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Effect of various post-irradiation treatments on ultraviolet irradiated *Shigella sonnei**

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INTRODUCTION

Post-irradiation treatments of ultraviolet irradiated bacteria cause changes in the percentage of survivors. Roberts & Aldous (1949) listed various factors which were important in the study of ultraviolet effects upon bacteria, including conditions employed following irradiation. They reported that the recovery of ultraviolet irradiated cells was greater on a chemically defined medium than on nutrient agar. The addition of nutrient broth to the chemically defined medium caused a decrease in the number of irradiated organisms that were capable of colony formation. Alper & Gillies (1958*a*) found that there was a greater survival of ultraviolet irradiated *Escherichia coli* B on Difco nutrient agar than on Oxoid blood agar base. They reported that if the Oxoid blood agar base was dialysed the non-dialysable fraction produced good recovery of the irradiated cells comparable to that on nutrient agar. They found that chloride ion and a factor in peptone reduced the recovery of irradiated bacteria. Alper & Gillies (1958*b*) reported that an increase in the concentration of peptone in nutrient agar or the addition of sodium chloride resulted in a decrease in the numbers of organisms surviving the irradiation treatment. It was pointed out that *E. coli* had a shorter lag phase and generation time when grown in nutrient broth supplemented with sodium chloride compared to the regular nutrient broth. Growth of the organisms was more rapid on Oxoid agar than on nutrient agar. It was postulated that suboptimal growth conditions favoured recovery by preventing imbalances in the synthetic processes of the ultraviolet injured cells (Alper & Gillies, 1960). Other workers have also observed similar results (Anderson, 1949; 1951; Stein & Meutzner, 1950).

The survival of ultraviolet irradiated cells increased when the irradiated cells were held in water or saline prior to plating on the recovery medium (Hollaender & Claus, 1937). On the other hand, prolonged holding in water resulted in a decrease in the surviving fractions (Hollaender, 1943; Barner & Cohen, 1956). Charles & Zimmerman (1956) termed this phenomenon 'dark' reactivation. It was found that maximum recovery of *E. coli* B occurred within 3–5 hr. when the

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cells were held in the dark (in appropriate culture media); this was then followed by a period in which there was a decrease in viable count (Woodside, Goucher & Kocholaty, 1960). Alper & Gillies (1960) reported that the colony-forming ability of irradiated cells increased as the time interval between irradiation and contact with the growth medium increased, up to a certain level.

In the present study of the effects of various post-irradiation treatments on the survival of ultraviolet irradiated *Shigella sonnei*, some of the factors investigated were the composition of the recovery media, temperature of incubation, and variations in the time interval between irradiation and plating upon recovery media.

METHODS

Two strains of *S. sonnei* were employed in our experiments: strain 5044–59 obtained from Dr W. H. Ewing, Laboratory Branch, Communicable Disease Center, Atlanta, Georgia, and strain F-6, obtained from Dr M. Finland, Boston City Hospital, Boston, Massachusetts. The cultures were periodically checked for purity. The source of ultraviolet light was a General Electric 15 W. germicidal lamp (G15T8) which emitted waves of approximately 2537 Å. The calculated intensity value under the conditions employed was 17·2 ergs/sec./mm.^{2}. The organisms were grown in the chemically defined medium (EM) of Erlandson & Mackey (1958). A 20–22 hr. culture of *S. sonnei* grown in EM medium was washed three times by centrifugation with 0·15 M phosphate buffer and resuspended in buffer to yield an optical density (OD) reading of 0·15 on the Bausch and Lomb 'Spectronic 20' spectrophotometer (wavelength set at 575 mμ). One ml. of this suspension contained approximately 10^8 cells. Ten ml. of the EM medium were inoculated with 1 ml. of this cell suspension. The tubes were incubated at 37° C. for 20–22 hr.; the growth was harvested and the cells were washed three times and resuspended in buffer to yield an OD reading of 0·15. Five-ml. aliquots of this cell suspension were irradiated in flat-bottom Petri dishes. The samples undergoing irradiation were shaken continuously to ensure uniform exposure of the cells. One-tenth ml. aliquots were withdrawn from the Petri dishes after 15 and 30 sec. of irradiation. An aliquot removed from the suspension prior to irradiation served as the unirradiated control. The unirradiated and irradiated samples were serially diluted; 0·1 ml. aliquots were plated upon the EM medium solidified with agar, nutrient agar (Difco), and brain heart infusion agar (BHI) (Difco). These plates were inverted and incubated for 24–48 hr. and the colonies were counted on the Quebec colony counter. This experiment was done in order to determine the effect of the composition of the recovery medium on the survival of the irradiated cells. In order to determine the effect of the temperature of incubation on the survival of the irradiated cells, nutrient agar plates were inoculated with aliquots of the cell suspensions and incubated at 15, 29, 37 and 44° C. for 24 hr. This was followed by an additional incubation period of 24 hr. at 37° C. to permit growth of cells which may have survived but did not form colonies, especially at the lower temperatures of incubation. In order to determine the effect of time intervals between irradiation and plating on recovery media, aliquots of irradiated cells were held in 0·15 M

phosphate buffer for $2\frac{1}{2}$, 5, and 8 hr. at 25° C. and then plated. Irradiated cells were also plated immediately to determine the change in population in the absence of any holding period. Unirradiated cells were also held at 25° C. for varying periods to determine the effect of the holding process alone. The percentage survivors after irradiation were calculated by dividing the number of viable irradiated bacteria per ml. by the number of viable unirradiated bacteria per ml. and multiplying the quotient by 100.

