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The role of gravity in chick embryogenesis

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Abstract

Thirty fertilized chick eggs preincubated for 0, 7 and 10 days on earth (10 eggs each) were flown in the space shuttle 'Endeavour' and further incubated for 7 days under microgravity. Twenty out of thirty eggs (9/10 ten-day-old; 10/10 seven-day-old; 1/10 zero-day-old) were recovered alive after landing. The only living embryo of the zero-day-old group died 24 days after launch, and was comparable to a 16-day-old embryo. The high mortality of the 0-day-old eggs appeared to be related to the specific inner structure of the egg. Simulation experiments performed on earth indicated that when yolk stayed in the albumen for more than 2 days, most of the embryos died. The subtle difference in specific gravity between the yolk (1.029) and albumen (1.040) plays a critical role in early chick embryogenesis.

Key words: Microgravity; Chick embryo; Embryogenesis; Space flight; Specific gravity

1. Introduction

It has been postulated that gravity plays a critical role in embryogenesis [1]. In case of *Xenopus* oocytes, the gravity appears essential for the determination of the developmental axes, especially for the determination of the dorso–ventral axis. In chicks, however, embryonic development is divided into three stages: fertilization to blastoderm (embryonic germ) formation, which lasts one day and takes place in the oviduct; embryogenesis, which lasts 3 days; and embryogenic growth, which lasts 18 days. The last two stages take place in the shelled egg. Fertilization of oocytes (yolk) occurs at infundibulum of the oviduct within 15 min after ovulation and the blastoderm grows to a level of 6×10^4 cells at oviposition. Therefore, the effect of microgravity on the determination of the developmental axes could be ignored in case of the 0-day-old fertilized laid eggs.

Vellinger et al. [2] conducted an experiment to test the effects of microgravity on the development of avian eggs, in which 16 two-day-old and 16 nine-day-old chick embryos were flown in the space shuttle 'Discovery'. All the 2-day-old embryos died during the space flight, whereas most of the 9-day-old embryos were recovered alive after landing [2]. From these results, they suggested that early chick embryogenesis, but not the late stage of embryogenesis, does not progress normally under microgravity. To confirm their results and to study in more details, we planned to ship three different age's chick embryos, 0-, 7- and 10-day-old fertilized eggs, in a shuttle with turning the eggs twice a day. Turning of eggs is essential for the normal development of embryos at least on the ground, in particular at an early stage of development, but this procedure was omitted in the Vellinger's experiment. We examined whether 1G gravity and turning of eggs in space are necessary for the early and late stages of chick embryogenesis.

2. Materials and methods

Thirty fertilized chick eggs (White Leghorn, Shaver strain, 55.8 ± 0.5 g (mean ± SD), American Selected Products, St. Lititz, PA) preincubated at 37.5°C for 0, 7 and 10 days (10 eggs each) on earth were flown in the space shuttle 'Endeavour' in 1992 and further incubated at 37.5°C for 7 days under microgravity. The developmental stage of the embryos preincubated for 7 and IO days was estimated to be stage 30 and 36, respectively, according to the criteria of Hamburger and Hamilton [3]. We used 0-day-old eggs rather than 2-day-old eggs, since the former appeared to be more resistant to the vibration of the space shuttle at launch. In each group, half of the eggs (5 eggs each) were dissected under CO_2 gas 3 h after landing. The rest of the eggs were further incubated until hatching. For the control group, another batch

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Table 1
Survival rate of chick embryos after 7 days under microgravity and hatchability after further incubation on earth

Embryonic age at launch	Survival rate		Embryonic age at launch	Hatchability	
	Flight group	Control group		Flight group	Control group
	(Number of surviving embryos/total embryos)			(Number of hatched embryos/total embryos)	
0-day-old	1/10 (2 were NF*)	7/10 (1 was NF*)	0-day-old	0/1	3/3
7-day-old	10/10	10/10	7-day-old	5/5	5/5
10-day-old	9/10	10/10	10-day-old	4/4	4/5
Total survival rate	20/30	27/30	Total hatchability	9/10	12/13

NF*, non-fertilized eggs.
For the control group, another batch of 30 fertilized eggs was incubated under 1G gravity using a flight model incubator. The embryo could not be inspected during space flight, but candling was performed 3 h after landing. In each group, half of the live embryos were immediately sacrificed and the rest of the eggs were further incubated until hatching and their hatchability was determined.

of 30 fertilized eggs was incubated on the ground under 1G gravity using the same incubator as the flight model. All the eggs were turned every 12 h. For turning eggs we gently shook each egg container three times, which contained 15 eggs, to the left and to the right from the 9 to 3 o'clock direction. During this operation the air chamber of each egg was always kept upward.

