

琉球大学学術リポジトリ

下垂体除去鶏における血中カルシウムレベルの変動と退縮卵胞の出現について(畜産学科)

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Changes in the Blood Calcium Level and the Rate of Follicular Atresia Following Hypophysectomy in the Domestic Fowl

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Summary

Changes in the concentration of the total calcium in the blood plasma and the rate of follicular atresia were examined to estimate the change of estrogen level after removal of hypophysis in the domestic fowl.

The total plasma calcium level of about 25 mg% prior to hypophysectomy decreased linearly to about 10 mg% by 2 days after operation, while in sham operated hens, the calcium level changed within the limit of about 25 mg% during the same period. On the process of the declination of the plasma calcium level by 2 days after hypophysectomy, the calcium values already showed a significant decrease at 30 min. after operation in comparison with that of the pre-operation ($P < 0.05$). From 2 days after operation throughout the experimental period for 5 days, the calcium level was maintained at a low level of about 10 mg%.

Ovarian follicles exhibited a differential rate of atresia due to follicular size following hypophysectomy. Atresia appeared first in the largest follicle at 14–16 hr. postoperatively, in 2nd by 17–19 hrs., and in 3rd by 24 hr.

These results suggest that the secretion of estrogen which is mainly responsible for raising the concentration of the blood calcium may be abruptly terminated by hypophysectomy whereas the rate of ovarian regression advanced with decreasing the level of the blood calcium which is closely associated with ovarian activity.

Introduction

The concentration of the blood plasma calcium in the domestic fowl changes in association with the reproductive status, e. g; of laying or non-laying. In laying hens, the calcium level in the blood plasma fluctuates from 25 mg% to about 30 mg% during the laying cycle²⁾, while it is uniformly maintained at a low level of about 10 mg% during the non-laying period⁶⁾. However, in non-laying hens, the administration of estrogen induced a remarkable increase in the blood plasma calcium⁵⁾.

On the other hand, it is well known in mammals that hypophysis is the master endocrine organ because it elaborates a number of hormones which in turn stimulate to secrete hormones. In the domestic fowl the production and the secretion of gonadal hormones are controlled by hypophysis. When hypophysis was removed in laying hens, the ovarian follicle shrank and eventually

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atrophied⁴⁾. It was recently reported that the injection of estrogen into the hypophysectomized hens delayed the onset of atresia⁷⁾. However, the change of estrogen level in blood in relation to ovarian regression following the removal of hypophysis remains unknown.

The purpose of this experiment was to know indirectly the change in estrogen level following hypophysectomy through the change in the blood plasma calcium level and the rate of follicular atresia in the domestic fowl.

Materials and Methods

Hens

White Leghorn hens (18 months of age) laying 2 or 3 eggs in a clutch were used. They were kept in individual cages on a commercial ration containing about 3% calcium and water given *ad libitum* and were exposed 14 hrs. of light from 05 : 00 to 19 : 00 each day.

Surgical procedure

Hypophysectomy was made on hens which were anesthetized with about 0.5 ml. of sodium pentobarbital (Somnopentyl, Pitman-Moor). Hens were placed in a dorsal position on a stand with the beak-and ears-holder, and then they were drilled from oral cavity up to the sella turcica in which hypophysis located, by means of handling a drill (1.5mm. in diameter). A tapered glass tube connected to a vinyl tube was inserted into the sella turcica through the bore, and then hypophysis was gently removed by sucking of syringe connected to a vinyl tube as mentioned above. The operation was conducted at 16 : 00—17 : 00 on the day of the lay of the terminal egg in a clutch. At the final stage of the experiment, hens were killed by the rapid injection of anesthetic and abdomen was opened to examine the morphological ovarian changes at various times of 11—13 hr., 14—16

