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Improved Hatch Rate in Helium–Oxygen by Reducing Shell Diffusion Area (38664)

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In our incubation system, fertile chicken eggs hatch only half as well as controls when helium (He) is substituted for nitrogen (N_2) in the ambient atmosphere, i.e., 79% He/21% O_2 vs. 79% N_2/21% O_2 (21, 22, 24). Since a depression of embryogenesis by He relates to the interesting question of a physiological or pharmacological role for the inert gases at ground level pressures, we have continued to seek for an explanation for the phenomenon. Our studies (21–24) have tended to rule out toxic or metabolic function of He, high heat loss in He and the absence of N_2 *per se*. The evidence nevertheless suggests that an inert gas is needed in the incubation environment and that whereas this need can be met adequately by N_2, Argon and Neon, it is only partly met by He.

As to what the need might be for an inert gas during incubation, or experimental data suggests it is related to moisture flux across the shell. Higher egg weight loss during incubation in He than in N_2 has been a persistent finding (21, 22, 24) and high weight loss can lead to depressed hatch (7). However, neither the increased egg weight loss nor the decreased hatch size could be controlled by elevating relative humidity (22, 24). In the present study, we report some success in decreasing weight loss and improving hatch in He through the expedient of covering portions of the shell with melted paraffin.

Procedure. The sealed plastic chambers in which these studies were carried out differed from our previous units (21, 22, 24) in being larger (900–1000 l) and in being recycling systems in which the O_2 and inert gas concentrations were automatically controlled (1). Two chambers were alternated between the control gas (79% N_2/21% O_2) and the helium mixture (79% He/21% O_2). Incubation temperature was set between 37 and 38° and relative humidity between 65 and 70%. Daily readings were taken from a Hg thermometer and a hair hygrometer. Gas composition in the chambers was also checked daily: O_2 on a Servomex OA 150 paramagnetic analyzer, CO_2 on a Beckman LB-1 infrared analyzer and He on a Cambridge instrument. The daily readings were averaged to provide a mean for a trial.

Routinely, 96 White Leghorn eggs were incubated in each chamber: two dozen without paraffin and two dozen each with one, two or three paraffin stripes painted on the shell. The eggs without paraffin in N_2 are considered the primary controls. All eggs were numbered, weighed and set pointed end down in wire baskets which were tilted thru 90° once a day for the first 18 days. Thereafter, the eggs were laid out horizontally for hatching, each paraffin stripe category in a separate wire mesh box. All chicks and unhatched eggs were removed on day 22 and weighed (pipped eggs were excluded). Five trials are reported here out of a total of eight run in this series; three trials were excluded because of a poor hatch (below 30%) in the primary controls.

A small camel's hair brush was used to paint the shell with the melted paraffin, producing a stripe varying from 1 to 1.5 cm in width. The one stripe category was produced by painting a single continuous band completely around the egg over its long diameter. The two stripe category by adding a second, continuous band around the long diameter at 90° to the first stripe; and the three stripe category by adding a band around the widest part of the egg at right angles to the other two stripes.

Percent shell surface area covered by paraffin was determined as follows: Each stripe in sequence was covered with transparent tape and the edges of the wax stripe traced onto the tape. The tape was then peeled off the egg, flattened out on paper,

TABLE I. CHARACTERISTICS OF EGGS INCUBATED IN He–O_2 AND COVERED WITH STRIPES OF MELTED PARAFFIN.

