A sequence of cytological changes occurring in both lysogenic and nonlysogenic strains of *Escherichia coli* following ultraviolet irradiation has been described in earlier publications by Kellenberger (1953), Hartman *et al.* (1955), Payne *et al.* (1955) and Kellenberger and Ryter (1955). This sequence involves aggregation of nuclear chromatin in the center and along the axis of the irradiated cell, followed by growth and a delayed increase in chromatin, without corresponding cell division. If the radiation has been sublethal and inducible prophage is absent, this sequence is reversible and the cells return to their normal dividing status. Following irradiation in lethal quantity or induction of prophage to maturation, the sequence described proceeds to irreversible fragmentation of the nuclear chromatin and lysis of the cells. Similar effects may be observed with X-rays (Kellenberger, 1955).

Whitfield and Murray (1956) have shown most recently that aggregation of nuclear chromatin in the center and along the axis of the cell can be brought about by injurious agencies as diverse as exposure to hypertonic solutions of sodium or potassium salts, low temperature or ultraviolet radiation, and the action of antibiotics and metabolic inhibitors. It appears from this work that this alteration in configuration of nuclear chromatin may be a secondary consequence of failure of a homeostatic mechanism regulating the ionic milieu within the cell. Ultraviolet irradiation is known to increase the permeability of the bacterial membrane (Heinemets and Lehman, 1955). Inhibition of cell division can also be brought about by many diverse injurious agents.

In the present study we have examined the cytological sequences following the action of ultraviolet radiation and "Furacin" upon wild-type *E. coli* strain B and its radiation- and furacin-resistant mutants. All three strains are found to exhibit the sequence of nuclear aggregation and growth without cell division following even minimal exposure, but the resistant mutants show much greater capacity to survive and return to normal. Capacity to resist these injurious agents is thus a function of genetic constitution, and may consist rather in the possession of efficient restorative capacity than in invulnerability to the primary injury. Capacity to recover is of course also a function of the degree of injury originally sustained.

**MATERIALS AND METHODS**

Wild type *E. coli* strain B and a radiation-resistant mutant selected as a survivor by the double irradiation screening procedure of Witkin (1947) were used. A furacin-resistant mutant was obtained from strain B by streaking resistant colonies on increasing concentrations of furacin (5-nitro-2-furaldehydesemiacbazone, Eaton Laboratories, Norwich, N. Y.) on Szybalski (1952) gradient plates.

Samples were removed from log phase roller tube cultures in nutrient broth (Difco) when the population had reached approximately $10^8$ cells/ml. About $10^7$ cells were spread on nutrient agar plates. The broth and nutrient agar contained 5 g/L of NaCl. They were then irradiated with the appropriate dose of ultraviolet from a 15-watt GE germicidal lamp at a distance of 47.5 cm (approximately 105 ultraviolet milliwatts per sq ft or 1330 ergs/sec/cm²). Although irradiation was carried out at room temperature, plates were prewarmed and returned to a 37°C incubator immediately after treatment. Precautions were taken to avoid photoactivation.

For study of the action of furacin similar
samples of about $10^7$ cells were spread on nutrient agar plates containing 5 g/L of NaCl and appropriate concentrations of furacin, and the plates were incubated at 37 C. Samples were also diluted and used to determine the numbers of survivors on similarly treated plates. Agar blocks were cut out of the more densely-seeded plates at appropriate intervals, fixed for 1 min in the vapors of 2 per cent osmium tetroxide, and impression smears were treated by the nuclear staining procedure described by DeLamater (1951, 1953).

RESULTS

Comparative resistance patterns. Ultraviolet resistance was determined by irradiating cells spread on nutrient agar plates as described. The relative sensitivities of the three test strains are presented in table 1.

