EXPERIMENTAL STUDIES INTO THE EFFECT OF NITROUS OXIDE ON TUMOUR CELL GROWTH

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SUMMARY

The effect of nitrous oxide on the growth of two types of ascites tumour in mice was investigated. The mice in the first two trials were injected with tumour cells, divided into three groups and exposed to one of the following atmospheres: (1) air; (2) 25% nitrous oxide; (3) 40% nitrous oxide. After treatment, the volume of ascites fluid and the concentration of cells were measured. It was found that nitrous oxide treatment significantly lowered the number of cells grown in the case of Ehrlich ascites tumour but had no effect on the growth of 2146 tumour. Two further studies were made in which treatment with nitrous oxide preceded injection of tumour cells; these failed to reveal any effect on tumour growth. Possible applications of nitrous oxide in the treatment of cancer are discussed.

Although nitrous oxide has been used therapeutically in the treatment of chronic leukaemia (Lassen and Kristensen, 1959) and acute leukaemia (Eastwood et al., 1963), little is known of its effect on other forms of tumour cell growth.

In considering the use of nitrous oxide for postoperative pain relief after cancer surgery the possible effects of the gas on tumour growth are of some importance. During surgery, manipulations of the tumour may release "showers" of cancer cells into the blood stream, and there is evidence that the prognosis depends to some extent on the resistance of the patient to these cells (Cole et al., 1961). Many factors, including the stress of the operation and anaesthesia, have been shown experimentally to lower the resistance of the host to tumour cells. Anaesthetic agents, such as barbiturates and ether (Trevino et al., 1961), and chloroform (Gaylord and Simpson, 1916) have been shown to affect adversely the prognosis in experimental studies, increasing either the tumour "take" rate or the rate of tumour growth, and, consequently, it appeared that nitrous oxide as an anaesthetic might have similar adverse effects. Conversely, in view of the anti-mitotic effect of the gas (Kieler, 1957), an inhibition of tumour growth is possible.

The present study was therefore designed to investigate the effect of nitrous oxide on the growth of ascites tumour in mice.

METHOD

Two forms of mouse ascites tumour were investigated, Ehrlich tumour and 2146 tumour. Both these tumours can be transferred readily from one mouse to another by intraperitoneal injection of the ascites fluid containing tumour cells. After a short latent period in the new host the tumour cells multiply and give rise to ascites and swelling of the abdomen. With both types of tumour a similar technique was used; the principles of such studies were described by Goldie and Felix (1951) but were modified slightly as follows. In all the studies female mice, of Tyler's Original strain, were used at a mean initial weight of 20 g. Four trials were made:

(1) Ehrlich tumour with nitrous oxide treatment during its growth (90 mice);
(2) 2146 tumour with nitrous oxide treatment during its growth (48 mice);
(3) Ehrlich tumour with previous nitrous oxide treatment (126 mice);
(4) 2146 tumour with previous nitrous oxide treatment (48 mice).
In each trial the mice were divided randomly into three groups and, except in experiment 3, they were individually coded with coloured dyes and individually weighed. In experiment 3, group weights were taken instead.

In the first two trials, each mouse was injected intraperitoneally with 0.1 ml of donor ascites fluid diluted to a standard concentration in each experiment. Individual mice were taken from each group in turn for injection, so as to avoid any bias arising from timing of the injection. The dose of tumour used was $5 \times 10^7$ cells.

Following injection of the cells each group of mice was exposed to one of the following atmospheres:

1. Air (controls);
2. 25% nitrous oxide, 21% oxygen and 54% nitrogen;
3. 40% nitrous oxide, 21% oxygen and 39% nitrogen.

The ventilation units have been described previously (Parbrook, 1967a). The duration of treatment was 6½ days in the experiments with Ehrlich ascites tumour, and 4 days in the experiments with 2146 tumour. Following treatment, individual mice were taken from each group in rotation to avoid risk of bias from timing of the subsequent tests. Each mouse was weighed and killed, and the volume of ascitic fluid was measured by draining it into a measuring cylinder. The tumour cell concentration in the ascitic fluid from each mouse was also measured using a haemocytometer, and the total number of cells was calculated in each case.