	79% He/21% O_2				79% N_2/21% O_2			
No. Paraffin stripes on shell	0	1	2	3	0	1	2	3
% Shell Covered by Paraffin	—	(7) 31.4	(7) 54.7	(6) 66.7	—	(7) 31.4	(7) 54.7	(6) 63.7
(No. eggs) % ± SE		± 2.0	± 2.2	± 1.8		± 2.0	± 2.2	± 1.8
No. Trials	5	5	5	4[a]	5	5	5	4[a]
No. Eggs per trial	24	24	24	24	24	24	24	24
Initial egg weight	(5) 55.4	(5) 56.5	(5) 55.3	(4) 55.3	(5) 56.0	(5) 56.3	(5) 56.0	(4) 55.4
(No. Trials) g ± SE	± 1.6	± 1.8	± 1.7	± 2.2	± 1.8	± 2.1	± 1.6	± 2.5
Incubator temperature (No Trials) °C ± SE	(5) 37.2 ± 0.13				(5) 37.1 ± 0.06			
Incubator O_2 concentration (No. trials) % ± SE	(5) 21.7 ± 0.09				(5) 22.5 ± 0.70			
Incubator CO_2 concentration (No. trials) % ± SE	(5) 0.04 ± 0.01				(5) 0.04 ± 0.01			
Incubator Relative Humidity (No. trials) % ± SE	(5) 69.1 ± 1.5				(5) 67.6 ± 0.7			
Hatch rate	(5) 20.2	(5) 37.8	(5) 67.2	(4) 25.2	(5) 74.2	(5) 66.2	(5) 21.0	(4) 1.0
(No. trials) % ± SE	± 9.0	± 14.2	± 6.5	± 9.2	± 4.6	± 6.6	± 7.6	± 1.0
Hatch relative to N_2 control	(5) 0.27	(5) 0.52	(5) 0.91	(4) 0.33	(5) 1.00	(5) 0.91	(5) 0.27	(4) 0.01
(No. trials) ratio ± SE	± 0.11	± 0.19	± 0.08	± 0.13	Control	± 0.11	± 0.22	± 0.01
Chick weight at Hatch	(3)[b] 30.0	(4) [b]35.6	(5) 35.2	(3) [b]35.9	(5) 35.0	(5) 35.1	(4) [b]37.6	(1) [b]33
(No. trials) g ± SE	± 2.2	± 1.5	± 2.5	± 3.8	± 1.4	± 1.3	± 3.2	—
22 Day Egg Weight Loss	(5) 17.8	(5) 11.5	(5) 8.1	(4) 7.4	(4)[c] 9.2	(5) 5.3	(5) 3.6	(4) 2.2
(No. trials) % ± SE	± 2.1	± 1.1	± 1.0	± 0.9	± 0.7	± 0.8	± 0.9	±0.9

[a] One trial did not include the three stripe category.

[b] No chicks hatched in some trials.

[c] No unhatched or unpipped eggs remained in one trial.

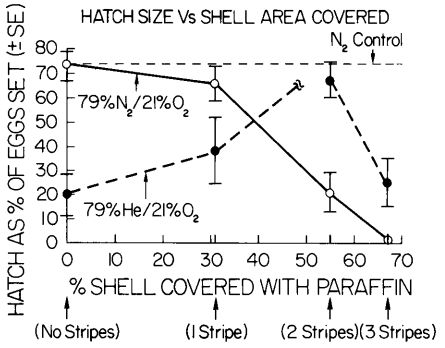

FIG. 1. Change in hatch rate of White Leghorn chicken eggs incubated in He–O_2 and N_2–O_2 as a function of % shell area covered with melted paraffin.

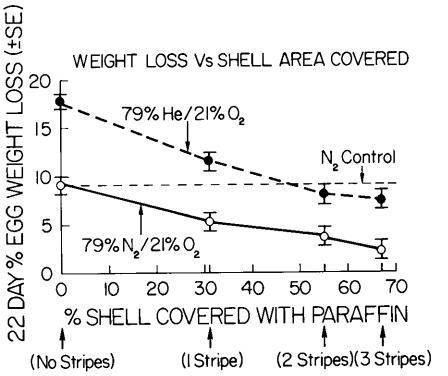

FIG. 2. Change in egg weight loss of White Leghorn chicken eggs incubated in He–O_2 and N_2–O_2 as a function of % shell covered with melted paraffin.

and the outline of the stripe transferred to a tracing paper overlay. The stripe area was cut out of the tracing paper, weighed on a microbalance and the area calculated from a weight/area calibration of the tracing paper. The total stripe area was then divided by the total egg area, in turn determined from the formula: $S = 3.14\ (B^2L)^{2/3}$ (12) where B = breadth and L = length of the egg, as measured by linear calipers. A sampling of six to seven eggs in each stripe category taken from several trials, was used to compute the average shell area covered by paraffin.