Comparative resistance patterns of the three strains to furacin were determined by streaking cell suspensions on gradient plates (Szybalski, 1932). Most of the E. coli strain B population was inhibited by 1 $\mu$g/ml of furacin, but a considerable portion of the cells were able to form colonies up to 2-3 $\mu$g/ml; rare scattered colonies developed up to 4-5 $\mu$g/ml. Strain B/r grew well up to about 5 $\mu$g/ml and produced scattered colonies up to 8-10 $\mu$g/ml. The B/F strain used grew well up to 50-60 $\mu$g/ml; this value seemed to represent the highest resistance level obtainable under our conditions with this strain.

The cytology of normal cells. E. coli strain B (figure 1). When spread from log phase nutrient broth culture onto nutrient agar, cells showed 2 to 4 nuclei per cell, with occasional elongated cells containing 8 or more nuclei. After 2 hr of incubation a small per cent of the cell population formed short filaments which by 4 hr had become long, thin filaments; some of these filaments formed twists and "skews" which by 6 hr ramified through the colonies to form a lacy network. These various forms are considered normal to this strain.

E. coli strain B/r (figure 20) contained mostly cells with 2 to 4 nuclei, with an occasional elongated cell; no filament formation was observed. Occasionally there were some underhydrolyzed or underhydrolyzed cells which might be confused with those cells that showed centralization of chromatin after irradiation.

E. coli strain B/F contained mostly cells with 2 to 4 nuclei and an occasional elongated cell with 8 to 16 nuclei; no filaments were seen. Strains B/r and B/F of E. coli presented similar pictures.

Sequences following ultraviolet irradiation. E. coli strain B, 1-sec exposure (figures 1-19). Within the first half hour after exposure to this dosage, the nuclei had paired and aggregated and assumed a central position within the cell (figure 2). The aggregated nuclei stained solidly. In an occasional 8- to 16-nucleated cell there was the suggestion that 3 nuclei fused together (figure 8). Polar granules were present at one or both poles. The cells began to elongate and the chromatinic material became granular and migrated toward the poles.

The next 30 min showed cells elongated 2 to 4 times the normal length. Some of these contained rather regularly spaced nuclei (figures 3 and 4), some were almost filled with aggregated chromatin (figure 9); some cells showed both conditions (figure 5). By 90 min (figures 10 and 11) some cells contained coarser, more intensely stained granules which, because of their compactness, tended to make these cells very dark. The population was now made up of filaments of various lengths which were filled with chromatin. Scattered along the filaments were very intensely stained granules.

Long "snakes," curled and twisted into clumps and bizarre shapes, showed up by 2 hr (figures 12-15). Chromatinic material was scattered along the lengths of the filaments. Some filaments were twisted into "skews" (figure 12). Some showed extensive widening in places (figures 13

<table>
<thead>
<tr>
<th>Time of Ultraviolet Irradiation</th>
<th>Per Cent Survivors (Colony Formers)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td>sec</td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>10-25</td>
</tr>
<tr>
<td>5–6</td>
<td>0.5-1.0</td>
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<tr>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>40</td>
<td>0.06</td>
</tr>
<tr>
<td>56</td>
<td>—</td>
</tr>
</tbody>
</table>

In resistance to ultraviolet, E. coli strains B/r and B/F are of the same order as strains K12 and K12S (Hartman et al., 1955). Strain B is more sensitive to ultraviolet and also more sensitive to x-rays than other strains of E. coli studied (Zelle and Hollaender, 1955, cf. pp. 374 and 390).
and 16). Radially enlarged cells persisted until at least the fourth hr after irradiation.

Small, normal-appearing cells or broken-off filaments (figure 17) became noticeable about 3 hr after irradiation. These increased in number (figures 17–19) until at 6 hr they made up the majority of the population. These cells arose from the breaking-up either of whole filaments or of large sections of them; this process was first observed at 4 hr. Occasionally some filaments showed very intensely stained granules. There were still some filaments visible among the normal-looking cells 6 hr after irradiation.

It is emphasized that the survivors of all 3 strains, whether recovering from the initial action of ultraviolet or furacin, showed elongated cells or filaments (where these occurred) which possessed nuclei at rather regularly spaced intervals, as if the cells or filaments were about to resume cell division.

