MUTATION OF AN AUXOTROPHIC STRAIN OF ESCHERICHIA COLI BY HIGH PRESSURE OXYGEN.

G.D.Gifford.

Department of Botany, University of Exeter, Exeter,Devon.

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Fenn et al. (1957) reported that relatively long, 16 hour, exposures to high pressure oxygen (HPO) are apparently capable of exerting a mutagenic effect in Escherichia coli which is similar to the mutagenic effect of X-rays. It was suggested that selective survival of the mutant fraction of the original bacterial population during HPO treatment might provide an alternative explanation, though the appearance of an absolute increase in the number of mutants following oxygen treatment argued against this. However, following the findings of Gifford (1968), which show that the resistance of bacteria to HPO is periodic, and related to the physiological state of the organisms at the time of exposure to HPO, the possibility of an explanation based upon selective survival seemed quite plausible. It might be postulated that at the time of pressure increase relatively more mutant than normal cells were in the resistant state and that during the period of exposure these might divide, thus giving an increase in mutant frequency and even an increase in the total number of mutants.

In order to clarify the situation experiments have been conducted in which the reversion to prototrophy of the tryptophan auxotroph E.coli WP2 hcr⁻ (Hill), (kindly supplied by Dr.B.A.Bridges), induced by brief exposures to oxygen at 10 atmospheres has been studied. The results support the hypothesis that high pressures of oxygen are mutagenic in E. coli.

METHODS.

Late exponential phase cultures in glucose-salts medium supplemented with 10μg/ml. tryptophan were exposed to oxygen at 10 atmospheres, in liquid films 1 - 2mm. thick, at room temperature, in pressure vessels similar to those described by Caldwell (1956). Following release of pressure, samples were plated for survival and prototroph determination on glucose-salts agar supple-

mented with 0.75μg/ml. tryptophan. Plates were incubated at 37°, either directly, or following a period of incubation at 16°. Colonies were counted after 48 hours incubation at 37°.

RESULTS AND DISCUSSION.

Following 15 minutes exposure to HPO no increase in the number of prototrophs was observed upon plating directly at 37°, nor after 3 hours incubation at 16° before transfer to 37°. However, with times in excess of 3 hours at 16° the number of prototrophs rose, to a maximum after 9 hours, and thereafter remained constant (fig. 1). Since the time of exposure is short, relative to the

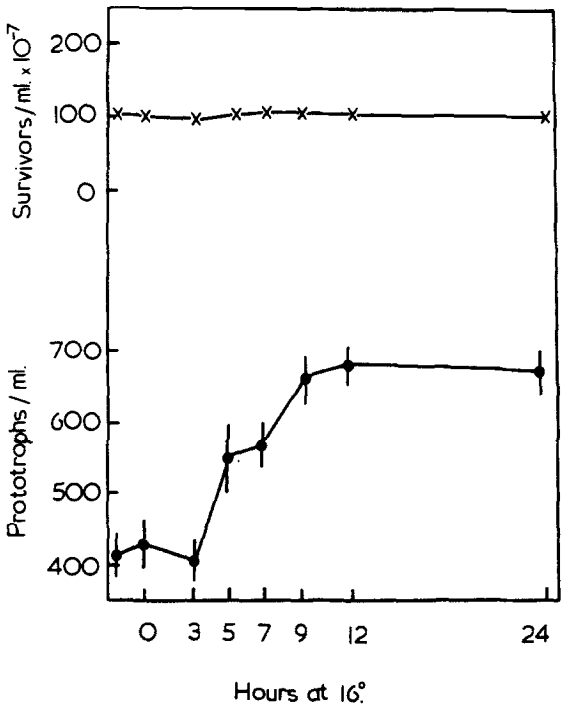

Fig. 1. Changes in survival and prototrophic frequency with incubation at 16° following 15 minutes exposure to HPO.
X ——— X , survival. ●——● , prototrophs.

generation time of approximately two hours in air, little division is likely to have occurred while under pressure. Therefore the observed increase in prototrophic frequency is attributed to a mutagenic effect of the high pressure oxygen. Presumably, at 16° the fixation of premutational lesions is faster than repair, whereas at 37° repair occurs at such a rate that even after 3 hours incubation at 16° there is sufficient time available for repair before fixation.

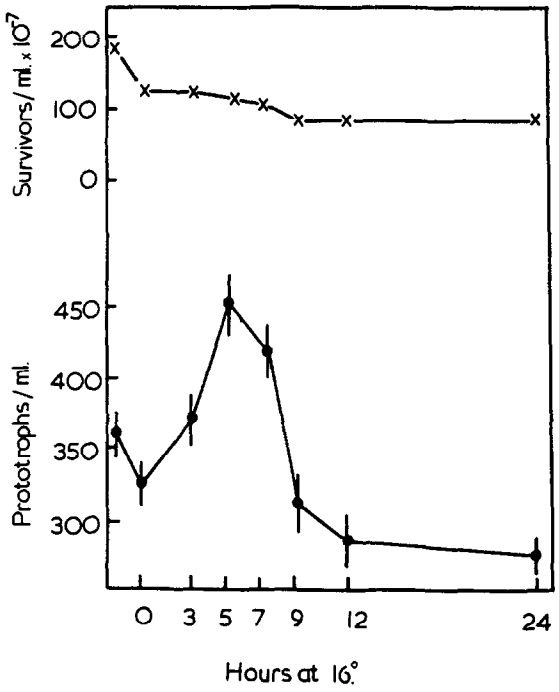

Fig. 2. Changes in survival and prototrophic frequency with incubation at 16° following 30 minutes exposure to HPO.
X ——— X , survival. ●———● , prototrophs.