To simulate the potential location of the yolk in the egg under microgravity, yolk isolated from freshly laid fertilized eggs was incubated in various mixtures of thin albumen and salt solution. Fertilized chicken eggs were broken after oviposition and only the yolk was transferred into an empty recipient eggshell. The recipient shells were then filled with culture media consisting of various mixtures of thin albumen (specific gravity; 1.040) and salt solution (specific activity; 1.011) containing of 50 mM $KHCO_3$, 30 mM $NaHCO_3$, 10 mM KCl, 2.5 mM $MgCl_2 \cdot 6H_2O$, 0.7 mM $CaCl_2 \cdot 2H_2O$ and 11 mM glucose [4], and they were sealed with cling film secured by plastic rings [4–6]. They were cultured for 3 days at 37.5°C with 60% humidity. The location of the yolk in the eggs and the viability of the embryos were recorded every day. Specific gravity of the culture media varied from 1.040 (albumen/salt solution = 100:0) to 1.026 (albumen/salt solution = 50:50).

3. Results and discussion

Table 1 shows the survival rate of chick embryos after landing and the hatchability after continuation of incubation on earth. Twenty out of thirty eggs (9/10 ten-day-old; 10/10 seven-day-old; 1/10 zero-day-old) were recovered alive after landing. In the control group, 27 out of 30 eggs (10/10 ten-day-old: 10/10 seven-day-old; 7/10 zero-day-old) survived after incubation for 7 days in the flight model incubator. Out of 10 zero-day-old eggs in

Table 2
Survival rate of chick embryos after culture in diluted albumen for 3 days

Type	Location of yolk (days in culture)		Culture medium: Thin albumen (%)/Salt solution (%)							Survival rate of embryos	
		Number of embryos	100/0	70/30	65/35	60/40	55/45	52.5/47.5	50/50	Total	%
1	up	survived	10	2	5	1				18	100
	down	dead	0	0	0	0				0	
2	up	survived		1	5	9	14	11	4	44	98
	down	dead		0	0	0	1	0	0	1	
3	up	survived					5	4	2	11	85
	down	dead					0	0	2	2	
4	up	survived					0	1		1	11
	down	dead					2	6		8	
5	up	survived					0	0	0	0	0
	down	dead					1	1	3	5	

Isolated yolks were cultured in various mixtures of thin albumen (specific gravity; 1.040) and salt solution (specific gravity; 1.011) for 3 days as described in section 2. The location of the yolk was classified into 5 types according to its sequential changes in the egg during culture.