\textit{E. coli} strain B, 5-sec exposure.\ The sequence of events was similar to that following 1-sec exposure to ultraviolet. Long snakes seemed to be slightly slower in appearing. At 3 hr many areas showed granular debris, as if enlarged cells had disintegrated there. Some filaments appeared faintly stained and seemed to be disintegrating. Quite a number of normal-appearing cells were observed by 5 hr. Here again they arose from the breaking up of filaments into normal-sized bacilli. Enlarged cells and filaments were still visible 6 hr after irradiation.

\textit{E. coli} strain B, 40-sec exposure.\ The initial pairing and fusion with centralization of chromatinic material of some cells was similar to 1-sec exposure to ultraviolet. However, many cells were darkly stained as though they were underhydrolyzed. Cells became slightly elongated by \(\frac{1}{2}\) hr, showing granulation to the poles, with intense polar or subpolar granules in some cases. Many cells were diffusely and not very intensely stained. Cells elongated only about 2 to 4 times normal length for the first 2 hr. At this time occasional short filaments were seen. Staining was faint although many cells contained intensely stained granules and some revealed a central, intensely stained chromatinic filament or “bar.”

At 3 hr occasional normal-appearing cells were
Figures 6-19. *Escherichia coli* strain B exposed to ultraviolet irradiation for 1 sec. Hydrolyzed in 1 N HCl at 60 C, stained by DeLamater's azur A-thionyl chloride method. The time given with each figure indicates time of incubation after irradiation. 6: *E. coli* strain B cells shortly after spreading on nutrient agar from a normal log-phase nutrient broth culture. 7: 15 min. Chromatinic material fused and centralized in some cells. 8: 45 min. Cells have elongated and DNA material has been replicated and spread throughout. 9: 60 min. 10: 75 min. Elongation continuing. 11: 90 min. Long filaments filled with densely staining material. 12: 105 min. Filaments and a cut-off portion of a long, twisted "skein." 13: 2 hr. Long, twisted filaments and an enlarged cell filled with densely staining chromatinic granules. 14: 2½ hr, showing one of the bizarre forms assumed by cells during filament formation. 15: 3 hr. A portion of a large, twisted mass of filamentous "jungle." 16: 4 hr. A vacuolated, radially enlarged cell, probably in a state of degeneration. 17: 4 hr. Another part of the preparation shown in figure 11. Long filaments breaking up into normal sized cells. 18 and 19: 4½ and 5 hr, respectively. Filaments producing normal-appearing cells.
seen. Some cells appeared to disintegrate into faintly staining debris. A few radially enlarged cells were visible at 4 hr, as were short filaments. These persisted through the sixth hour. By 5 hr it was apparent that some filaments were breaking up into normal-sized cells.

_E. coli strain B/r, 1-sec exposure_ (figures 20–29).

Some centralization and fusion of chromatinic material with subsequent elongation of cells and granulation of chromatins occurred within 1/2 hr after ultraviolet (figure 21). By 1 hr the cells were 2 to 4 times the normal length and were filled with granular material (figures 22 and 23).

Elongation continued until about 1 1/2 hr after irradiation when the cells had attained lengths 4 to 8 times the length of unaffected cells (figures 24 and 25). Further elongation did not take place, although such elongated cells were still seen at 2 1/2 hr after ultraviolet (figures 27 and 28). Normal-looking cells were visible 1 1/2 hr after irradiation (figure 26); these increased in numbers until by 3 hr they formed the majority of the population (figure 29). Snakes, long filaments, and radially enlarged cells were not observed.

_E. coli strain B/r, 5-sec exposure._ Events similar to 1-sec exposure.