RESULTS

Greater recovery of irradiated *S. sonnei* occurred on the chemically defined medium than on nutrient agar or BHI agar. Recovery was slightly better on nutrient agar than on BHI agar (Fig. 1). The difference in the ability to recover colony-forming cells diminished at the higher ultraviolet dosage used. Colonies appeared larger on the BHI medium than on the EM medium; colonies on nutrient

Table 1. *Effect of the composition of the plating medium on the recovery of ultraviolet irradiated* Shigella sonnei

Composition of plating medium	Percentage survivors (258 ergs/mm.^{2})
Brain heart infusion (BHI)	17·1
BHI plus thiamin	16·0
BHI plus niacin	16·7
BHI plus $MgSO_4$	17·9
BHI plus glucose	18·0
BHI plus aspartic acid	22·2
Erlandson-Mackey chemically defined medium (EM)	38·2
EM plus BHI ingredients	30·1

agar were intermediate in size. In order to determine whether some constituent of the EM medium favoured the recovery of irradiated cells, the ingredients of the EM medium were added singly to the BHI medium. Furthermore, ingredients of the BHI medium were added to the EM medium to learn if the components of the BHI medium inhibited the recovery of irradiated cells. Table 1 summarizes the data. The addition of thiamin, niacin, $MgSO_4$, and glucose to the BHI medium did not materially alter the ability of the medium to recover irradiated cells. However, the addition of aspartic acid may have produced a slight increase in the surviving fractions that were able to form colonies. On the other hand, the addition of BHI ingredients to the EM medium produced a significant reduction in the ability of the medium to grow the irradiated bacteria. We varied the concentration of peptone in the nutrient agar to see if the peptone concentration affected the recovery of the bacteria exposed to ultraviolet light. We tested 5, 10, and 15 g. of peptone per litre of media. There was no difference in the numbers of cells that could be recovered.

Since Alper & Gillies (1960) found that conditions which were more favourable for the growth of unirradiated bacteria proved to be less favourable for the recovery of irradiated organisms, we determined the growth curve of *S. sonnei* in various liquid media. The results are illustrated in Fig. 2. The lag phase was shortest in the

BHI liquid medium, longest in the EM medium, and intermediate in nutrient broth. Essentially, the growth rate of *S. sonnei* was greater and more rapid in the medium which yielded the poorest recovery of the ultraviolet-treated cells.

A post-irradiation incubation temperature of 37° C. for 24 hr. resulted in greater

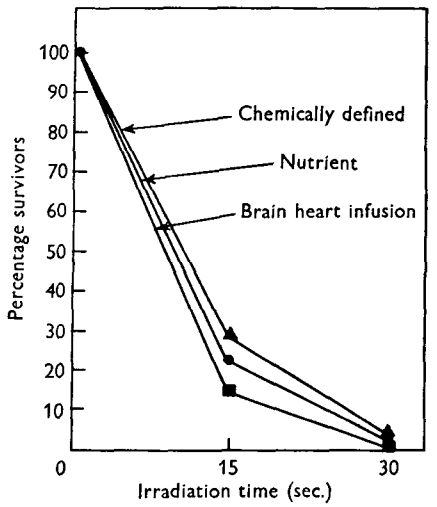

Fig. 1

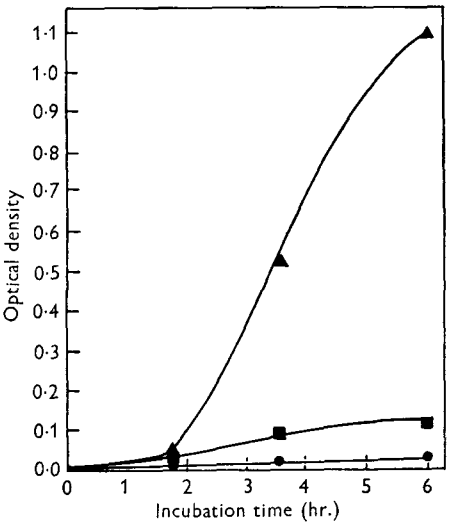

Fig. 2

Fig. 1. Recovery of ultraviolet irradiated *S. sonnei* on three different media.

Fig. 2. Growth rate of unirradiated *S. sonnei* in liquid media: ▲, Brain heart infusion; ■, nutrient broth; ●, Erlandson–Mackey chemically defined broth.