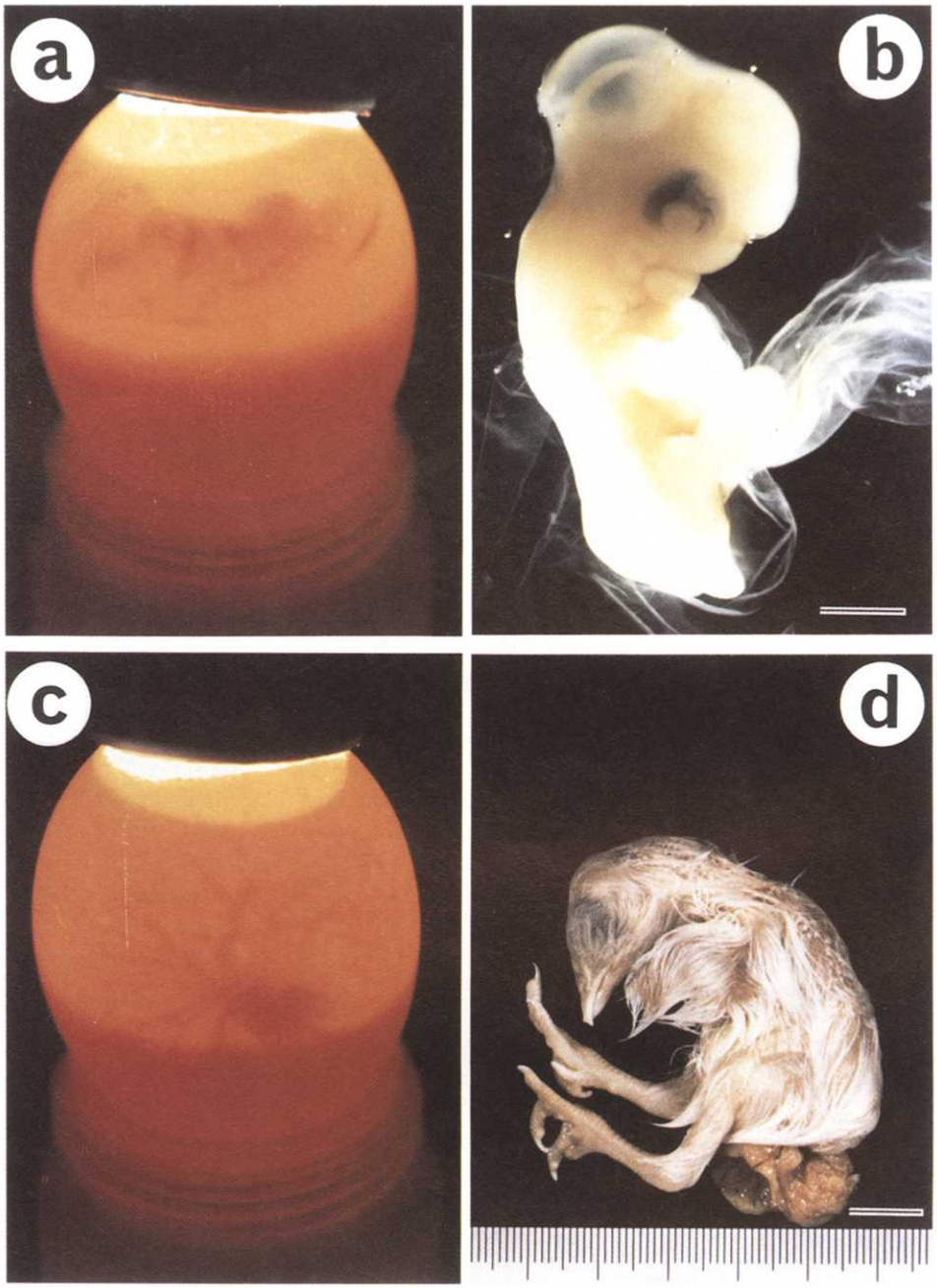

Fig. 1. Candling of a dead and a live 0-day-old chick embryo after landing and their development. Three hours after the space shuttle landed, survival of the 0-day-old chick embryos was examined by candling. Candling (a) and morphology (b) of the dead embryo, which was estimated to be 5 days old according to the criteria of Hamburger and Hamilton [3]. Candling (c) and inorphology (d) of the live embryo (space embryo), which died at the stage of 16 days old after incubation for 24 days in total (7 days in space and 17 days on earth after landing). Bars in b and d indicate 2 mm and 1 cm, respectively.

the flight group, 2 were non-fertilized eggs. Of the remaining 7 dead embryos, 2 survived for 5 days (Fig. 1a and b) and the rest died within 3 days. Candling performed 3 h after landing showed that angiogenesis had started in all of the 7 dead embryos as well as in the living embryo, but its development was delayed compared to that in the age-matched control embryos. The only living embryo (Fig. 1c) died 24 days after launch, and was comparable to a 16-day-old embryo according to the criteria of Hamburger and Hamilton [3] (Fig. 1d). Morphologically that embryo appeared normal. The other dead 0-day-old embryos also appeared almost normal. The detailed morphology of the 0-day-old embryos is currently under investigation. Most of the 7- and 10-day-old embryos in the flight group and 0-, 7- and 10-day-old embryos in the control group hatched normally after continuation of incubation on earth.

