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사료섭취가 병아리의 간세포증식 리듬에 미치는 영향

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Circadian DNA-synthetic Rhythm Accompanied Mitotic Rhythm in Newly Hatched in Chicken Liver : Possible Role of Feeding Regiment

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Abstract

The division and circadian DNA-synthesis rtythm are studied in chick liver immediately after hatching. The division function is tested by the mitotic and the ^{3}H-thymidine labelling index. The DNA synthesis exhibited a cyclic variation of 12 hours immediately after hatching. The rhythmic changes of DNA synthesis was maintained in the liver of meal-fed chickens, but the DNA synthetic activities decreased gradually in the starved chicken liver. From this time, the rhythm of DNA synthesis was greatly afffected by lighting schedule, all these DNA synthesis of chicken liver was accompained by mitosis.

Introduction

It has long been known from histological studies that there is a circadian rhythm in mitotic activity in the liver of rats and mice[1]. Food appears to be an important factor in the occurrence of the circadian rhythm since the rhythm of cell division and DNA synthesis in the liver can be modified by both diet and schedule of feeding[2-4]. In the liver of neonatal rats, however, there is no mitotic circadian rhythm during the first 20 days of life and the rhythm appears progressively from the 20th day onwards[5].

The present paper reports that the circadian DNA-synthetic rhythm is observed in chicken liver immediately after hatching and that the rhythm is modulated by both feeding and lighting regimens.

Materials and Methods

Animals

Male White Leghorn chickens were obtained from Ishii Hatchery, Tokushimn, Japan, on the day of hatching and kept either under a continueus light condition or in a light-dark cycle of 12 h of light(6 : 00-18 : 00) fellowed by 12 h of dark(18 : 00-6 : 00) period. Chickens were meal-fed "Chick A"(Marubeni, Japan) from the day after hatching for 3 h(9 : 00-12 : 00) unless otherwise stated. The composition of "Chick A" was 21.5% protein, 12.0% carbohydrate and 2.5% lipid. Tap water was available to the fed and starved animals *ad libitum*.

Determination of DNA Synthesis.

[^{3}H]Thymidine (New England Nuclear, specific

activity, 15 Ci/mmole) was used to evaluate DNA synthesis. At different times during the 24-h day, the chickens were injected intraperitoneally with [^{3}H]thymidine (10 μCi in 1 ml of saline) and killed 1 h later. The livers were quickly removed and homogenized in 5 volumes of distilled water with a Potter-Elvehjem homogenizer. Perchloric acid was added to the homogenate to a final molarity of 0.5. After centrifugation, the residue was washed once more with cold 0.5 M perchloric acid. DNA in the pellet was separated from RNA as described by Munro and Fleck[6] and was assayed by the Ceriotti procedure. Radioactivity was measured on a Packard Tri-Carb liquid scintillation spectrometer using a dioxane-based scintillator.

Determination fo Mitotic Activity

Chickens were injected intraperitoneally with colchicine(1 mg/kg of body weight in 0.2 mℓ of saline) and killed by decapitation 4 h later. A portion of the median lobe of the liver was immediately excised and the mitotic activity was determined by the method of Echave Llanos and Sadnik[7]. Arrested metaphases were counted in at least 1,000 hepatocytes.

Results and Discussion

Fig. 1 shows the 1-h incorporation rate of labeled thymidine into DNA in the liver of fed and starved chickens under the condition of light-dark cycle. In the meal-fed animals, DNA synthesis exhibited a definite 12-h cycle ; the peak incorporation occurring at 9 : 00 and 21 : 00. In the starved animals, a similad 12-h cycle was observed, but the 21 : 00 peak tended to be decreased until it disappeared completely on the 4th day after hatching. Thus it appears that the nature of 9 : 00 peak and 21 : 00 peak is different ; the former is independent of feeding while the latter is dependent on the feeding schedule.

