MODIFICATION OF THE LETHAL AND MUTAGENIC EFFECTS OF HIGH PRESSURE OXYGEN ON ESCHERICHIA COLI BY TREATMENT AFTER EXPOSURE.

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Summary.

It has been shown that high pressure oxygen (HPO) is mutagenic to Escherichia coli. The mutagenic effect is enhanced by acriflavine or a period of incubation at 16° following the HPO treatment. The presence of nutrient broth in the plating medium apparently facilitates repair of the premutational lesion and enhances survival. It is suggested that the results are due to the effects of the treatments on protein synthesis. There is an indication that the nuclear lesion induced by HPO has the form of a single stranded chromosome break.

Introduction.

It has been reported that high pressure oxygen (HPO) is mutagenic to Escherichia coli WP2 hcr⁻ and that incubation at 16° following HPO treatment enhances the yield of mutants (Gifford, 1968a). Gerschmann et al. (1954) suggested that the mutagenic action of HPO and X-rays may have a common mechanism. Thus the increase in mutant frequency of E.coli WP2 hcr with incubation at 16° was unexpected as it has been observed that the number of mutants induced in E.coli B/r WP2 by X-irradiation declines with incubation at 16° (Bridges and Munson, 1966). Experiments have now been conducted in which the effects of other treatments capable of influencing mutation establishment have been investigated.

Methods.

Late exponential phase cultures in glucose-salts medium supplemented with

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10.0 μg/ml. tryptophan were exposed to oxygen at 10 atmospheres, in liquid films 1-2 mm. thick, at room temperature, in pressure vessels similar to those described by Caldwell (1956). Following the release of pressure, samples were plated for survival and prototroph determination on three media:

1). Glucose-salts agar supplemented with 0.75 μg/ml. tryptophan (M+T).

2). As above with the addition of 5 μg/ml. acriflavine (M+T+A).

3). Glucose-salts agar supplemented with 2.5% (v/v) nutrient broth (M+B).

Plates were incubated at 37°, either directly, or following a period of incubation at 16°. Colonies were counted after 48 hours incubation at 37°.

Bacteria used in the investigation were Escherichia coli B/r WP2 and WP2 hcr (Hill), kindly supplied by Dr.B.A.Bridges.

TABLE 1

Survival and prototrophic frequency of E.coli WP2 hcr⁻ after 15 minutes exposure to HPO.

Treatment.	Medium.	Viable Count /ml.x 10^8.	Prototrophs /ml.
Control.	M+T	3.1	173
"	M+B	3.1	180
"	M+T+A	3.0	187
15 min.HPO.	M+T	3.0	175
" " "	M+B	3.1	175
" " "	M+T+A	2.9	490
15 min.HPO+ 12hr.at 16°.	M+T	3.0	465
" " "	M+B	3.1	435
" " "	M+T+A	3.0	510

TABLE 2

Survival and prototrophic frequency of E.coli B/r WP2 after 15 minutes exposure to HPO.

Treatment.	Medium.	Viable Count /ml.x 10^8.	Prototrophs /ml.
Control.	M+T	13.3	159
"	M+B	13.6	172
"	M+T+A	13.2	169
15 min.HPO.	M+T	12.2	170
" " "	M+B	13.6	163
" " "	M+T+A	6.9	209
15 min.HPO+ 12hr.at 16^o.	M+T	8.9	120
" " "	M+B	13.5	178
" " "	M+T+A	7.1	240

Results.

Cultures of E.coli B/r WP2 and WP2 hcr$^-$ were exposed to HPO for 15 minutes, then plated on the three media (Tables 1 and 2).

The results shown in Table 1, WP2 hcr$^-$, show that when incubated directly at 37^o there is an increase in prototrophic frequency only on M+T+A plates. However, following a period of incubation at 16^o there is an increase in mutant frequency on all media. It appears that the amount of damage induced by the HPO exposure was small since there is no fall in viability in any of the treatments.

