. They therefore examined the kinetics of PR in the parent strain, *E. coli* B. Studies similar to those conducted with the mutant indicated that PR in the parent strain is almost entirely direct at 405 nm, but has a large indirect component at 334 nm. Recent support for these conclusions comes from their finding that thymine dimers in the DNA of irradiated cells are split by PR at 334 nm to a smaller extent than by PR at 405 nm.

One consequence of this work is that existing action spectra for PR must be re-examined in the light of possible contributions of indirect PR. For example, the spectrum of JAGGER and LATARJET (1956) was done with cells starved in the logarithmic growth phase. Such cells are photoprotectable, and hence probably show indirect PR. Therefore, their action spectrum may not correspond to the absorption spectrum of the chromophore of the PR enzyme.

This work should also serve to alert people to the fact that, although it is likely that thymine-dimer splitting is the major event in most photoreactivation, rarely is it the only mechanism of photoreactivation.

Several laboratories in the past few years have directed their efforts toward location on the bacterial chromosome of the genes controlling sensitivity to u.v. VAN DE PUTTE, VAN SLUIS and RÖRSCH have been active in this effort. A summary of their findings is shown in Fig. 7, where the circle represents the *E. coli* chromosome and biochemical markers are indicated by symbols connected to the circle by short radial lines (e.g., *threo* and *try*). Markers affecting u.v. sensitivity in strain B are shown inside the circle (*syn*, *B*$_{s-2}$, *phr*, *fil*) and those affecting the u.v. sensitivity of strain K-12 are shown outside the circle (*her*, *dir*$_{3}$, *dar*$_{3}$).

In *E. coli* B, the marker controlling production of the PR enzyme is located near the galactose marker. The marker controlling the shift to the high sensitivity of strain *B*$_{s-2}$ is located between the markers for methionine and threonine, while the marker *syn*, also controlling a shift to greater u.v. sensitivity, is located some distance away, between the markers for xylose and streptomycin resistance. Finally, a marker controlling filament production (*fil*), which is believed to be related to radiation sensitivity, is located near the marker for the PR enzyme. Clearly, markers for various factors that influence u.v. sensitivity are scattered widely on the genetic map.

In K-12 strains, the marker for host cell reactivation (*her*), which appears to be phenotypically related to the *syn* marker, does indeed
lie in the same general region of the chromosome. On the other hand, another mutant to u.v. sensitivity (\textit{dar}_5), which is phenotypically similar to \textit{hcr}, is some distance away from this region. The marker concerned with filament formation in K-12 (\textit{dir}_3) is not too far away from the \textit{fil} marker for strain B.

![Genetic map for chromosome of \textit{E. coli}](image)

**Fig. 7.** Genetic map for chromosome of \textit{E. coli}. Biochemical markers represented by symbols connected to circle by short radial lines. Markers affecting u.v. sensitivity (no radial lines) shown inside the circle for \textit{E. coli} B and outside the circle for \textit{E. coli} K-12 (\textit{Van de Putte}, \textit{van Sluis} and \textit{Rörsch}).

The findings just described are from only one laboratory. If I were to include those of other laboratories, I could draw a quite complicated picture. This would reveal the same characteristics that are demonstrated by the simpler diagram that we have here, namely (1) that markers affecting u.v. sensitivity are scattered over the entire genetic map, (2) that quite similar markers in the same strain (e.g., \textit{hcr} and \textit{dar}_5) may be widely separated, and (3) that apparently identical
markers in different strains (e.g. fil and dir) generally occur in the same regions but not in identical positions. I think the interpretation we may draw from this is that (1) sensitivity to u.v. is affected by many genetic factors, and (2) either some of these factors interact, or the same sensitivity may result from different patterns of mutation.

We cannot leave the work of van de Putte, van Sluis and Rörsch without mention of their recent demonstration of a dark recovery \textit{in vitro}. Their test system was the replication of bacteriophage \( \Phi \times 174 \) in bacterial spheroplasts. The DNA of this phage was isolated in the double-stranded form, irradiated with u.v., held for various times in an extract of cells of \textit{Micrococcus lysodeikticus}, then allowed to infect the host spheroplasts. The host cells were \( \textit{her}^- \), that is, unable to conduct host cell reactivation, and therefore probably lacked a dark recovery enzyme. However, the phage DNA incubated with the lysodeikticus extract showed higher survival, in proportion to the time of incubation with the extract. Therefore, the extract contains a repair factor. This factor is precipitable with ammonium sulphate, is inactivated by trypsin or by heat, and is non-dialyzable. Thus it appears to be a protein and, since it catalyses a biological reaction, may be further considered to be an enzyme. Thus, we now have systems that can conduct photoreactivation \textit{in vitro} and also systems that can conduct dark repair \textit{in vitro}, and all these systems appear to utilize enzymes. Although the active components are probably present in only very small concentrations in cells, it nevertheless appears to be only a matter of time before they will be isolated and characterized.