In trials 3 and 4, the injection of 0.1 ml of tumour cell fluid was delayed until after exposure of the animals to the experimental atmospheres. The animals were then allowed to live for a further period of either 6½ days (Ehrlich tumour) or 4 days (2146 tumour), after which they were killed and the tumour cell counts were made as previously. In trial 3, $10^7$ Ehrlich ascites cells were injected, and $5 \times 10^7$ cells in trial 4.

**RESULTS**

**Trial 1. Ehrlich ascites tumour. Concurrent treatment with nitrous oxide.**

In this trial 90 mice in three groups of 30 were treated with the special atmospheres during the 6½ days period of growth allowed for the ascites tumour. It was found that the weight gain varied in the three groups, being greatest in the control group, and least in the 40% nitrous oxide group; these changes were not statistically significant ($0.1 > P > 0.05$). This was not due to reduced food intake in the nitrous oxide groups, the food consumption being, in fact, slightly greater in these groups. This observation was in contrast with studies of normal mice previously reported (Parbrook, 1967a), in which a slight depression of food intake was seen with nitrous oxide treatment.

Owing to the large number of mice involved, the ascites fluid measurements were made in two stages. In the first stage (Series 1), 20 mice were taken randomly from each of the three cages and measurements were made. The remaining mice, 10 in each cage (Series 2) were assessed 12 hours later and are, therefore, given as a separate group in table I. The volumes of ascitic fluid recovered from the mice varied in the three groups in both series, being greatest in the control animals, and least in the 40% nitrous oxide group. In Series 1 this difference did not reach statistical significance, but in Series 2 the difference was significant at the 2% level. An overall test of significance indicates a significant nitrous oxide effect ($t_{14} = 2.75$, $P < 0.01$). The calculated numbers of tumour cells were regarded as a more satisfactory index of the effect of nitrous oxide on tumour growth and this showed that a highly significant effect was present with treatment (table I), the number of cells grown being reduced by nitrous oxide treatment.

It is seen that the total number of cells grown is higher in Series 2, owing to the fact that an extra twelve hours was allowed for tumour growth in this case.

**Trial 2. 2146 tumour. Concurrent treatment with nitrous oxide.**

This study was similar to the previous one but three groups of 16 mice were used and 2146 tumour cells were injected in place of Ehrlich ascites tumour. As this tumour grows more rapidly than Ehrlich tumour, the duration of nitrous oxide treatment was limited to 4½ days, after which the animals were killed, and the tumour cell counts and other measurements made.
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TABLE I

Effect of concurrent nitrous oxide treatment on the growth of Ehrlich ascites tumour in mice.

<table>
<thead>
<tr>
<th></th>
<th>Controls (air)</th>
<th>25% nitrous oxide</th>
<th>40% nitrous oxide</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>X</td>
<td>SE</td>
<td>N</td>
</tr>
<tr>
<td>First 60 mice (Series 1)</td>
<td>20</td>
<td>289</td>
<td>51</td>
<td>20</td>
</tr>
<tr>
<td>Final 30 mice (Series 2)</td>
<td>9</td>
<td>433</td>
<td>87</td>
<td>10</td>
</tr>
</tbody>
</table>

X = mean number of tumour cells (in millions) per mouse recovered from the ascites fluid.
N = number of mice in each group.
SE = standard error.

Results in Series 2 were obtained 12 hours later than those in Series 1.

Significance of overall results. An analysis of variance showed that there was a highly significant reduction of tumour cell counts with nitrous oxide treatment, \( t_{0.01} = 6.698, P<0.001 \).

* One mouse died of tumour cell growth in the control group prior to completion of the trial.

TABLE II

Effect of concurrent nitrous oxide treatment on the growth of 2146 tumour in mice.

