EMBRYOGENESIS IN 100% O₂ AT REDUCED PRESSURE*

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Abstract. Fertile chicken eggs were incubated in an altitude chamber in a near 100% O₂ atmosphere at 225 torr. Both N₂ and CO₂ were kept under 0.5%. Temperature was a standard 37.5° but a high relative humidity of 90% was required to prevent dehydration. In ten trials involving 382 eggs, hatchability averaged 21% of controls and weight of chicks was 11% less than controls, but embryo mortality was distributed similarly. Low pressure per se and small differences in O₂ tension may have affected the results, but similarities to incubation in 21% O₂-79% He call attention to absence of nitrogen as a possible explanation.

1. Introduction

Embryo development has served as a sensitive test in the search for artificial atmospheres suitable for life support in sealed systems. In connection with the need for inert gas in such atmospheres, incubation studies with fertile chicken eggs have suggested that He may not adequately replace N₂ inasmuch as embryonic mortality was doubled in 21% O₂-79% He compared to air (Weiss et al., 1965). The increased mortality could not be corrected by environmental manipulations involving temperature, humidity or insulation which were designed to compensate for the higher heat conductivity of He compared to N₂ (Weiss and Wright, 1968). One of the questions raised by these results was whether the depression in hatchability was related to the presence of He or the absence of N₂. In the present study, the question was examined by incubating fertile eggs in an atmosphere essentially devoid of all inert gas, accomplished by using 100% O₂ at reduced pressure. Prior studies of this type seem to have been limited to 3-4 days of embryonic development (Cook, 1945; Allen, 1963).

2. Procedure

The low pressure incubation system consisted of a cylindrical metal altitude chamber approximately 2 m long and 1 m diam. Sliding in and out of the metal cylinder on a set of tracks was a sealed polyvinyl chamber (modified after gnotobotic isolators) in which the eggs were held. Before insertion into the altitude chamber, the isolator was loaded with fertile eggs, a supply of water for maintaining humidity and a tray of soda lime for adsorption of CO₂. It was then flushed with 100% O₂ until less than 1% N₂ remained. Thereafter the isolator was supplied with 100% O₂ from a pressurized cylinder thru a demand-type oxygen mask regulator located within the altitude chamber. The regulator kept the flexible plastic isolator inflated with a pressure 1–2

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Any leaks in the isolator were therefore outboard, insuring a near 100% O₂ atmosphere (Grimard, 1970).

A pressure of 225 torr was selected as a P₀₂ likely to avoid problems from O₂ toxicity and still provide a margin of safety against hypoxia (see discussion). Pressure was maintained in the altitude chamber by means of a sensor (I²R Therm-o-Watch) mounted on a Hg manometer. Standard incubation temperature of 37.5° was used (thermistor controlled, YSI) but initial studies showed that a relative humidity around 90% (by hair hygrometer calibrated in room air) was required to prevent excessive dehydration of eggs (Taylor, 1949). The high moisture levels were obtained by water pans placed in the bottom of the isolator. A small, continuously operating fan within the isolator aided in gas mixing and heat distribution.

Fungal and bacterial growth appeared to be promoted in and on the eggs by the isolator conditions and caused at least 5 runs to be discarded. The problem was reduced to a manageable level by: (1) using unwashed eggs; (2) preventing moisture which condensed on the isolator walls from dripping onto the eggs; (3) dusting the eggs (including controls) with nystatin (Mycostatin), and anti-fungal agent; and (4) spraying the isolator before use with a peracetic acid solution.

Fertile eggs in the 40–60 g range were obtained from the University Poultry Dept’s White Leghorn flock, weighed, and set pointed end down. They were turned thru 90° once a day for the first 18 days by a remote operating linear actuator (Grimard, 1970) thus avoiding the need for daily decompression and recompression of the altitude chamber. On the 18th day, the chamber was returned to ground level, the isolator wheeled out, and the eggs laid horizontal. A test of gas composition was made at this time, using a Beckman E-2 for O₂, Beckman LB-1 for CO₂ and a Barber-Coleman gas chromatograph for N₂. All manipulations were carried out by means of rubber gloves incorporated in the isolator wall and a flushable gas lock, insuring minimal disturbance of the 100% O₂ atmosphere. The system was returned to altitude until the 22nd day, when the trial terminated.

A typical trial involved 18–30 eggs each in the isolator and in the control system, which was a small, commercial, forced-draft incubator (American-Lincoln) located on an adjacent lab bench. Unhatched eggs were weighed after incubation to evaluate weight loss. All unhatched eggs were opened and if fertile, time of death of embryo estimated as during the first, second or third week of incubation. Chicks which hatched were weighed and some were raised for short periods to observe performance. Hatchability was determined as the percent of fertile eggs which produced live chicks, and relative hatchability was the ratio of the hatchability in the isolator system to control hatchability. Mortality distribution was calculated as a percent of the number of unhatched fertile eggs. Contingency Chi Square, ‘t’ tests between means and correlation analyses were used to evaluate results (Snedecor, 1956).

A small series of additional 'control' runs was carried out in the isolator-altitude chamber incubation system. The system was loaded and sealed but not taken to altitude and the atmosphere remained room air. Relative humidity was held between 45 cm H₂O above that in the altitude chamber per se. Any leaks in the isolator were therefore outboard, insuring a near 100% O₂ atmosphere (Grimard, 1970).