Table 2, B/r WP2, shows results of an essentially similar pattern. There is an increase in mutant frequency only on M+T+A plates even after incubation at 16^o. The exposure induced a considerable fall in viability on M+T and M+T+A plates but not on M+B plates.

TABLE 3

Survival and prototrophic frequency of E.coli WP2 hcr$^-$ after 30 minutes exposure to HPO.

Treatment.	Medium.	Viable Count /ml.x 10^8.	Prototrophs /ml.
Control.	M+T	3.9	142
"	M+B	3.9	133
"	M+T+A	3.8	136
30 min.HPO.	M+T	2.7	165
" " "	M+B	3.8	137
" " "	M+T+A	1.4	158
30 min.HPO+ 5hr.at 16^o.	M+T	1.7	140
" " "	M+B	3.8	208
" " "	M+T+A	0.5	227

The experiments were then repeated using a longer exposure to HPO, of 30 minutes (Tables 3 and 4).

The results of Table 3, WP2 hcr$^-$, show the same pattern of response with the greatest mutation establishment after incubation at 16^o. There is no change in viability on M+B plates though there are marked falls on M+T and M+T+A plates.

Table 4, B/r WP2, shows the same pattern though there is also a fall in viability on M+B plates after incubation at 16^o.

The effect of one other treatment upon survival has been investigated. After exposure to HPO a culture of E.coli B/r WP2 was dispensed into two glass Petri dishes; one was wrapped in aluminium foil and both were placed beneath a 40 watt fluorescent lamp for 15 minutes. After this time they were serially diluted and plated on M+B medium (Table 5).

TABLE 4

Survival and prototrophic frequency of E.coli B/r WP2 after 30 minutes exposure to HPO.

Treatment.	Medium.	Viable Count /ml.x 10^8.	Prototrophs /ml.
Control.	M+T	19.7	140
"	M+B	21.2	151
"	M+T+A	20.2	143
30 min.HPO.	M+T	13.0	252
" " "	M+B	19.3	150
" " "	M+T+A	6.0	263
30 min.HPO+ 5hr.at 16^0.	M+T	8.1	190
" " "	M+B	12.7	108
" " "	M+T+A	2.7	275

TABLE 5

Survival of E.coli B/r WP2 incubated in light and dark for 15 minutes after exposure to HPO.

Treatment.	Viable Count/ml.
Control.	80.0×10^6
HPO + light.	7.2×10^5
HPO + dark.	13.0×10^5

It was noted that exposure to light reduced survival. The result was confirmed in other experiments with Escherichia coli and Bacillus subtilis.

Discussion.

It has been suggested that exposure to HPO may lead to the inactivation of nuclear repair enzymes (Gifford, 1968a) thus recovery from HPO-induced damage

may be dependent upon the de novo synthesis of the appropriate enzymes. Therefore treatments retarding protein synthesis may enhance mutation establishment and lethality, and vice versa. Of the treatments investigated both low temperature and acriflavine should retard protein synthesis and they have been found to enhance mutation and reduce survival. In contrast the presence of nutrient broth, which will encourage rapid protein synthesis, markedly reduces mutation and enhances survival.

It has been suggested that E.coli WP2 hcr is unable to make incisions into the chromosome in order to commence excision-repair (Bridges and Munson, 1966). Thus the observation that, under appropriate conditions, E.coli WP2 hcr$^-$ is able to repair all the induced damage, and exhibits a similar sensitivity to HPO to E.coli B/r WP2, suggests that the HPO-induced nuclear lesion may have the form of a single stranded chromosome break.

The effect of light upon survival may result from a light mediated delay in protein synthesis, as in indirect photoreactivation (Witkin, 1964). This observation is of interest in relation to the results of Doudney (1968) which showed that bacteria are sensitive to the lethal effects of hydrogen peroxide after exposure to photoreversing light. It was suggested that the light caused some sublethal damage which is made lethal by agents such as organic peroxides. It may be that the sublethal damage is an inhibition of protein synthesis and that bacteria are then unable to synthesise the appropriate enzymes to protect themselves against the occurrence of hydrogen peroxide in the environment (Gifford, 1968b).

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