THE EFFECT OF VARIOUS CONCENTRATIONS OF NITROUS OXIDE ON THE 24-HOUR EXPLANTED CHICK EMBRYO

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SUMMARY

The effects of nitrous oxide on the early explanted chick embryo were investigated using the technique of New (1955). After 22 hours of incubation the embryos were explanted and exposed for a further 24 hours to the following gases: air, nitrous oxide (25 per cent), nitrous oxide (60 per cent), nitrous oxide (79 per cent). The oxygen concentration was maintained at normal levels (20.9 per cent) and the balance of the gases was nitrogen. In a fifth trial a 60 per cent nitrous oxide 40 per cent oxygen mixture was used. After exposure the embryos were examined under the dissecting microscope and in histological sections. These revealed no effect on the chick embryo after treatment with nitrous oxide for 24 hours.

These investigations, undertaken as part of a general study into the toxicity of atmospheres containing nitrous oxide, appeared essential in view of current interest in the long-term use of nitrous oxide for postoperative analgesia in patients (Parbrook, Rees and Robertson, 1964; Petrovsky and Yefuni, 1965).

Not only is long-term nitrous oxide toxic to the bone marrow (Lassen et al., 1956; Green and Eastwood, 1963) but studies by Kieler (1957) using cultures of embryonic myoblasts indicated that nitrous oxide was a mitotic poison. Nitrous oxide has also been reported to reduce the sensitivity of tissues (Evans and Orkin, 1962) and of tumour cells (Ebert and Hornsey, 1958) to irradiation and such effects are also in keeping with the hypothesis of a general antimitotic effect of the gas. More recently, Rector and Eastwood (1964) showed a reduction of fertility in whole eggs exposed to nitrous oxide.

In our own studies the explanted chick embryo underwent rapid development from the primitive streak stage to differentiation of brain, neural tube, heart, somites, notochord and vitelline circulation.

MATERIALS AND METHODS

Fresh farm eggs from free range hens were incubated at 37.5°C for 24 hours. The embryonic discs from these eggs were then explanted using the technique of New (1955). After opening the egg and decanting the thin albumin into a beaker the egg yolk was tipped into sterile saline. The vitelline membrane was cut around the equator of the yolk and, with the blastodisc still adherent to it, carefully removed and mounted on a glass ring in a watch glass (figs. 1 and 2).

Thin albumin was then run beneath the vitelline membrane to provide nourishment for the embryo for a further 24 hours. After explantation the embryos were divided into control and treated groups of 12 to 14 each, and each embryo of the batch was catalogued according to the stage of development (Hamburger and Hamilton, 1951).

Two incubators were required. One was of a standard type and was used for the control batch of eggs whilst the second (fig. 3) was modified to permit exposure of the experimental group of embryos to nitrous oxide atmospheres.

For the treated embryos the lid of each petri dish was raised slightly by three sterile wood
blocks (fig. 1) to allow freer circulation of the atmosphere and these dishes were stacked in two plastic "cake box" containers which in turn were placed in the incubator and coupled to the gas flow system. The overall flow of gas was about 1 litre per minute and was balanced to the two containers by means of the two light clamps. The gases were humidified by passage through a water bottle outside the incubator and through water in the plastic containers.

The following treatments were given to five batches of embryos:

1. air (preliminary trial);
2. 25 per cent nitrous oxide, 20.9 per cent oxygen and 54.1 per cent nitrogen;
3. 60 per cent nitrous oxide, 20.9 per cent oxygen and 19.1 per cent nitrogen;
4. 79.1 per cent nitrous oxide and 20.9 per cent oxygen;
5. 60 per cent nitrous oxide and 40 per cent oxygen.

The preliminary trial (1), conducted with only air flowing through the incubator, confirmed that continual replacement of the atmosphere round the embryo did not affect its development when compared with the untreated controls. The final
trial of a 60 per cent nitrous oxide and 40 per cent oxygen mixture was included to determine whether any toxicity of the nitrous oxide might be exacerbated by a raised oxygen tension because nitrous oxide is normally administered (therapeutically or in anaesthesia) with added oxygen.

The gases used were provided from cylinders in the case of nitrous oxide and oxygen and from a compressor in the case of air, the three gases being metered in appropriate proportions by means of three flowmeters (fig. 3).

Because of its high accuracy, a Servomex Paramagnetic Oxygen Meter (Nunn et al., 1964) was used to achieve an accurate balance of the three flowmeters. In the case of the 25 and 60 per cent nitrous oxide mixtures, the air and nitrous oxide flows were initially adjusted until the appropriate oxygen depression was indicated by the reading on the meter (15.3 per cent oxygen for the 25 per cent nitrous oxide, and 6 per cent oxygen for the 60 per cent nitrous oxide mixture). Oxygen was then added in a flow sufficient to return the oxygen concentration to 20.9 per cent.