_E. coli strain B/r, 40-sec exposure._ Some cells showed fusion and centralization of nuclear material within 1/2 hr after exposure. Slight elongation of cells began before 1 hr after ultraviolet, but cells did not attain lengths more than 2 to 3 times that of normal controls during the time of observation. The cells remained diffusely stained or finely granular throughout, beginning from 1 hr. Prominent granules were seen from 2 1/2 hr through 3 hr. Central chromatins filaments or “bars” were observed from 2 hr onward. A few normal-looking cells were seen throughout the period of observation; these increased in number from 3 hr after exposure until they comprised the majority of the cell population.

_E. coli strain B/r, longer exposures in an attempt to overcome ultraviolet resistance_ (60, 60, 70, 100 sec, 3 min, 5 min). The initial cellular responses were seen with the lower doses, viz., fusion and centralization of chromatinsic material. Cells elongated up to about twice the normal length. Cells remained faintly or diffusely stained throughout. Intensely stained prominent granules appeared in many cells subjected to the lower doses. Axial filaments and bars were observed in cells after longer exposures. Long filaments and radially enlarged cells were not seen in B/r exposed to ultraviolet.

_E. coli strain B/F, 1-sec exposure._ The cytopathological sequences were practically identical with those described under B/r above.

_E. coli strain B/F, 5-sec exposure._ The nuclear material followed a sequence of events similar to cells exposed to 1 sec ultraviolet. The population was predominantly normal-looking by 4 hr.

_E. coli strain B/F, 40-sec exposure._ Nuclei were broken down, fused and centralized 15 min after exposure. Elongation was evident at 1 1/2 hr. Granular material was spreading toward the poles. By 1 hr cells were 2 to 3 times normal length, with finely granular material spread toward the poles, and prominent polar and cytoplasmic granules were evident in some cells. By 90 min some cells were about 4 times the normal length. Most cells showed prominent polar and cytoplasmic granules. The finely granular material was faintly stained at 2 hr; prominent granules were evident. By 4 hr some of the cells had reached lengths 6 to 8 times that of normal cells. Prominent granules were still visible. Central densely staining chromatinsic “bars” and filaments were visible in many cells. Occasional normal-looking cells could be seen. At 5 1/2 hr cells were about 8 times normal length. Intensely stained central bars and granules were observed in most cells. Rarely there was a radially enlarged cell. Prominent granules and central bars still persisted at 6 hr; some normal-appearing cells were present.

_E. coli strain B/F, longer exposure_ (50, 60, 100 sec). Centralization of chromatinsic material occurred within 1 1/2 hr; elongation was beginning. Cells were 2 to 3 times normal length by 1 hr. Occasional polar granules. Nuclear material was finely granular or diffuse, faintly stained, and moving toward the poles. Chromatinsic bars showed up 2 to 3 hr after exposure. Cells still 2 to 3 times normal length; chromatinsic substance poorly stained. With the lowest dose, some cells were 6 to 8 times normal length at 4 1/2 hr. Some long cells showed up later in the other dosages. An occasional radially enlarged cell was seen 5 to 6 hr after ultraviolet. Prominent bars and granules persisted. Whole areas of normal-looking cells were observed at 5 hr. It appeared that some elongated cells were breaking up into normal cells.

Sequences Following Exposure to Furacin. _E. coli strain B, 1 μg furacin/ml_ (figure 1). One-half
hr after spreading on furacin agar some cells revealed fusion and centralization of chromatinic material. Cells had elongated 2 to 4 times the normal length by 1 hr, at which time many of them contained granular material spreading toward the poles. Polar granules were seen in some cells. Many cells seemed to contain normal-looking nuclei. By 2 hr cells were 2 to 8 times the normal length, with occasional short filaments. Some nuclei seemed to be recovering and returning to a normal appearance. Long filaments, snakes, and "skains" were visible at 3 hr. Radially enlarged cells were seen at 3½ hr. These aberrant forms persisted for at least 8½ hr after plating. Some areas showed faintly staining enlarged cells and filaments, from 6½ hr, as if they were degenerating. Prominent granules were visible in some filaments from the seventh to the eighth hr. Normal-looking cells appeared at about 7 hr and increased in numbers with time. Whole filaments resolved themselves into normal-sized cells at 7½ hr.