<table>
<thead>
<tr>
<th></th>
<th>Controls (air)</th>
<th>25% nitrous oxide</th>
<th>40% nitrous oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>X</td>
<td>SE</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>132</td>
<td>22</td>
</tr>
</tbody>
</table>

X = mean number of tumour cells (in millions) per mouse recovered from the ascites fluid.
N = number of mice in each group.
SE = standard error.

Two mice died from tumour cell growth in the 40% -treated group and one in the control group.

TABLE III

Effect of previous nitrous oxide treatment on the growth of Ehrlich ascites tumour in mice.

<table>
<thead>
<tr>
<th></th>
<th>Controls (air)</th>
<th>25% nitrous oxide</th>
<th>40% nitrous oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>X</td>
<td>SE</td>
</tr>
<tr>
<td>Preliminary trial</td>
<td>8</td>
<td>157</td>
<td>33</td>
</tr>
<tr>
<td>Final trial</td>
<td>30</td>
<td>367*</td>
<td>35</td>
</tr>
</tbody>
</table>

X = mean number of tumour cells (in millions) per mouse recovered from the ascites fluid.
N = number of mice in each group.
SE = standard error.

* In the final trial two mice in the "control" and the "25% nitrous oxide" groups died due to tumour growth prior to completion of the trial. There is therefore a slight bias in the results tending to lower the means in these two groups. The overall results give no evidence of any effect from prior treatment with nitrous oxide.

TABLE IV

Effect of previous nitrous oxide treatment on the growth of 2146 tumour in mice.

<table>
<thead>
<tr>
<th></th>
<th>Controls (air)</th>
<th>25% nitrous oxide</th>
<th>40% nitrous oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>X</td>
<td>SE</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>82</td>
<td>18</td>
</tr>
</tbody>
</table>

X = mean number of tumour cells (in millions) per mouse recovered from the ascites fluid.
N = number of mice in each group.
SE = standard error.

One mouse died (from tumour cell growth) in each of the treated groups.
The results (table II) showed no significant effect of the treatments on the tumour growth.

**Trial 3. Ehrlich ascites tumour. Prior treatment with nitrous oxide.**

In this study mice were treated with the special atmospheres for 6 days prior to injection of tumour cells. A further 6½ days later the animals were killed and the usual recordings made. In a preliminary trial three groups of 10 mice were used but the results were inconclusive. The trial was therefore repeated using three groups of 32 mice each and the results (table III) revealed no significant effect from prior treatment with nitrous oxide.

**Trial 4. 2146 tumour. Prior treatment with nitrous oxide.**

Three groups of 16 mice participated in the final trial, and the injection of tumour cells was made after 4½ days exposure to the atmospheres. Four days were allowed for the tumour growth in this study. No significant effect was seen from the nitrous oxide treatment (table IV).

**DISCUSSION**

It is seen that exposure to a nitrous-oxide-containing atmosphere produced a significant effect on the growth of Ehrlich ascites tumour in mice. This was not due to the secondary toxic effect of the gas, such as leucopenia, as no effect was seen when the treatment preceded the injection of tumour cells. It has previously been demonstrated that the toxic effects of such treatment can readily be detected and can outlast completion of the exposure to nitrous oxide by several days (Parbrook, 1967b).

The results are best explained in terms of a suppression of mitosis in the tumour cells associated with exposure to nitrous oxide with the result that growth of the tumour is slower in treated animals. This would be consistent with the work of Kleer (1957) who showed an anti-mitotic effect of nitrous oxide on embryonic myoblasts. Similarly, an anti-mitotic effect of nitrous oxide could explain the decreased sensitivity of Ehrlich ascites tumour cells to irradiation during exposure to nitrous oxide (Ebert and Hornsey, 1958). An anti-mitotic effect may also underlie the marrow toxicity of the gas, as explained in a previous paper (Parbrook, 1967b).

The absence of an effect on the growth of 2146 tumour was surprising, as this tumour normally shows a similar pattern of growth to the Ehrlich tumour. Nevertheless, slight differences do exist between the two types of ascites tumour, as the Ehrlich tumour originated as a breast carcinoma, while 2146 tumour originated as a skin carcinoma. It was felt that this slight difference in cell type was more likely to account for the different results than the slight difference in duration of nitrous oxide treatment in the two cases.