In the liver of immature growing rodents, rhyth-

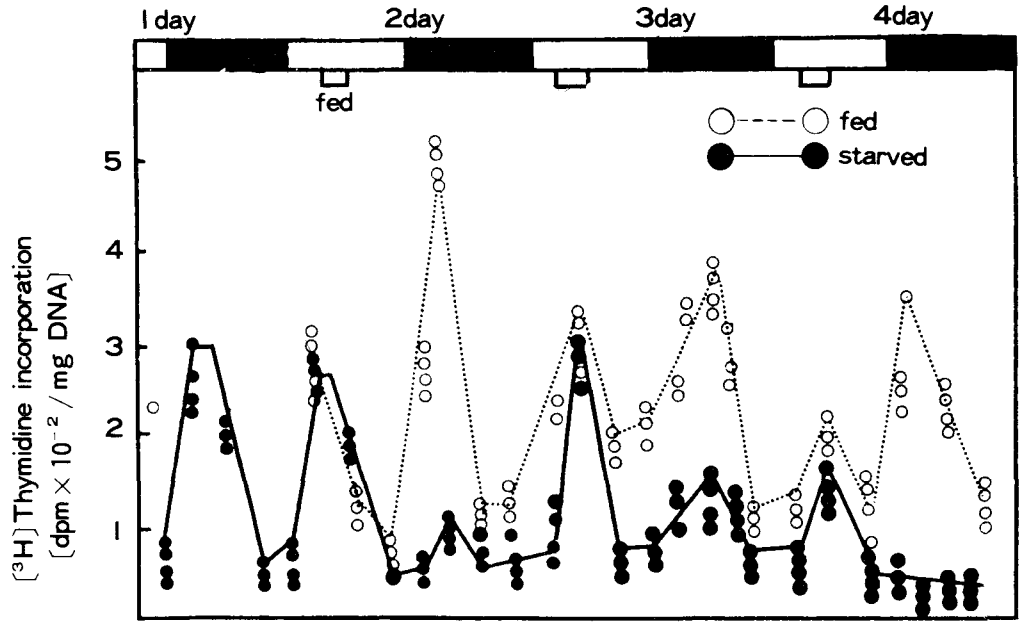

Fig. 1. Changes in hepatic DNA synthesis during chicken development in dark-light condition.

mic periodicity of mitosis at 24-h intervals has been observed[8-9]. Since the reversal of feeding schedule from dark period to light period shifts the mitotic rhythm in parallel with the feeding regimen [10], the rhythm of mitosis appears to be generated not by the rhythm of lighting but by the rhythm of food intake. Halberg et al[11] indicated the importance of lighting regimen in controlling the mitotic rhythm in mouse liver, but their experimental scheme did not exclude the possibility that light acted indirectly by affecxting the feeding pattern of the animals.

When the lighting regimen was changed to 24-h continuous lighting, the DNA-synthetic rhythm in the chicken liver underwent a profound change. As shown in Fig. 2, DNA synthesis in the meal-fed chicken liver displayed a 24-h rhythm with the peak at 24 : 00. The 9 : 00 peak was not recognizable. In the starved animals, the 12-h rhythm was maintained but the peaks were shifted to 3 : 00 and 15 : 00.

Fig. 3 shows the variations of the DNA-synthetic activity and the mitotic index in the liver of chickens kept under the continuously lighting condition. DNA synthesis correlated well with the changes in the mitotic index occurring about 12 h later. A similar lag period between DNA synthesis and the initiation of mitotic activity has been observed in mouse[12] and rat[13] livers.

The nature of the stimulus or stimuli responsible for the circadian rhythm of DNA synthesis in chicken liver is not known. Nevertheless, the present study suggests that the stimuli may vary not only in relation to food intake but also in response to changes in lighting environment of the animals. Regarding the effect of light, it is interesting to note that the circadian rhythm of melatonin synthesis in chicken pineal gland is greatly affected by light[5, 9]. Whether or not the DNA synthesis in chicken liver is under the control of pineal function remains to be determined.

In the liver of neonatal rats, there is no mitotic

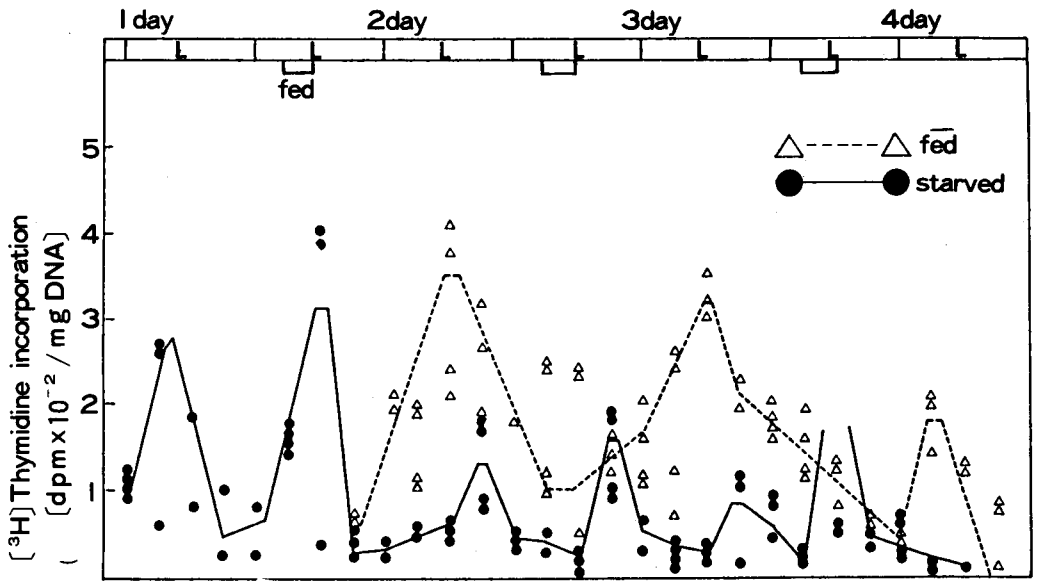

Fig. 2. Changes in hepatic DNA synthesis during chicken development in light-light condition.