E. coli strain B, 3 µg furacin/ml. Nuclear events took longer to show up than with 1 µg furacin/ml and they were not so dramatic. Snakes and radially enlarged cells were not seen until 4 hr after plating. Fewer cells seemed to follow these sequences than on 1 µg furacin/ml, although they persisted until at least 9½ hr after plating. Prominent granules were observed in some of the filaments from 8 to 10 hr. Small normal-looking cells began to appear about 8 hr after plating.

E. coli strain B, 5 µg furacin/ml. Centralization and fusion occurred and seemed to be extended over a period of about 3 hr in many cells. Chromatinic material was spread poleward in some cells by 2 hr. Many cells stained diffusely or finely granularly over the 6-hr period of observation. Elongation was slow to begin and was not very dramatic. By 4 hr there were some short filaments, although most cells did not exceed 2 to 6 times the normal length. Prominent granules were seen in some cells at 5½ hr. Radially enlarged cells were not observed. Large numbers of smaller, normal-sized cells showed up from the ninth to tenth hr after spreading.

E. coli strain B, 10 µg furacin/ml. Initial centralization and fusion of chromatinic material occurred. Many cells contained centralized chromatin 6 hr after plating. Some cells were granular; the granules spread to the poles by 2 hr, while many remained poorly and diffusely stained at 6 hr. A few elongated cells, 6 to 8 times normal length, were seen at 2½ hr. Other than this there was no noticeable elongation.

E. coli strain B/r, 50 µg furacin/ml. Centralization of nuclear material occurred by 1½ hr, and many cells showed this condition at the end of 6 hr. Also, the chromatinic materials appeared as a solid bar, or as coarsely granular substance, in the central area of many cells through the 6 hr of observation. Elongation did not take place.

E. coli strain B/r, 1 µg furacin/ml. Cytological sequences were essentially as on 1 µg furacin/ml.

E. coli strain B/r, 10 µg furacin/ml. Chromatinic material centralized by 1½ hr. Cellar elongation did not occur up to 10 hr after plating. The chromatinic material remained centralized and often faintly and diffusely stained throughout the time of observation (for 10 hr).

E. coli strain B/r, 50 µg furacin/ml. Cells contained centralized chromatin by ½ hr, and this condition persisted for 10 hr. Staining was often faint and diffuse. Elongation did not take place over a period of 10 hr.

E. coli strain B/F, 1 µg furacin/ml. Fusion and centralization in some cells by ½ hr. Centralization was visible in a few cells up to 4 hr. Some cells were slightly elongated by 1 hr. Cells were granular to poles. Elongation did not exceed about twice normal length. At 3 hr many cells appeared to be normal. The normal population increased and prevailed from 4 to 6 hr.

E. coli strain B/F, 3 µg furacin/ml. There appeared to be less chromatinic fusion and centralization than on 1 µg furacin/ml. Otherwise the sequences seemed to be the same. Cells looked as though they were returning to normal at 3 hr, and normal cells existed from 4 hr onward.

E. coli strain B/F, 5 µg furacin/ml. Only a few fused and centralized nuclei were seen at ½ to 1 hr; most of the nuclei remained discrete and separate and appeared to be unaffected. Slight
elongation occurred at 1 hr but did not progress further. Polar and central granules were visible at 2 hr. Many cells were returning to their normal morphology at 2 hr. The numbers of normal-appearing cells increased until they comprised the population by 5 to 6 hr.

E. coli strain B/F, 10 μg furacin/ml. Some of the nuclei were fusing at 1½ hr; 1 or 2 chromatin masses were present in the cells. At 1 hr many discrete, normal nuclei could be seen, while other cells contained either granular or diffusely stained chromatin. Slight elongation took place. By 1½ hr granules were spreading toward the poles in those cells showing granulation. Polar granules were occasionally seen. Small, normal-appearing cells were seen at 3 hr; these increased in number until they made up the whole population in 4 to 6 hr.