The high mortality of the 0-day-old chick eggs ap-

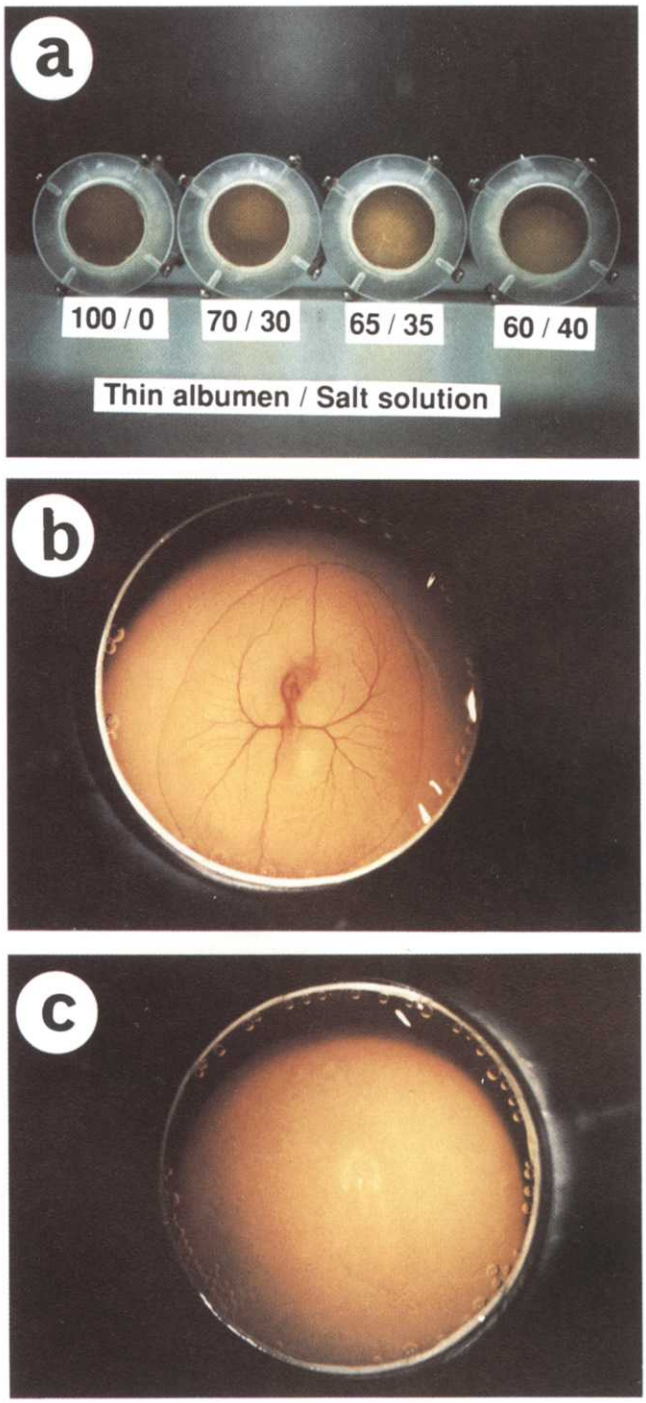

Fig. 2. Survival of chick embryos incubated for 3 days in various mixtures of thin albumen and salt solution. Isolated yolks were cultured in various mixtures of thin albumen (specific gravity; 1.040) and salt solution (specific gravity; 1.011) for 3 days as described in section 2. Panel a shows the location of yolks in the different mixtures on day 3. In the culture with 100% thin albumen (specific gravity; 1.040), the yolk always floated on the albumen (a) and all the embryos survived (b). In contrast, in the culture with a 55/45 mixture (specific gravity; 1.027) of thin albumen and salt solution, some of the yolk sank to the bottom (a) and died (c).

peared to be related to the specific inner structure of the egg which consists of the albumen and the yolk, having different specific gravities. The specific gravity of the yolk (1.029) is significantly lower than that of the albumen (1.040). Thus, under 1G gravity, the yolk always floats on the albumen and the blastoderm is situated at the top of the yolk. This allows the blastoderm to lie near the eggshell membrabe. Such a position of the embryo appears important for inducing angiogenesis in the chorioallantoic membrane [7], which develops along the inside of the eggshell.

In order to simulate the potential location of the yolk in the egg under micmgravity, yolk isolated from freshly laid fertilized eggs was incubated in various mixtures of thin albumen (specific gravity; 1.040) and salt solution (specific gravity; 1.011). In the culture with 100% thin albumen, the yolk always floated on the albumen and all embryos survived (Fig. 2a and b). In contrast, in the culture with a 55/45 mixture of thin albumen and salt solution, some of the yolk sank to the bottom and they died (Fig. 2a and c). Table 2 summarizes the survival rate of chick embryos after culture for 3 days. The location of the yolk was classified into 5 types according to its sequential changes in the egg during culture. In type 1, the yolk always floated on the albumen. In type 2, the yolk sank to the bottom for the first few hours but floated for the rest of the culture period. In type 3, the yolk sank for the first 24 h but floated on days 2 and 3. In type 4, the yolk sank for the first 2 days but floated on day 3. In type 5, the yolk sank to the bottom for the entire period of 3 day culture. In types 1–3, the survival rate was 85% or higher. In contrast, in types 4 and 5, almost all of the embryos died. These results indicate that early embryogenesis progresses normally if the yolk floats on days 2 and 3. When the yolk sinks to the bottom for more than 2 days, most of the embryos die. Under microgravity, the yolk would not be expected to float on the albumen and embryogenesis would cease within 3 days. In our experiment, turning of eggs was performed twice a day during the space flight, since it stimulates the development of the chorioallantoic membrane at least on earth. This may have resulted accidentally in the embryo achieving the correct position, especially in the case of our one surviving 'space embryo'.

In summary, the high mortality of the 0-day-old fertilized chick eggs in space appeared to be due to the lack of separation of yolk from albumen in microgravity. In contrast, the high survival rate of the 7- and 10-day-old embryos may have been due to the fact that these embryos were already fixed in the correct position in the egg and angiogenesis had started prior to the time of launch. The subtle difference in specific gravity between the yolk and albumen plays a critical role in early chick embryogenesis.

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Addendum

At the 10th International Academy of Astronautics (IAA) Man in Space Symposium held in Tokyo in April 1993, Wassersug et al. [8] reported that the entire processes from the fertilization to tadpole formation in *Xenopus laevis* progress normally under microgravity.