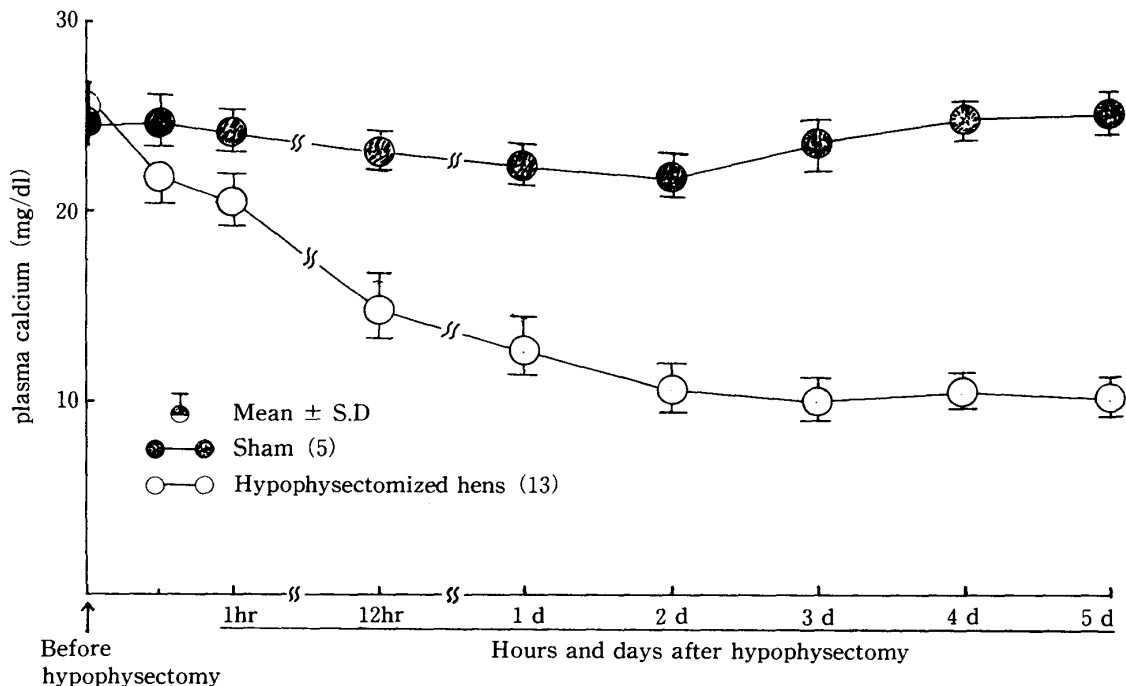


Fig. 1. Changes of total plasma calcium level before and after hypophysectomy
hr=hours, d=day, ()=No of hens used

hr., 17–19 hr. and 24 hr. after hypophysectomy. Only those hens that were verified on a complete removal of the organ by autopsy were used for the data of this work.

Determination of total plasma calcium

Approximately 0.5 ml. of blood was taken from the wing vein with a heparinized syringe, and the plasma was obtained by centrifugation (3,000 r.p.m. for 10 min.). The collection of blood samples were conducted just before operation, 0.5 hr., 1 hr., 6 hr., and 12 hr. postoperatively, and then every 24 hour for 5 days after hypophysectomy. The calcium concentration was measured in 0.05 ml. aliquot of plasma with a calcium estimation kit (C-test wako, Wako Pure Chemical Industries, LTD).

Results

The changes in the concentration of blood plasma calcium before and after hypophysectomy in laying hens are shown in Figure 1. The calcium concentration strikingly decreased from about 25 mg%, prior to the removal, to about 10 mg% by 2 days following operation. It was also found that