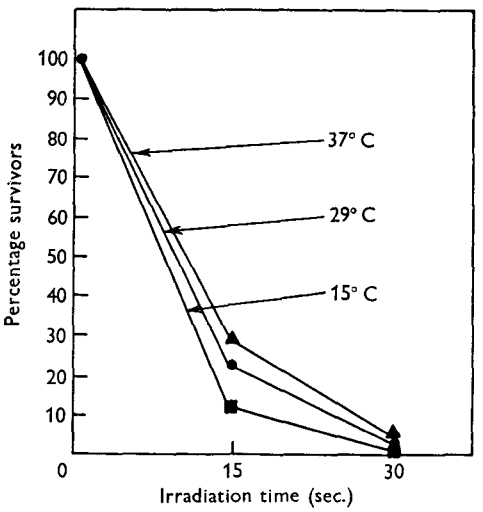

Fig. 3

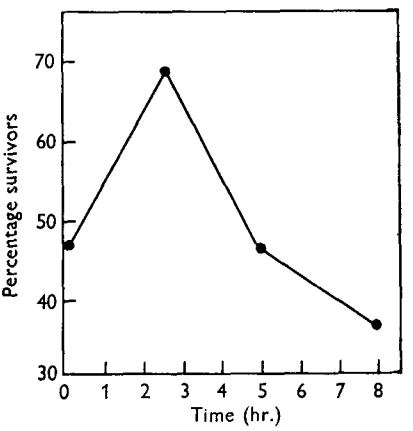

Fig. 4

Fig. 3. Survival of ultraviolet irradiated *S. sonnei* cells which were incubated at various temperatures for 24 hr. and then reincubated for 24 hr. at 37° C.

Fig. 4. Survival of ultraviolet irradiated *S. sonnei* held in buffer at 25° C. for varying periods of time prior to plating upon recovery media.

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recovery of ultraviolet irradiated cells than incubation at 29 or 15° C. when the cells were exposed to the lower level irradiation (258 ergs/mm.^{2}). However, there was little difference in the ability to recover cells when exposed to the higher level of irradiation (516 ergs/mm.^{2}). Fig. 3 summarizes the results. A post-irradiation incubation temperature of 44° C. resulted in fewer colony-forming cells than at the post-irradiation temperature of 37° C.

Irradiated cells held in buffer at 25° C. for $2\frac{1}{2}$ hr. possessed a greater potential for recovery from the effects of ultraviolet light than cells which were not held following irradiation or cells which were held in buffer for 5 and 8 hr. As a matter of fact the recovery of cells held for 8 hr. was poorer than cells which were plated immediately (Fig. 4).

DISCUSSION

The survival of ultraviolet irradiated *S. sonnei* varied considerably with different post-irradiation treatments. In the present study the ability to recover irradiated cells was affected by the composition of the recovery medium, the temperature of incubation following irradiation, and the time interval between exposure to ultraviolet light and plating upon recovery media. Recovery of irradiated cells was greater on simple synthetic media than on complex media. One possible explanation for this is that cell growth in the chemically defined medium may be sufficiently slower than on complex media thereby allowing a longer period of repair from the damaging effects of the irradiation. It also appeared that some component in the BHI medium actually inhibited or delayed the growth of the ultraviolet-treated cells. Although Alper & Gillies (1958*a*) found a factor associated with peptone that was inhibitory towards recovery, we did not find any differences in the ability to recover cells when the peptone concentrations varied from 5 to 15 g. per litre. However, we agree with Alper & Gillies (1960) with regard to the fact that conditions optimal for growth were unfavourable for recovery of the irradiated cells.

It is difficult to explain why cells incubated at 37° C. following irradiation permitted better recovery than cells incubated at 15 and 27° C. A reduction in the colony-forming cells when the irradiated cells were incubated at 44° C. could have been due to the fact that at this temperature the cells metabolized too rapidly to permit proper repair of injured sites. It is even conceivable that this temperature had a direct inhibitory effect upon the growth of irradiated cells.

Several suggestions have been offered to explain the increase in recovery of irradiated cells when the cells were held in a liquid medium prior to plating upon the recovery medium. Roberts & Aldous (1949) felt that during this holding period some cell 'poison' produced by the irradiation was destroyed. Alper & Gillies (1960) suggested that the increase in survival was due to the retardation of metabolism which protected the irradiated bacteria against the action of the 'peptone factor' in the plating medium. Furthermore, it has been reported that some protein synthesis was necessary for repair and that an exogenous source of nitrogen inhibited the recovery of irradiated cells (Doudney, 1959; Sawada & Suzuki, 1961). Immediate contact between irradiated cells and nutrients may

favour unbalanced growth which leads to death while holding in a liquid such as phosphate buffer before plating favours reparative processes. Billen (1957) reported that the release of 260 mμ-absorbing material from irradiated cells was inhibited in the absence of exogenous metabolites. The prevention of the loss of this fraction by incubation in a nutrient-free medium might play a role in preventing death.

SUMMARY

More cells of *Shigella sonnei* were recovered on a chemically defined medium than on complex media after the cells were irradiated with ultraviolet light. The temperature of incubation affected the numbers of colony-forming cells that could be recovered. A temperature of 37° C. yielded the largest number of colonies compared with temperatures of 15, 27 and 44° C. Recovery of irradiated cells was favoured when the cells were held in buffer at 25° C. for $2\frac{1}{2}$ hr. before plating on solid media.

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