To check that the gases given would reach the embryo a special “dummy run” was made giving oxygen only and sampling from the petri dishes by means of fine tubing. Analysis of the gas from the petri dishes for nitrogen showed that it was over 90 per cent equilibrated after 1 hour (table I).

**Table I**

<table>
<thead>
<tr>
<th>Equilibration of petri dishes.</th>
<th>Initial</th>
<th>½ hour</th>
<th>1 hour</th>
<th>1½ hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen meter readings (%)</td>
<td>79.1</td>
<td>8.8</td>
<td>6.1</td>
<td>2.9</td>
</tr>
<tr>
<td>(mean of 4 readings)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6.8-10.2</td>
<td>2.0-9.1</td>
<td>2.5-3.5</td>
<td></td>
</tr>
</tbody>
</table>

The results were obtained using a flow of pure oxygen (1 l./min) into the plastic containers which held the petri dishes.

After incubation, the control and treated embryos were once more staged and then harvested by gently shaking the glass mounting ring in physiological saline to detach the disc from the vitelline membrane. Embryos were studied for

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**FIG. 4A**
Whole mount view of control embryo with air flowing at a rate of 1 l./min.

**FIG. 4B**
Whole mount view of a chick embryo treated for 24 hours with 79.1 per cent nitrous oxide.
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Gross abnormalities under a dissecting microscope, and thereafter fixed in Boulin's fluid for approximately 24 hours. The specimen was dehydrated, cleared in cedar wood oil and embedded in paraffin wax. Serial sections of each embryo were cut at 8μ and stained with Delafield's haematoxylin and eosin.

RESULTS

No abnormalities were found on examination under the dissecting microscope. It was noted in particular that a normal beating heart was present in the treated embryos despite the reported toxicity of nitrous oxide to embryonic heart tissue in mice (Kieler, 1957); a typical treated embryo is shown in figure 4B. Microscopic examination of sections at magnifications of ×400 and inspection under oil immersion gave the same negative result. A representative section is shown in figure 5 (low power). A preliminary count of the number of mitotic figures present was carried out, but there was no evidence of any suppression of mitosis with nitrous oxide treatment. Mortality in the treated embryos, was the same as for the controls, being about 1 to 3 per batch of 12 to 14 and related to technical factors, e.g. trauma to embryo or vitelline membrane. It was noted that the mortality rate was less in the final series (0 to 1 per batch) for both control and treated groups, owing to a steady improvement in technique.

DISCUSSION

The use of explanted chick embryos presented several advantages for this study. It allowed the exposure of a known viable embryo directly to the nitrous oxide atmospheres and avoided indirect secondary maternal effects possibly arising from treatment with nitrous oxide. The latter problems were exemplified in a preliminary report of this work (Parbrook, Mobbs and McKenzie, 1965) in which pregnant rats also were exposed for 48 hours to an atmosphere containing 60 per cent nitrous oxide. A significant reduction in the number of live births was seen in the treated animals but it was also noted that a weight loss of just under 5 per cent had occurred during the period of treatment. Such weight losses, related to a reduction of appetite, could affect pregnancy and make it difficult to interpret results.

Rector and Eastwood (1964) in their studies exposed whole eggs to 80 per cent nitrous oxide for the full period of gestation and found a marked reduction of fertility in treated eggs and spastic paralysis in some of the chicks in the treated group. They point out that these adverse effects could be related either to nitrous oxide toxicity or to nitrogen lack.

These results are not necessarily inconsistent with our findings in view of the different duration of exposure of the eggs. A recently published study of the effects of exposure of eggs to nitrous oxide for periods of 6 hours (Smith, Gaub and Moya, 1965) also showed no effects from an 80 per cent nitrous oxide and 20 per cent oxygen mixture, but showed that hypoxic mixtures, e.g. 90 per cent nitrous oxide 10 per cent oxygen, were more toxic to the chick embryo than a mixture of 90 per cent nitrogen and 10 per cent oxygen. A survey of these two studies and our own suggests that toxicity of nitrous oxide to the chick embryo is mild and only evident if exposure is very prolonged, or if concurrent hypoxia is present.

FIG. 5
Lower power view of a transverse section through the neural tube and heart of a chick embryo treated for 24 hours with 79.1 per cent nitrous oxide.
It is difficult to draw firm conclusions regarding human toxicity of nitrous oxide from these investigations on the chick embryo but the results do suggest that short-term exposure of early embryos to nitrous oxide may be surprisingly well tolerated despite the proven cytotoxic and antimitotic effects of this gas.

It is concluded that exposure of the early chick embryo in culture to various concentrations of nitrous oxide up to 79 per cent for 24 hours produced no alteration in its development.

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REFERENCES