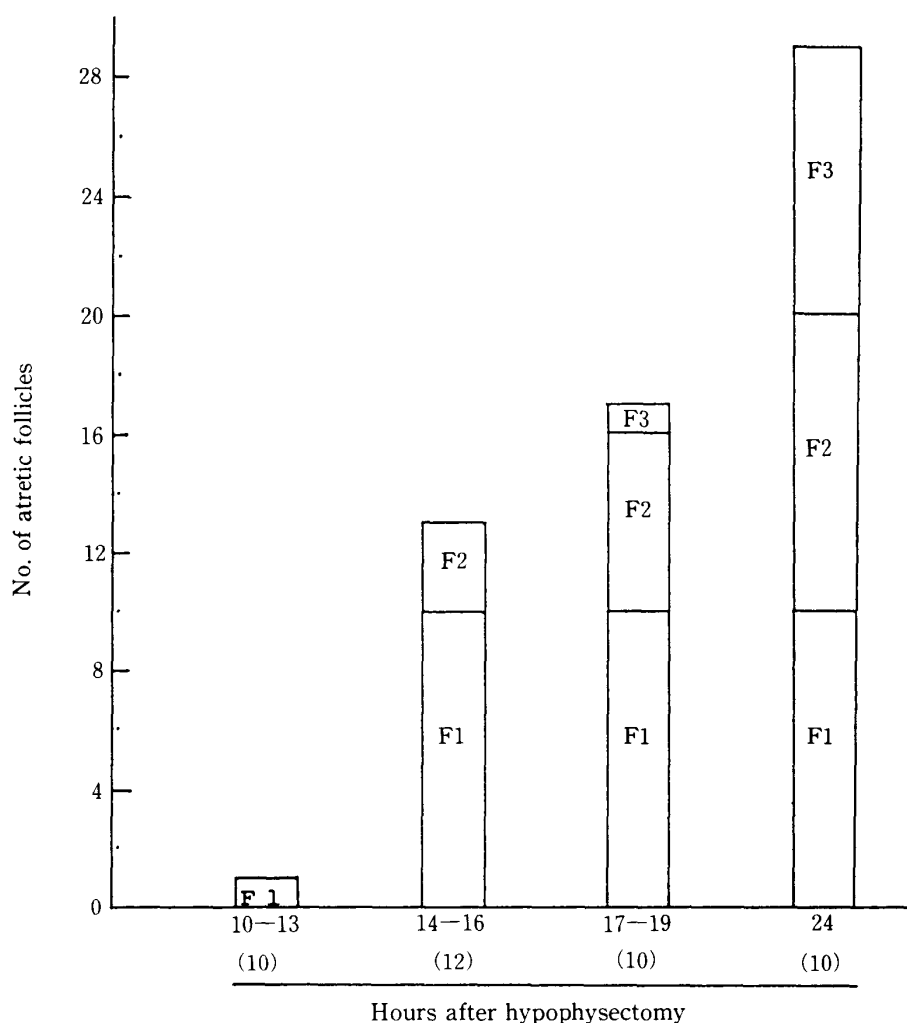
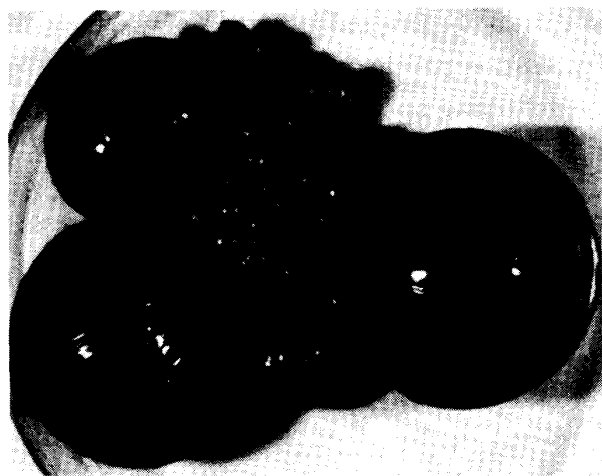


Fig. 2. Number of atretic follicles at various hours after hypophysectomy

() = No. of hens, F₁ = the largest follicle, F₂ = the 2nd largest follicle, F₃ = the 3rd largest follicle

the calcium values at 30 min. after operation significantly decreased in comparison to that of the pre-operation ($P < 0.05$). In sham operated hens, the calcium levels before and after operation did not change significantly during the experimental period of 5 days.