E. coli strain B/F, 50 μg furacin/ml. At ½ hr the nuclei looked large and coarse, or diffusely stained. Centralization was not noted. Discrete nuclei prevailed at 1 hr. Cellular elongation was very slight at most. Polar granules and some central chromatinic bars occurred at 1½ hr. At 2 hr nuclei were diffuse, granular, or apparently unaffected. The edges of the nuclei were often fuzzy and indistinct. Nuclei appeared to be normal by 4 hr. These forms increased in numbers through 5 to 6 hr.

The cytological sequences following exposure of E. coli strains B, B/r and B/F to ultraviolet radiation and to furacin are summarized in tables 2 and 3.

**Table 2**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time of Exposure to Ultraviolet</th>
<th>Aggregation of Chromatin</th>
<th>Elongation of Cells</th>
<th>Filament Formation</th>
<th>Snakes and Bizarre Forms</th>
<th>Radially Enlarged Cells</th>
<th>Return to Normal Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli B</td>
<td>1 and 5 sec</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>40 sec</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E. coli B/r</td>
<td>1, 5 and 40 sec</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Longer exposures to 5 min</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>E. coli B/F</td>
<td>1 and 5 sec</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40 sec</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Longer exposures to 100 sec</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Phenomenon was observed in at least some of the cells; − phenomenon was not observed.

**Table 3**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Concentration of Furacin</th>
<th>Aggregation of Chromatin</th>
<th>Elongation of Cells</th>
<th>Filament Formation</th>
<th>Snakes and Bizarre Forms</th>
<th>Radially Enlarged Cells</th>
<th>Return to Normal Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli B</td>
<td>1 and 3 μg/ml</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5 μg/ml</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>10 μg/ml</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>50 μg/ml</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>E. coli B/r</td>
<td>1, 3 and 5 μg/ml</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10 and 50 μg/ml</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>E. coli B/F</td>
<td>1, 3, 5, 10 and 50 μg/ml</td>
<td>+</td>
<td>+</td>
<td>–</td>
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concentrations of salt. Cultures were grown overnight in roller tubes in nutrient broth containing 5 g/L NaCl. Subcultures were prepared by inoculating 0.1 ml into 5.0 ml of nutrient broth containing 5 g/L NaCl. Tubes were placed on roller apparatus for 2 hr. For survival experiments the 2-hr culture was diluted in distilled water or 0.9 per cent NaCl solution and plated promptly on nutrient agar (Difco Noble agar 1.5 per cent) containing the concentration of NaCl under test. For cytological observation 0.15 ml of the 2-hr culture was spread on the surface of a thick agar plate (Noble agar 1.5 per cent) containing the specified concentration of NaCl. The plates were incubated at 37 C and blocks were removed at the intervals indicated for making impression smears, and staining as in the earlier experiments.

In experiments in which the 2-hr cultures were plated on nutrient Noble agar containing 5 and 10 g/L NaCl there was 100 per cent survival and, on agar containing 80 and 100 g/L NaCl no colonies grew out. Plating on agar containing 20, 30, 40, 50, and 60 g/L NaCl gave intermediate survival values, which were significantly higher for the resistant strains B/r and B/F than for wild type B.

Cytological sequences are summarized in table 4. On agar containing 0 and 10 g/L NaCl the cytological appearances differed little if at all from the controls on agar containing 5 g/L NaCl. On agar containing 100 g/L NaCl the chromatin was irreversibly condensed into deeply staining granules, and no further change followed. At 40, 50, and 60 g/L NaCl, many of the cells showed irreversible condensation of chromatin. The survivors, however, showed sequences of cytologic change similar to those following brief irradiation with ultraviolet and exposure to low doses of furacin, and, as with ultraviolet and furacin, the aberrations in E. coli B were more extreme and of longer duration than in strains B/r and B/F.