Results. Listed in Table I are the pertinent data related to the incubation of eggs whose shell surface has been covered with stripes of paraffin. In Fig. 1, hatch size has been plotted against % shell surface area covered by paraffin. The 31% of shell surface covered by the single stripe (see row 2, Table I) had minimal effects on the N_2 hatch (decreasing from the control level of 74% down to 66%), but almost doubled the hatch in He (from 20% to 38%). Covering an additional 24% of the shell with the second paraffin stripe (to a total of 55% of shell) raised the hatch in He to 67%, close to normal, but reduced the hatch in N_2 to 21%. A small additional covering of shell surface from 55 to 67% by the third stripe sharply reversed the improvement pattern in He, bringing the hatch down to 25%. In N_2, the 3rd stripe virtually eliminated hatching. From the sharp fall off of hatch in He between the second and third stripe, one might estimate that the He results would be closest to N_2 control levels at about 50% coverage of the shell with paraffin.

Egg weight changes during incubation are graphed as % loss vs. % shell surface area covered by paraffin in Fig. 2. For both He and N_2, egg weight loss decreases as the shell surface area covered by paraffin increases, but the loss in He remains about twice that in N_2 for all groups. The loss in He approximates the 9% found for the N_2 control close to the 50% shell area coverage estimated to give a hatch in He equal to that of the N_2 control (Fig. 1).

From Table I, it may be noted that smaller chick size in He, another persistent finding in our previous results (21, 22, 24), was eliminated by the paraffin stripes (row 12). The data on initial egg weight (row 5) and incubator conditions (rows 6–9) show that these variables were similar for He and N_2 eggs and therefore did not bias the results.

Discussion. In our plastic chamber incubation system, covering about 50% of shell with melted paraffin permits eggs to hatch as well in a 79% He/21% O_2 as in 79% N_2/21% O_2 (air). Eggs in He not so covered with paraffin hatch at a rate far below the N_2 control, as we have reported in numerous previous trials (21, 22, 24). Covering 50% of the shell of eggs incubating in N_2, however, sharply reduces the hatch, in agreement with data reported by others for air incubation (16, 18).

The improvement of hatch in He appears to be closely associated with reduction in egg weight loss during incubation. At about 50%

coverage of the shell, weight loss of the He eggs was about equal to N_2 controls, whereas in the absence of paraffin the weight loss in He is about double that in N_2. Excess or deficient weight loss is known to affect hatching adversely (7). Weight loss is undoubtedly due to water loss, although humidity was the same in the two environments. Since water loss presumably takes place by diffusion thru gas filled pores in the shell (6, 19), it will be affected by nature of the gases in the pore. Savin (13) calculates that the diffusion coefficient for water vapor in He-O_2 is 145% of that in air whereas Sibbons (15) indicates it to be 230% of air.

It is entirely possible that the high moisture flux and weight loss are not in themselves the cause of the He effect on embryogenesis, but rather are indicators of a disturbance in the diffusion of other gaseous components important in incubation, most likely CO_2. Similar equations describe diffusion of H_2O, O_2 and CO_2 across the shell (20). Erasmus and Rahn (3) have reported that a He environment markedly decreases the CO_2 content in the air cell of incubating eggs. Openings in the egg shell similarly reduce CO_2 (17, 18) and may be expected to elevate pH (18). Covering portions of the shell with paraffin (2, 17) or epoxy (16) can increase the CO_2 in the air cell for eggs incubating in air and presumably would do the same for eggs incubating in He.

Return of hatch size in He to near control levels by the simple expedient of covering some 50% of the shell with paraffin would seem to rule out any direct pharmacological or metabolic role for He in the embryo. It also refutes the claim that the He effect is an artifact of incubation in plastic chambers (4). The inert gases would appear to be important indirectly via their effect on gaseous diffusion across the shell. What role the reported production of N_2 by the embryo (8) might play in this is unclear but would seem to be minimal. In other species, however, workers continue to report that He in the one atmosphere range appears to have direct effectson physiological function, (e.g. 5, 9–11, 14).

It should be of some interest, practical as well as theoretical, to see if similar parafining of eggs incubating at normal O_2 tension but at reduced pressure also improves hatch, since increased embryo mortality and high weight loss were observed under those conditions (23).

Summary. For eggs incubating in a He atmosphere (79% He/21% O_2), covering approximately 50% of the shell with melted paraffin improves hatch rate to control values (from 20% to 74%) and decreases egg weight loss to control values (from 17% to 9%). In air (79% N_2/21% O_2) the same paraffin treatment depresses hatch rate. The role of the inert gases in incubation appears to be an indirect one related to their modification of the rate of gaseous flux across the shell with the adverse effects of He due to excessively rapid diffusion.

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