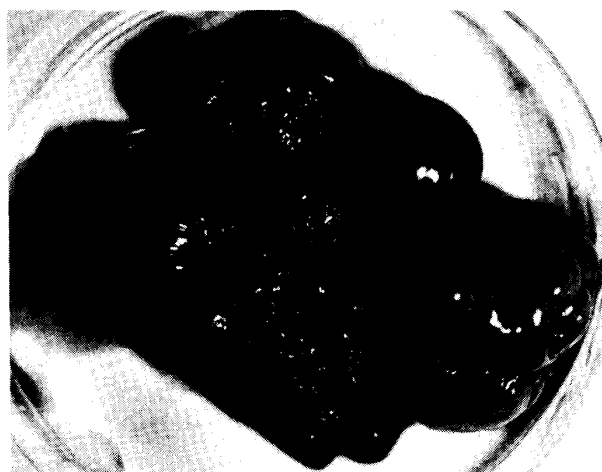
The rate of the incidence of atretic follicle at various times after hypophysectomy is shown in Figure 2. Until 12 hrs. after the operation, the atretic follicle was observed in just one hen out of 10. By 14–16 hrs., most of the largest follicle (more than 83%) atrophied, by 17–19 hrs., it spread to most of 2nd follicle, and then finally extended to the 3rd follicle by 24 hrs. These atretic follicles are shown in Figure 3.



Normal



14–16 hr after hypox



17–19 hr after hypox



24 hr after hypox

Fig. 3. Follicular atresia following hypophysectomy
hypox ; hypophysectomy

Discussion

Hypophysectomy creates endocrine vacuum, for all the other glands depend on hypophysis secretions for normal functioning, and eventually brings about regression in these hypophysis-dependent organs. In the hypophysectomized hens, ovary could be maintained or restored by the injection of mammalian gonadotrophic hormones or chicken pituitary materials^{3,4)}. This indicates that ovary is completely dependent upon gonadotrophic hormones. Armstrong¹⁾ reported that the aromatase activity, which participated in estrogen biosynthesis, was reduced in atretic follicles. In hen's ovary, the atretic follicle appeared first in the largest follicle and spread to small follicles with increasing time after hypophysectomy. This shows that the quick disappearance of gonadotrophic hormones required for ovarian growth may be brought about by means of hypophysectomy, and accompanied with the termination in the production of ovarian estrogen.

On the other hand, the calcium level in the blood plasma fluctuates in association with the change in ovarian activity in laying hens. The calcium level is normally maintained at levels from 20 mg% to 30 mg% in the blood plasma²⁾. When they ceased to lay at the onset of molt and brooding, the blood plasma calcium level soon returns to the non-laying calcium level of about 10 mg%⁶⁾. However, the administration of estrogen into non-laying hens causes a marked elevation of blood calcium⁵⁾. It was accordingly indicated that the blood plasma calcium levels mainly controlled by estrogen secreted by ovary. From them, it appears that the fall in the production of ovarian estrogen after hypophysectomy may be a fast process because of the abrupt drop of the concentration of plasma calcium, which is governed by ovarian estrogen, and the decreasing pattern of blood calcium agrees with that of ovarian regression.

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下垂体除去鶏における血中カルシウムレベルの変動と 退縮卵胞の出現について

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要 約

産卵鶏において血中カルシウムレベルの調節は主に卵巣から分泌されるエストロゲンによって行われている。一方、卵巣機能は下垂体前葉より分泌される性腺刺激ホルモンによって支配されている。

本実験は産卵鶏において卵巣の上位支配器官である下垂体前葉を除去し、以後血中カルシウムの変動と卵胞の形態的变化から卵巣のエストロゲン分泌機能の消退状況を推定することにした。

血中総カルシウムレベルは、下垂体除去前において約25mg%であったものが除去後2日目までに直線的な減少を示し、約10mg%レベルになった。以後実験期間中このような低レベルが維持された。なお、除去後2日目までの血中カルシウムの急激な低下過程において、除去30分後の血中カルシウム値はすでに除去前のそれと比較して有意な ($P < 0.05$) 減少を示した。

下垂体除去後の卵胞の退縮は14~16時間後において殆どの最大卵胞に出現し、17~19時間後および24時間後においては第二および第三卵胞にまで波及した。

これらの結果から下垂体除去後卵巣のエストロゲン分泌能力は速かに低下し、それに伴って卵胞の退縮が進行するものと思われた。