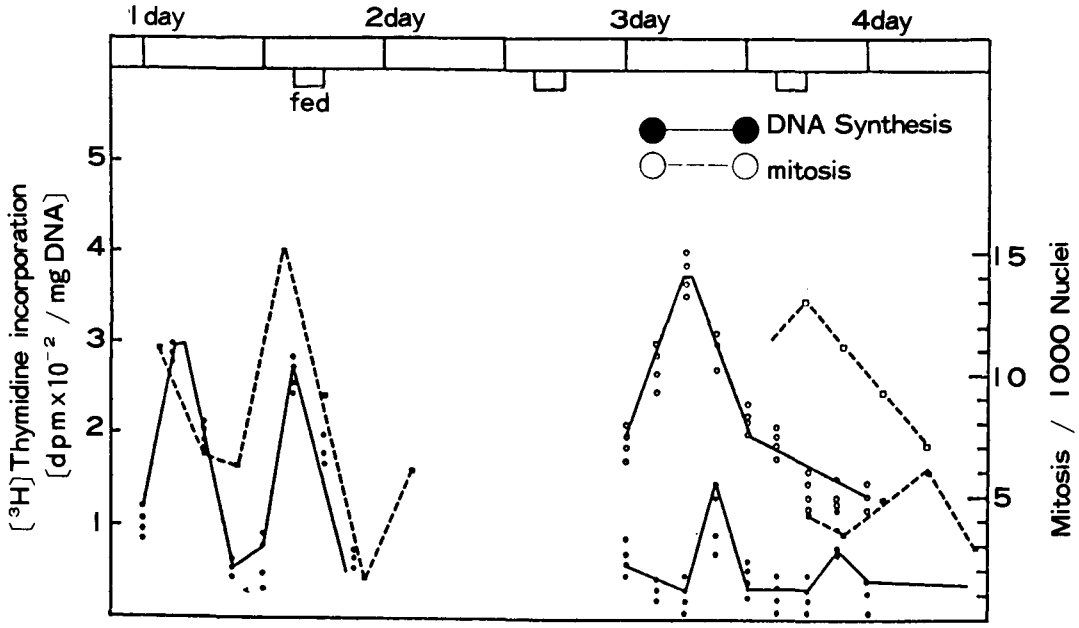

Fig. 3. Changes in mitotic indices in chicken liver.

circadian rhythm during the first 20 days of life and the rhythm appears progressively form the 20th day onwards. It is of interest to note that the appearance of the rhythm slightly preceded weaning which took place at 22 days.

In the case of chickens, peaks of DNA-synthetic rhythm occur at 21 : 00 on the lst day and at 9 : 00 on the 2nd day after hatching(Figs. 1 and 2). It should be pointed out that these two peaks occur without any feeding experience. Moreover, the timing of these peaks is identical for both light-dark cycle and continuous light groups. The fertilized chicken eggs were incubated for 21 days in complete darkness and the newly hatched chickens were exposed to light for the first time in their life at the day of hatching. It is thus possible that the biological clock that controls DNA-synthetic rhythm in chicken liver is set *in ovo*, independent of feeding or environmental lighting. Studies *in ovo* are necessary to clarify the mechanism of ontogenesis of the DNA-synthetic rhythm.

요 약

세포증식에 관한 개체발생의 일주리듬을 검토하기 위하여 부화직후의 병아리 간장 DNA 합성 리듬 및 간 세포증식의 개체발생에 대해서 검토한 결과 포유류에 있어서 DNA 합성 리듬은 이유기 후기에 출현하는데 비하여 조류(병아리)에 있어서 DNA 합성 리듬은 부화 12시간후 바로 출현하였고 간장의 DNA 합성 리듬은 사료섭취에 의해 리듬주기가 유지되었다. 한편 DNA 합성 활성은 절식에 의해 감소되었으며 DNA 합성의 리듬변화는 사료섭취에 의해서도 변화하였지만 빛의 조건에 의해서도 많은 영향을 받았다. 이러한 간 DNA 합성은 12시간 늦은 위상에서 유사분열도 동반하였다.

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