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# A Remarkable Discovery by Mr. Yutaka Tahara, a Japanese High School Head Teacher for Biology, has Opened a New Possibility for the Culture of Avian Embryos in Eggs Denuded of their Shells

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## Introduction

Embryonic developments of chicken microvascular network can now be observed microscopically by Taharas method, suggesting that the exposure to pure oxygen caused no observable effects on microvascular system of hatched chicken. Mr. Yutaka TAHARA, a well-known Japanese high school teacher (Oihama high school, Central ward, Chiba prefecture, Japan) has concentrated his teaching and research for the past 40 years, on the challenge of how to grow chick embryos to maturity in cultures and continuously observe their development, in eggs deprived of their shells. He selected this difficult task as the central plank in the biological training of his high school students. His life's work was directed to encouraging these students recognize with their own eyes in real time, and record how the microvascular, respiratory and skeletal systems were established at the beginning of avian life. To achieve this, the efforts of his students were focused on the hatching of small domestic birds in the school class room. During this endeavour he established a novel technique for hatching domestic chickens and quail from eggs that had been denuded of their shells. The hatchability of the chicks in these cultures is greater than 30% for quails and 67% for domestic chickens [1], at present. Furthermore after hatching, these chicks have grown into normal adult birds which lived full, uneventful lives, laid eggs and raised chicks of their own at the homes of their "breeder students". Tahara's successful methods and interesting observations are summarized below. A particularly interesting feature of the technique is that it involved culturing the embryos in pure, moistened oxygen which the embryos tolerated unharmed. Tahara's recent findings described below indicate that the main function of the eggshell is not to modulate the oxygen supply to embryos but to protect them from physical injuries caused by nesting and brooding movements of their parents and attack by predators, while avian embryos themselves are unaffected by hyperoxia.

## Materials and Methods

Using his technique the shells of fertilized eggs were carefully and quickly removed according to Tahara's protocol [2,3]. First spray with 70% alcohol and wipe off the eggshell surface quickly with a sheet of paper towel, and then open the egg by the use of an eggshell cutter into the plastic sheet hammock [1].

### Materials required for the construction of a transparent artificial hammock for a shell-less egg

Food-grade extensive plastic film-wrap and transparent plastic cups for domestic use (polymethyl penten, Riken Technos.Co.) The wrapping film was shaped into a smooth, fold-less, concave hammock by stretching it over an intact egg, held upright in an eggcup. The hammock was suspended in a plastic cup to accept a deshelled egg, so that the culture chamber permitted three-dimensional observation through the transparent egg holder.

Materials required for the culture and hatching of chicks in plastic cups, as described in 1 above and for experiments in the classroom [2].

Thermostatic chamber for fertilized eggs to be maintained at 15°C. (This may seem very cool for avian embryos. But it is recommended by Japanese traders for fertilized eggs)

Calcium lactate $Ca(CH_3CHOHCOO)2.5H_2O$, which is essential for formation of the embryonic skeleton because there is no shell to act as a calcium reservoir (Figure 1).

## Comments

Hyperoxia is harmful to mammalian tissues since excess oxygen generates various bioactive chemicals in biological tissues, which disturb normal metabolic pathways and destroy living cells. On the other hand, oxygen deficit results in slowing, distortion or complete inhibition of oxidative energy-producing chain reactions. Thus, the normal physiology of the body depends on the maintenance of a critical balance between the merits and demerits of its systemic oxygen supply.