\textbf{V. SUMMARY}

Many of the papers we have just reviewed concern actions of u.v. on molecules other than DNA. Because of its genetic role, as well as its ability to absorb the radiation, DNA is certainly the most important biological target for u.v. However, RNA absorbs u.v. quite as well as DNA, and must therefore also be considered. Two of the papers have dealt with effects of u.v. on RNA. One supports the interesting generality that every RNA plant virus that has been studied in this regard shows PR under the appropriate conditions, while the other suggests that it may be possible to photoreactivate mutations produced in the RNA of yeast cells. Of course, there is a good deal of other evidence suggesting that damage to cellular RNA can be photoreactivated.

The involvement of protein in u.v. effects on cells is a very difficult
thing to study. The papers we have considered here show clearly that u.v. irradiation of bacteria does make their DNA susceptible to cross-linking with protein, but they have not provided the final proof that this actually occurs inside cells. The non-photoreactivability of the phenomenon implies that such cross-linking cannot make up more than a minor fraction of the u.v. damage. Nevertheless, it could be a significant fraction, and we may hope that these studies will be actively pursued.

The matter of extrapolation from one biological system to another arises frequently. For example, do our findings with bacteria apply to mammalian cells? PR studies suggest that yeast and bacteria behave in somewhat parallel fashion, and, even more striking, that echinoderm eggs and bacteria behave similarly with regard to BUdR sensitization and photoreactivation. These limited observations tend to support the idea that one can extrapolate from one biological system to another in a surprising number of instances.

We have considered some papers that deal with both light and dark recovery in bacteria. It has been shown that an effect that apparently encourages dark recovery is caused by PR light, and that, therefore, there are two entirely different mechanisms of PR in some bacteria. This points up the error that people frequently fall into, namely that of equating PR and thymine dimer splitting. PR does split thymine dimers in most systems, but it is rarely, if ever, the only PR effect.

Dark repair enzymes have now clearly been found in cell extracts. At least some dark recovery may act by cutting out thymine dimers and patching up the holes with complementary DNA, and we are now beginning to see more and more evidence that this amazing process actually does occur. We have seen also that the genetic basis of u.v. sensitivity is complex and this is a problem that is not likely to be solved without a great deal more work.

VI. FINAL REMARKS

We have considered in this session a rather heterogeneous group of papers. It is difficult, therefore, to draw general factual conclusions. I think, however, that we might make some observations on the course of research in this field and the direction it may take in the future.

The u.v. photochemistry of the nucleic acids is now virtually a subject in itself. Much has been learned about specific in vitro mechanisms in the last 5 years, and vigorous work in this direction continues. On the other hand, u.v. photobiology of a descriptive type has also
reached a certain maturity, as the result of some 30 years of research. The problem now is to bring the two together, and it is a problem indeed. Many sophisticated studies have been made using all the modern methods of biochemistry and physical chemistry that are applicable. The workers who conduct these studies are to be congratulated for their perseverance and ingenuity. Nevertheless, a large proportion of these studies run into serious stumbling blocks when it comes to proof that such-and-such a molecular reaction studied in vitro plays such-and-such a role in vivo. Such proofs are not at all easy to come by. I feel that their demonstration in most instances requires an attitude of respect for and thorough knowledge of both the physical and the biological facts. Experts entirely on one side or the other are not likely to bridge the gap.

A second point I should like to make is the necessity of considering all biological effects, regardless of how small or unimportant they may seem at the moment. In 1937, Hollaender and Claus observed a recovery of cells from u.v. by holding them in distilled water. It was a small effect. Judging from subsequent literature, hardly anyone noticed it. In 1949, Roberts and Aldous rediscovered the effect, showed it could be quite large, but found that it occurred only in certain bacterial strains. It then began to be discussed by a few photo-biologists who did not mind being accused of esoteric interests, but it seems quite certain that photochemists disregarded it. Now, essentially this same phenomenon is being used to explain one hundred-fold differences in u.v. sensitivity, and, as I have noted in the Introduction, it promises to be one of the most important and far-reaching discoveries of u.v. photobiology.

REFERENCES