**DISCUSSION**

Our observations show that the initial sequence of cytological changes, comprising the aggregation of nuclear chromatin in the center and along the axis of the cell and growth of the cell without corresponding division, occurs similarly in wild-type *E. coli* strain B and its resistant mutants B/r and B/F following exposure to ultraviolet radiation; a similar sequence follows exposure of strains B and B/r to furacin. This sequence occurs with minimal exposure to the injurious agents, as well as with exposure to larger dosages; when exposure exceeds the threshold of survival for most of the cells the sequence of change may be retarded and reduced and may be superseded by the changes characteristic of cell-death. The initial sequence of change here described for *E. coli* strains B, B/r and B/F is the same as previously described (Hartman et al., 1955; Payne et al., 1955) for *E. coli* strains K12 and K128. The early changes in nuclear chromatin described by us appear to be essentially the same as those described by Whitfield and Murray (1956) in a variety of bacteria subjected to various injurious agencies, all of which seem to interfere with the normal functioning of a homeostatic mechanism regulating the ionic milieu within the cells.

The cytological sequences following low doses of ultraviolet or furacin may be imitated by the survivors of strains B, B/r and B/F when plated on critical concentrations of hypertonic NaCl-agar.

The cytological aberrations following exposure to ultraviolet radiation, furacin or hypertonic NaCl we find to be continued longer and to go to greater extremes in the sensitive wild type strain B than in its mutants B/r and B/F. Demerec and Latarjet (1946) earlier found that at a given dosage of ultraviolet radiation the induced mutation rates to a particular bacteriophage-resistant mutant were the same for strains B and B/r, whereas the bactericidal effects were greatly different. Inhibition of deoxyribonucleic acid synthesis for about 40 min after a given dose of ultraviolet irradiation was found to occur equally in *E. coli* strain B and B/r (Kanazir and Errera, 1955). Various post-treatments of *E. coli* B after irradiation with ultraviolet in the experiments of Roberts and Aldous (1949) permitted as much as 90 per cent recovery from the "radiation sickness" so produced; these treatments did not promote recovery of B/r. Anderson (1951) found that strain B was also restored by increased temperature of incubation whereas *E. coli* strain B/r was not heat-reactivated. A particularly thorough study of reactivation effects on strains of *E. coli* including B and B/r has been presented and analysed by Harm and Stein (1953, a, b). Strain B was found to be much more readily inactivated than B/r by direct and indirect ultraviolet effects, By peroxide and by X-irradiation, and strain B was correspondingly more heat-reactivable after all these effects than B/r. Stein and Harm (1953) conclude that the difference in resistance between strains B and B/r is to be sought pre-eminent in heat-reactivable, indirect, non-genetic lethal effects. Our results lead us similarly to the conclusion that B/r and B/F are relatively resistant to ultraviolet radiation not by virtue of escaping the primary direct injury but rather by the efficacy with which reparative forces restore the injured cells to their normal status.

In an earlier communication (Payne et al., 1955) the hypothesis was proposed that the described aberrations in distribution of chromatin after ultraviolet irradiation might "bear some causal relationship to such genetic phenomena as induction, mutation and certain aspects of recombination and segregation." (also Kellenberger 1955). In view of the above discussion it now seems more likely that the cytological sequences described are results of more primary injuries.

Szybalski and Nelson (1954) observed, and we have confirmed, that mutation to a high degree of furacin resistance in *E. coli* strain B occurs in several steps. The first step of furacin resistance (and comparative resistance to a number of other toxic chemicals and X-rays) of strain B and the B to B/r change has been shown to be due to mutation at a single gene locus (Witkin, 1947; Bryson, 1948; Bryson et al., 1951; Szybalski and Nelson, 1954; Bryson et al., 1955).

Szybalski and Nelson (1954) reported that B/r was about 40 times more resistant than radiation-sensitive strain B to the nitrofuran derivatives, furadroxyl and furacin. Thirty independently isolated strains of B/r exhibited the same property, and all independent strains of B...
resistant to furadroxyyl were also radiation resistant. The first step to furacin resistance of strain B and the B to B/r change was shown to be identical (Szybalski, personal communication).

