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# The study of protoporphyrin IX metabolism in cell proliferation using photoluminescence method

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## Abstract

Protoporphyrin IX metabolism was studied in the cell proliferation process in chick embryos by measuring photoluminescence. Characteristic emission of protoporphyrin IX at 625 nm was measured under the excitation at 405 nm. It was observed that the relative emission intensity increases during the proliferation process, indicating an increase in the concentration or in the metabolism level of protoporphyrin IX. This model might be used to study the protoporphyrin metabolism during the proliferation process of cancer cells. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Protoporphyrin IX metabolism; Cell proliferation; Chick embryo; Fluorescence

## 1. Introduction

Abnormal metabolism of protoporphyrin IX (PpIX) has been observed in the blood of cancerous patients. In many situations both cancer cells in vitro and tumor tissue in vivo do accumulate substantially more PpIX than the normal cells and tissues [1]. PpIX is an intermediate in the biosynthesis of heme, and can be accumulated since the conversion into heme is slow. The factors which control the biosynthesis and accumulation of PpIX have been investigated, but are as yet not fully understood [2–5]. The cells of a cancerous growth

are descendants of a single cell that reproduces uncontrolledly, indicating that the accumulation of PpIX may be due to the proliferation of cells.

The egg of a chick embryo is originally a single cell; the placenta is on the membrane of the yolk. Under appropriate temperature and humidity, the fertilized eggs will start mitosis. The embryo will obtain the nutrition and energy from the albumin and release the products of metabolism into it. By analyzing the albumin, the metabolic level of PpIX could be investigated. In this paper, cell proliferation in young chick embryos was used to simulate the growth of cancer cells. Photoluminescence of albumin from different developmental stages was measured and the increase in the relative intensity of PpIX characteristic emission observed. This suggests that chick embryo may be a useful model for cancer research.

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## 2. Sample preparation and experimental methods

The protocol for the chick embryo preparation was a modification of a previously described technique [6]. Fertilized eggs (Abor-Acres,  $60 \pm 5$  g) were washed with 70% alcohol, incubated at  $38^\circ\text{C}$  in 60% humidity, and then rolled over hourly. Starting on day three of embryonic development, a hole was drilled in the smaller apex and 1 ml of albumin was aspirated from a group of three eggs. This procedure will be repeated every three days for subsequent groups. A non-incubated group was used as a control. The sample was placed in a quartz cell for fluorescence measurement. Emission spectra were measured using Hitachi F-4000 spectrofluorometer with excitation at a wavelength of 405 nm.

## 3. Results and discussion

Fig. 1 shows the spectra of the albumin from the non-incubated (dashed line) and 3-day-incubated eggs (solid line), respectively. A fluorescence peak at 625 nm is the characteristic emission of PpIX [7]. It was observed that PpIX content in the albumin from the incubated eggs is substantially high compared to that from the non-incubated eggs, assuming that the relative emission intensity of PpIX is proportional to its concentration. This indicates that the cell proliferation obviously affects the PpIX metabolism. If we use the ratio of relative emission intensity of PpIX to define the level of PpIX metabolism,  $L$ , then we have

$$L = \frac{I_a - I_b}{I_b}, \quad (1)$$

where  $I_a$  is the peak fluorescence intensity of PpIX at point a;  $I_b$  is the fluorescence intensity of background at point b. Fig. 2 shows the development of the PpIX metabolic level vs. the time of cell proliferation. During the initial phase of mitosis and embryonic development, the cell proliferation rate is very high and PpIX accumulates, the PpIX metabolism level increases with time. While nutrition and energy are consumed from the albumin, especially as the organ development starts, the

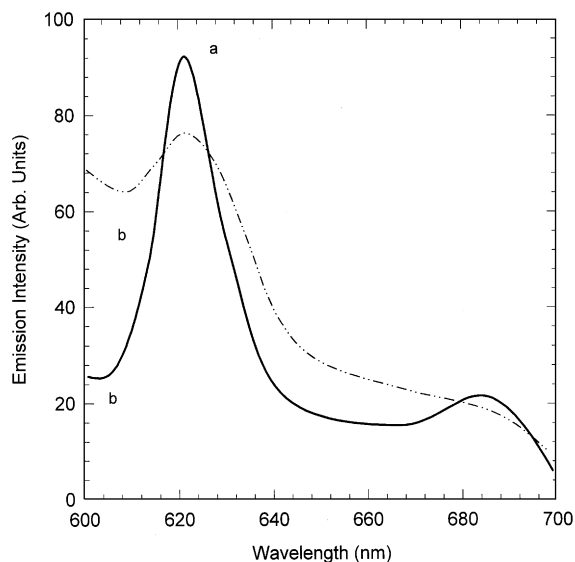


Fig. 1. Emission spectra from PpIX. Solid line represents the emission from albumin of fertilized egg, while the dashed line represents that from non-fertilized egg.

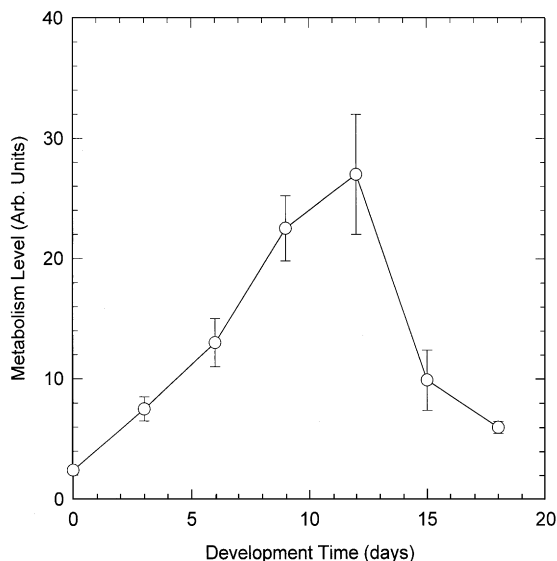


Fig. 2. PpIX Metabolism level vs. the embryonic development time. The line connecting the data points is added as a visual aid.

rate of cell proliferation will slow down. The PpIX metabolic level reached its maximum around day 12.

PpIX is an intermediate in the biosynthesis of heme (complex of PpIX with  $\text{Fe}^{2+}$ ), a prosthetic group of proteins, such as myoglobin, hemoglobin, catalase, peroxidase, and cytochrome. Heme biosynthesis is an essential pathway in cell metabolism. Due to the aggressive cell proliferation and division and the slow conversion into heme from PpIX, both cancer cells in vitro and tumor tissue in vivo will accumulate substantially more PpIX than the normal cells and tissues. The process is very similar to the initial phase of the chick embryonic development.

In summary, PpIX metabolism was studied in the cell multiplication process of chick embryo by measuring photoluminescence. Characteristic emission of PpIX at 625 nm was used to monitor its metabolism level. It was observed that the relative emission intensity increases during the initial phase of cell proliferation and division, indicating the PpIX accumulation occurs. The model might be used to study the PpIX metabolism of cancerous growth, especially for the initial phase.

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