《Research Note》

Changes in Radioactive Phosphorus Uptake by the Ovarian Follicular Yolk of Laying Hens during an Ovulatory Cycle

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The aspect of day to day accumulation of yolk into ovarian follicles of the hen prior to ovulation was already demonstrated by a daily incorporation of either fat-soluble dyes¹⁻⁵⁾ or radioactive phosphorus⁶⁾. Using the technique of injection of the radioactive phosphorus solution, the present experiments were performed to elucidate whether the yolk accumulation by the ovarian follicles of the hen changes during a 24-hour period prior to ovulation.

Experimental

White Leghorn hens (21 months of age, 1.8-2.2 kg body wt.) were kept in individual cages under 14 hour (05:00-19:00 hours) light per day with feed and water available at all times. Only hens which had been laying 4-5 eggs in a clutch with one day pause between clutches were used. In these hens, time of ovulation of the initial ovum (C_1) of the clutch was regarded as being 05:00-06:00 hours and the second ovum (C_2) about 08:00 hours. This was repeatedly confirmed in the birds of the same flock under the same conditions by autopsies at a time determined by the record of the time of ovipositions of previous clutch.

Carrier-free $H_3^{32}PO_4$ solution was injected into the wing vein of the hens at nine different times (07:00, 10:00, 13:00, 17:00, 20:00, 23:00, 01:00, 04:00, and 06:00 hours) in the period between ovulations of C_1 and C_2 . The dose of ^{32}P injected into each hen was 25 μCi per kg body weight. At each time, the injection was made into 5 hens. The hens were killed 2 hr after the injection, and the largest follicle and the second largest follicle in the ovary were removed. Each ovum (yolk) was excised, weighed and placed in a flask. After stirring the yolk thoroughly with a glass bar, 100-120 mg of the yolk were placed on a counting dish with a few drops of 1N NaOH solution, and then placed under a heat lamp until dryness. The radioactivity of the yolk was counted by GM-counter (Kobe Kogyo: SA-20). The range of countings was 1,000-2,000 cpm. Counts of each sample were taken in duplicate. The superficial pectoral muscle (200-400 mg) of the same hen was also taken and its radioactivity was measured in the same way. The radioactivity of the whole yolk was expressed as:

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$$\frac{\text{cpm per mg of yolk}}{\text{cpm per mg of muscle}} \times \text{yolk weight (mg)}.$$

Differences between means were analyzed by the use of DUNCAN's multiple range test⁷⁾.

Results and Discussion

The results are shown in Table 1 in which the time of the uptake was expressed as the middle time of the 2-hr interval between the injection of ^{32}P and the removal of the yolk. In both the largest and the second largest follicles, the uptake of ^{32}P by the yolk was greater at 14:00 hours (18 hr before C_2 ovulation) than at 11:00 hours (21 hr before C_2 ovulation). Although the values listed in Table 1 were obtained after dividing the cpm values of the yolk by the cpm values of the muscle, similar results were obtained without the dividing. The results indicate that the ^{32}P -uptake by the ovarian follicular yolk of the hen increases some 21-18 hr before ovulation of the largest follicle, suggesting that a factor(s) which stimulate the uptake may act at the restricted time before ovulation.

Table 1. Uptake of ^{32}P by ovarian follicular yolk of the hen at various times during the period between the first and the second (C_2) ovulation of a clutch

Hour of day ¹⁾	Hour before C_2 ovulation ¹⁾	Radioactivity ² of yolk	
		Largest follicle	Second largest follicle
08 : 00	24	5638 $\pm$ 499* abc**	5587 $\pm$ 1285 ab
11 : 00	21	6260 $\pm$ 431 abc	5772 $\pm$ 552 ab
14 : 00	18	9665 $\pm$ 935 d	8354 $\pm$ 717 d
18 : 00	14	7755 $\pm$ 235 cd	7885 $\pm$ 571 cd
21 : 00	11	7465 $\pm$ 197 abc	7729 $\pm$ 738 abcd
00 : 00	8	5667 $\pm$ 1120 abc	6156 $\pm$ 1009 abcd
02 : 00	6	4740 $\pm$ 737 ab	5852 $\pm$ 494 abc
05 : 00	3	4594 $\pm$ 282 a	6453 $\pm$ 605 abcd
07 : 00	1	4202 $\pm$ 1296 a	5037 $\pm$ 865 a

1) Expressed as the middle time between ^{32}P injection and removal of ova

2) $\frac{\text{cpm per mg yolk}}{\text{cpm per mg muscle}} \times \text{yolk wt (mg)}$

* Mean of 5 hens $\pm$ SE

** Means followed by different letter are statistically significant ($P < 0.05$).

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産卵鶏の卵胞における放射性リン取込量の排卵周期中の変動

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白色レグホーン種の産卵鶏（21 ヶ月齢，体重 1.8-2.2 kg）に体重 1 kg 当り $25 \mu\text{Ci}$ の投与量となるように $\text{H}_3^{32}\text{PO}_4$ 溶液をクラッチ第 1 卵の排卵時から第 2 卵排卵時までの期間のいろいろな時間に注射し，注射 2 時間後に卵巢中の最大卵胞（クラッチ第 2 卵）と大きさが第 2 番目の卵胞（クラッチ第 3 卵）とを採取して，それぞれの卵黄の放射能をガイガーミュラーカウンターで測定した。また同時に浅胸筋組織を採取して放射能を測定し，卵黄 1 mg 当りの放射能値を浅胸筋 1 mg 当りの放射能値で除し卵黄重量 (mg) 値を乗じて得られた値を卵黄 1 個当

りの放射能値とした。

最大卵胞（クラッチ第 2 卵）の卵黄 1 個当りの放射能値は排卵予定時刻の 14-18 時間前にピークを示し，以後排卵時刻に近づくに従って減少した。クラッチ第 3 卵の卵黄もこれとほぼ同様な傾向を示した。従って，排卵周期中においては排卵の約 21 時間前から約 18 時間前までの期間に卵黄の ^{32}P 取込量を増加させるような要因が卵巢に作用するのであらうと推察される。

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