The enzymatic difference in furacin susceptible and resistant strains demonstrated by Asnis (1953, 1955, 1956) appears only in one of the higher steps to full furacin resistance rather than in the B to B/r change. This leaves still unclear the precise nature of the presumed primary biochemical alteration which results from mutation at a single locus and confers upon the cell the B/r phenotype. The initial cell damages caused both by furacin and by ultraviolet rapidly lead to common alterations in cell structure. It is unlikely that the chain of reactions leading to the observable cellular alterations is alike in both cases, however, since further mutation to furacin resistance can protect the cell against changes undergone in low furacin concentrations without similar protection against survival or nuclear changes effected by ultraviolet.

In seeking the primary sites of action of injurious agents upon bacterial cells it becomes clear that we must seek interactions antecedent to the somewhat non-specific consequences of injury manifested in the cytological sequences we have been studying. In addition, our results indicate that an understanding of the underlying mechanisms of the lethal action of radiations must come both from identification of these primary sites of action and from a fuller knowledge of the structural physiology and biochemistry of the cells we are dealing with. Lethal action of radiation on E. coli strain B and B/r is reviewed in detail by Heinmets and Kathan (1954), Heinmets et al. (1954), Heinmets and Lehman (1955), Kelner et al. (1955) and Zelle and Hollaender (1955).

**SUMMARY**

Sequences of cytological change following the action of ultraviolet irradiation, of "Furacin" and of hypertonic NaCl-agar are described for wild-type Escherichia coli strain B and its resistant mutants B/r and B/F. Ultraviolet irradiation is followed by an initial sequence of change, including aggregation of nuclear chromatin in the centers and along the axes of the cells and growth without cell division, in all 3 strains. In strain B these sequences go on to extremes and result in long filaments, skeins and radially enlarged cells. Not until 6 or more hours after exposure do the surviving cells and filaments regenerate a culture of normal morphology. In strains B/r and B/F the sequences of change are less extreme; shorter filaments are formed and the culture returns to normal morphology sooner. The survivors of cultures plated on critical concentrations of hypertonic NaCl-agar show equivalent aberrations.

Exposure to sublethal concentrations of furacin is followed by similar sequences in strains B and B/r. Again the changes in B proceed to extreme elongation of filaments and to radially enlarged cells, and in B/r are less extreme and of shorter duration. In strain B/F, brought to high resistance to furacin by prolonged growth in the drug, changes following exposure to any tolerated dose of furacin are minimal.

The initial aggregation of nuclear chromatin in the centers and along the axes of the cells is compatible with a mechanism described by others for several microorganisms subjected to a variety of injurious agents. This alteration in configuration of chromatin appears to be a secondary consequence of failure of a homeostatic mechanism regulating the ionic milieu within the cell. Inhibition of cell division appears also to be a secondary consequence of a primary injury.

The differences between the wild-type strain B and its mutant resistant strains (excepting the resistance of B/F to furacin) seem clearly therefore to be in the greater efficacy of reparative forces in the resistant strains rather than in any invulnerability to the primary injury. It is probably significant that others have found the frequencies of gene mutations in strains B and B/r to be similar with similar doses of ultraviolet or X-rays, whereas survival for B/r was much greater. It seems compatible with available evidence to suppose that initial injury, at least to 2537 A ultraviolet radiation, is sustained by nuclear DNA and perhaps other sites, and that the cytological changes are secondary consequences to which wild-type strain B is peculiarly sensitive. Strain B/F may have mutated to a degree of true invulnerability to this drug.

**REFERENCES**


Asnis, R. E. 1953 TPN-linked reduction of


Asnis, R. E. 1956 The reduction of furacin by cell-free extracts of furacin-resistant and parent susceptible strains of *Escherichia coli*. Arch. Biochem. & Biophys. (accepted for publication).