A similar pattern of response was observed after 30 minutes exposure (fig. 2), though here the number of prototrophs rose to a maximum after 5 hours at 16°, then declined and reached a stable value after 12 hours at 16° . This decline may be due to the presence of potentially lethal mutagenic damage, which follows the premutational lesion at the tryptophan locus in the sequence of replication and thus fixation. Incubation at 37° after 5 hours or less at 16° allows the repair of this lethal damage even after fixation of the premutational lesion at the tryptophan locus. However, with incubation times in excess of 5 hours at 16° the lethal damage is fixed and the organism dies.

Longer exposures, of 90 minutes, have given variable results. Figure 3a shows the results of an experiment in which the exposure induced a large fall in viability, especially of the prototrophs; this is probably due to relatively more prototrophs than auxotrophs being in the sensitive state at the time of exposure to HPO. Upon incubation at 16° the number of prototrophs rose, to a stable value after 3 hours. This rapid rise may be due to the expression of premutational lesions which are too close to fixation to allow repair after a short period of incubation at 16°.

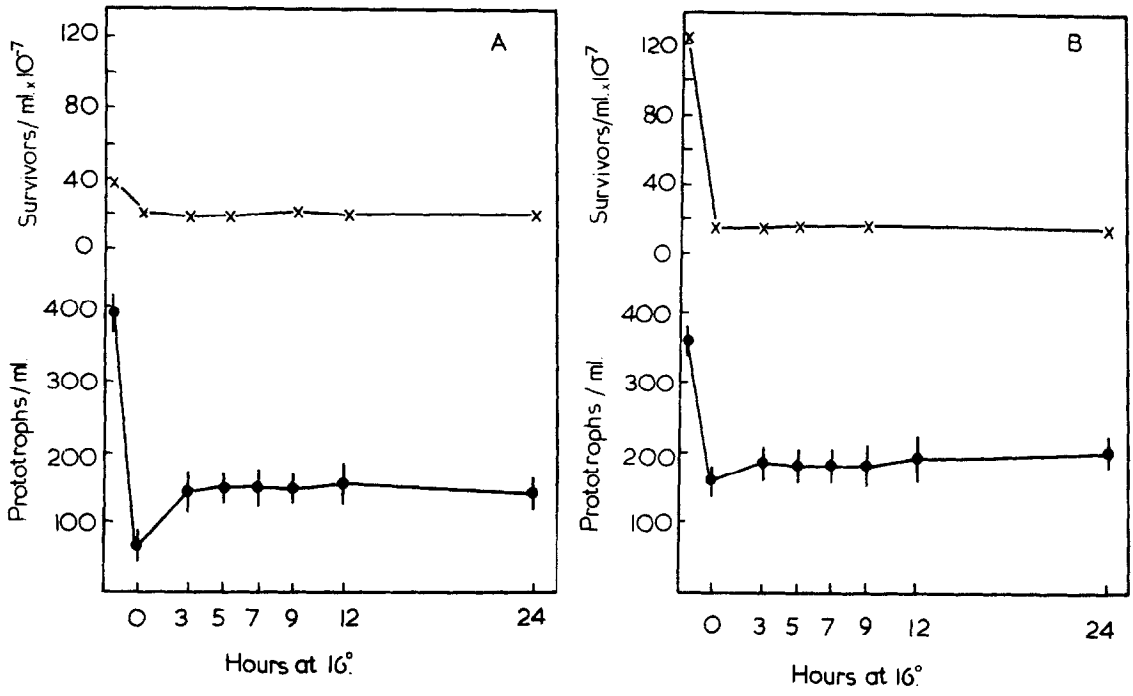

Fig. 3. a and b. Changes in survival and prototrophic frequency with incubation at 16° following 90 minutes exposure to HPO.
X ——— X , survival. ●——● , prototrophs.

A further experiment (fig. 3b), resulted in a large fall in viability associated with a smaller fall in the number of prototrophs per ml. . There was no change with incubation at 16°. This may be explained by assuming that the majority of the prototrophs were in the resistant state at the time of pressure increase, the apparent increase in prototrophic frequency being due to selective survival. Since it is reasonable to assume that one of the causes of death was the establishment of potentially lethal premutational lesions it is strange that there is no increase in prototrophic frequency with incubation at 16°. This suggests that the well known enzyme inhibitory effects of high pressure oxygen may extend to nuclear-repair enzymes and that following this exposure no repair of nuclear damage could occur.

It has been suggested that the mutagenic action of high pressure oxygen and X-rays may have a common mechanism (Gerschmann et al. 1954). In this event it is surprising that incubation at 16° following exposure to high pressure oxygen increases the number of prototrophs, for it has been shown

that the number of prototrophs induced in E. coli B/r WP2 by X-irradiation decreases with post irradiation incubation at 16° (Bridges and Munson 1964). E. coli B/r WP2 is the strain from which E. coli WP2 hcr⁻ was derived and it has been concluded that it does not possess any means of removing X-ray mutational lesions which are not also possessed by E. coli WP2 hcr⁻ (Bridges and Munson 1966). The reasons for this apparent anomaly are being investigated.

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