Cytotoxic drugs may improve prognosis after certain types of cancer surgery, and it is possible that nitrous oxide may have some therapeutic value if tumours in humans can be shown to be sensitive to its anti-mitotic action. Paradoxically, however, it is the absence of an effect in the case of 2146 tumour that offers the better prospects of therapeutic use of nitrous oxide in cancer treatment. Evans and his co-workers (1964) have shown that a nitrous oxide-oxygen mixture at 2 atmospheres pressure protects mice against lethal doses of irradiation, and their histological study demonstrated this protection particularly in the cell population of the bone marrow.

The increased resistance of bone marrow cells to irradiation during exposure to nitrous oxide may well be associated with a normal radiosensitivity of tumour cells such as 2146 tumour, which are unaffected by the gas. Consequently, there may well be a therapeutic application for hyperbaric nitrous oxide and irradiation in treatment of selected tumours, and it will be of interest to learn the results of further studies of this aspect which Evans and his colleagues are undertaking.

Since completion of this investigation a “work in progress” report of the effect of nitrous oxide on tumour growth has been published by Fink (1966). In this study it has been shown that treatment with 75% to 80% nitrous oxide in oxygen slowed the growth of fibrosarcomas in mice. Fink reported, too, that the treated mice showed a 10% weight loss which could be attributed to the high concentration of nitrous oxide used in his study.

**ACKNOWLEDGEMENTS**

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REFERENCES


ETUDES EXPERIMENTALES DE L'EFFET DU PROTOXYDE D'AZOTE SUR LA CROISSANCE DE CELLULES TUMORALES

SOMMAIRE
L'effet du protoxyde d'azote sur la croissance de deux types de tumeur d'ascite a ete etudi, chez la souris. Les animaux furent injects au cours des deux premiers essais avec des cellules tumorales, partages en trois groupes et soumis à une des atmosphères suivantes: (1) air; (2) 25 pourcent de protoxyde d'azote; (3) 40 pourcent de protoxyde d'azote. Après traitement, le volume du liquide d'ascite et la concentration des cellules furent mesurées. On notait que le traitement au protoxyde d'azote réduisait significativement le nombre de cellules formées en cas de tumeur d'ascite Ehrlich, mais n'influénçait nullement la croissance de la tumeur 2146. Dans deux autres études, le traitement au protoxyde d'azote précédait l'injection des cellules tumorales; dans ce cas, il n'y avait aucun effet sur la croissance tumorale. Les applications possibles du protoxyde d'azote dans le traitement du cancer sont discutées.

EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DIE WIRKUNG VON LACHGAS AUF DAS TUMORZELLENWACHSTUM

ZUSAMMENFASSUNG
Es wurde die Wirkung von Lachgas auf das Wachstum zweier Arten von Ascitestumoren bei der Maus untersucht. Die Mäuse in den beiden ersten Versuchsreihen erhielten eine Injektion von Tumorzellen und wurden dann in drei Gruppen einge teilt und einer der folgenden Atmosphären ausgesetzt: (1) Luft; (2) 25%iges Lachgas; (3) 40%iges Lachgas. Nach der Behandlung wurden das Volumen der Ascitesflüssigkeit und die Konzentration der Zellen gemessen. Es wurde gefunden, daß die Lachgasbehandlung die Zahl der gewachsenen Tumorzellen im Falle des Ehrlich Ascitestumors deutlich herabsetzte, dagegen auf das Wachstum des 2146 Tumors keine Wirkung hatte. In zwei weiteren Untersuchungsreihen wurde die Lachgasbehandlung vor der Injektion der Tumorzellen durchgeführt; hierbei zeigte sich keinerlei Wirkung auf das Tumorzellenwachstum. Mögliche Anwendungen des Lachgases bei der Behandlung des Karzinoms werden diskutiert.