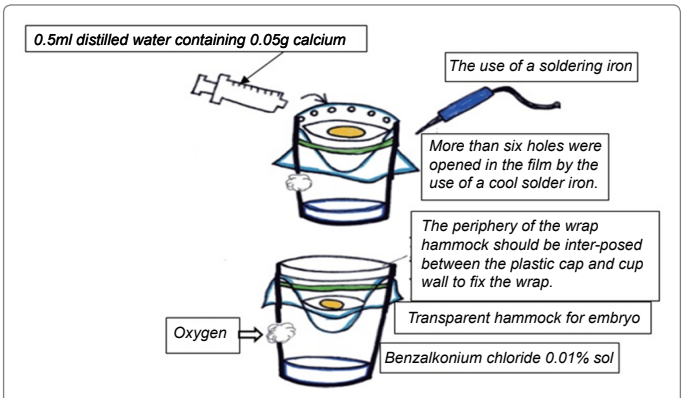


**Figure1:** Shows the following details: Domestic (disposable) transparent plastic cup and film for wrapping food. Vent hole, Pledget (oxygen inlet), Plastic cup, Benzalkonium chloride solution, Plastic cover, Air space, Yolk, Albumin, Distilled water+Calcium lactate 0.01% Benzalkonium chloride solution (osvan solution). Do not let any of this fall onto the egg yolk [3].

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## Results and Discussion

Preterm human babies were at one time maintained in hyperoxic incubators, a practice which often enabled them to survive but caused many babies to lose visual acuity because of damage to the immature retina caused by hyperoxia [4-6]. Similarly in treatment for the alleviation of obstructive (hypoxic) coronary artery disease, too sudden an opening of a previously obstructed vessel often causes fatal arrhythmia. Comprehensive studies have shown that oxygen supply to living tissue is tightly controlled to the necessary minimum for physiological function, and from this it was assumed that the avian eggshell (and its membranes) must play an essential role in protection against any excessive inflow of oxygen to the developing embryo. The present technique permits to observe micro-vessels from every direction. However Tahara's somewhat surprising recent findings described here indicate that the main function of the eggshell is to protect embryos from physical injuries caused by nesting movements of avian parents but play no part in regulating oxygen supply to the embryos. Even more surprising is the demonstration that avian embryos themselves are apparently unaffected by hyperoxia. As well as enabling continuous, non-destructive observation and recording of avian embryonic and pre-hatching development, the method should be of considerable value for production of transgenic chicks for immunological studies on the sequential steps of embryonic development (Figure 2).

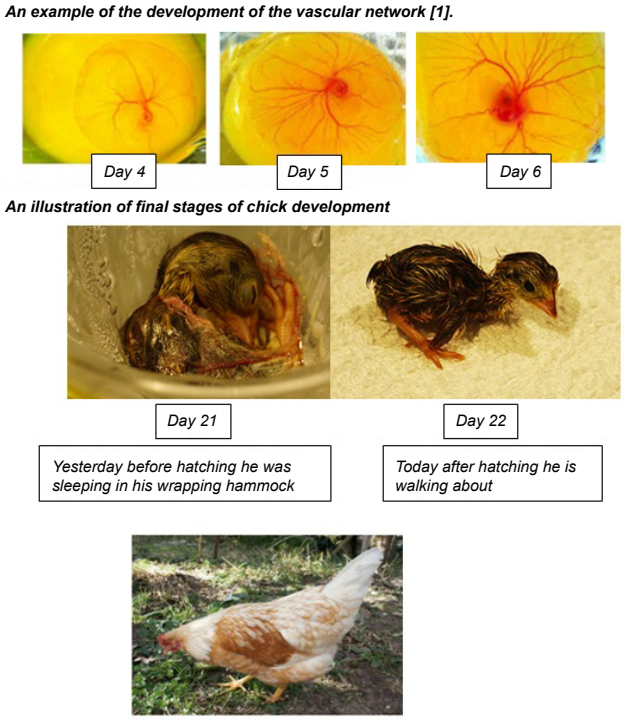


**Figure 2:** This hen has laid eggs almost every day since 2013 [2], some of the chickens were kept with a cockerel and raised chicks from their own fertilized eggs.

## Conclusion

Tahara's studies have demonstrated that avian embryos are not damaged by elevated oxygen tension. This finding opens another research field closely related to human pathophysiology.

### Acknowledgment

Finally, T. Koyama wishes to express his cordial thanks to Prof. IA Silver, Bristol University, for his suggestions, and also to Mr. Y Tahara for his kind permission to reuse the figures.

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