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著者	TORYU Yoshiyuki, HOSHINO Tadahiko, TAMATE Hideo
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# HISTOLOGICAL STUDY OF THE LINGUAL SALIVARY GLANDS IN THE CHICKEN WITH SPECIAL REFERENCE TO THE OCCURRENCE OF THE GLYCOGEN IN THE GLAND CELL NUCLEI

By

Yoshiyuki TORYU, Tadahiko HOSHINO, and Hideo TAMATE

*Department of Animal Husbandry, Faculty of Agriculture,  
Tohoku University, Sendai, Japan*

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## Introduction

It has been shown that the lingual salivary glands of the domestic animals contain three types, the serous, the mucous, and the mixed glands, which may be distinguished by their secretion and the cellular characteristics of the glandular epithelium. In the bird, however, the two lingual glands, the anterior and the posterior, have been regarded as the mucous gland (1, 4). The present study was planned to examine the secretive type of the lingual salivary glands in the chicken, since serous type-like glands were found in the preliminary observation of the chicken tongue histology by the present authors (3).

No one has found the glycogen in the nuclei. In the present investigation, a special attention was also paid to the presence of the glycogen in the nuclei of the anterior lingual gland cells of the hen, because in the same observation the deposition of the glycogen in the cells was demonstrated.

## Materials and Methods

Nine cocks and seven laying hens of White Leghorn were used. The total tongues from these birds were fixed either in Helly's or in Carnoy's fluid, embedded in paraffin, and cut into  $8\mu$  serial sections. The stains employed were as follows: Hematoxylin-eosin stain and Mallory-azan stain, for general histology; PAS-hematoxylin stain with or without saliva digestion, for the mucin and the glycogen; Heidenhain's iron hematoxylin-acid fuchsin stain, Feulgen-light green stain, and 0.1 per cent thionin stain, for the nucleic acids; Mallory-azan stain and Heidenhain's iron hematoxylin, for the demonstration of the zymogen granules.

### Results

Examination of the serial sections indicated that the anterior lingual glands were present in the body of the tongues, and the posterior glands at the base of the organ. The gland cells of the posterior lingual showed lighter cytoplasm with dark, small, and flattened nuclei in the Hematoxylin-eosin preparations. In the anterior lingual glands, however, the cells had somewhat darker cytoplasm which was stained by hematoxylin. The round nuclei of those cells were larger and lighter than those in the posterior glands. After PAS stain, the posterior lingual glands showed a brilliant red color in the whole cytoplasm, indicating that they contained a large amount of the mucin. A small amount of the mucin was demonstrated in the cytoplasm of the anterior gland cells (Fig. 1). No zymogen granules were found in these two glands after Mallory-azan stain or Heidenhain's iron hematoxylin stain.

It was noticed that a large number of the nuclei of the anterior gland cells in the laying hens showed a strong positive reaction to PAS stain (Fig. 2). This reaction became negative in the sections previously treated with saliva for less than one hour at 37°C (Fig. 3). This indicated that the PAS-positive substance in the nuclei was glycogen. The remaining part of the karyoplasm also contained a small amount of glycogen, and was faintly PAS reactive (Fig. 5). PAS-positive substance, polysaccharide other than glycogen was found in the Golgi apparatus (Fig. 5).

As shown in Figure 5, various forms of the nuclei were arranged in an order according to their shape and the amount of the intra-nuclear glycogen mentioned above. The nucleus, which might be taken as "normal", was approximately  $5.3\mu$  in diameter, containing one or two nucleoli and chromatin granules (Fig. 5, A). They contained no glycogen. Some of the glycogen-free nuclei included a few numbers of vacuoles (Fig. 5, B). When the vacuoles became larger, they nearly occupied the entire space of the nucleus, pushing aside all chromatin granules towards the nuclear membrane. Consequently, the chromatin granules were found attached to the membrane (Fig. 5, C, D). In such nuclei glycogen deposition occurred.

The intra-nuclear glycogen demonstrated by PAS stain was limited in the largest vacuoles occupying most of the nuclear space. The process of the glycogen deposition in the vacuoles may be traced as follows. At first, a few glycogen granules, small in size, appeared in the periphery of the vacuoles close to the chromatin granules attached to the nuclear membrane (Fig. 5, E~F). The size of the glycogen granules remarkably increased, united with each other, and the whole vacuolar cavity was finally filled with them (Fig. 5, G~J). Sometimes the glycogen was found as a layer deposited in the periphery of the vacuoles.

The process of the nuclear vacuolation and the migration of the chromatin granules towards the nuclear membrane, just stated, were also ascertained in the sections stained by Feulgen's technique or by thionin stain. It was concluded that the vacuoles contained no nucleic acids, since they remained unstained after these stainings. The vacuoles also appeared transparent after hematoxylin-eosin stain, Heidenhain's iron hematoxylin stain, and Mallory-azan stain.

In spite of the presence of the vacuoles and the glycogen in the nucleus, no demonstrable difference was observed in the cytoplasm of these cells from those with the "normal" nuclei in the anterior lingual glands stained with the methods as already mentioned.

The occurrence of the glycogen-containing nuclei was examined in the various loci of the glands. Generally speaking, the nuclei were more abundant in the upper part than in the lower part of the glands. The percentages of the cells with the glycogen-containing nuclei were estimated by counting their numbers per 1000 cells in the four loci of the glands, the sinus epithelium facing the common sinus cavity, the neck, the body, and the bottom of the glands. The percentages were, 74.2, 43.0, 34.3, and 5.8 in these four loci, respectively. This showed that the cells with the glycogen-containing nuclei increased their numbers towards the sinus epithelium. It was also noticed that the percentages varied in the various glandular systems of the anterior lingual gland. Accordingly, the percentages were estimated by counting the numbers of the cells with the glycogen-containing nuclei per 1000 gland cells in fifty glandular systems. The results are shown in Table 1.

**Table 1.** The percentages of the cells with glycogen-containing nuclei in the anterior lingual glands of the laying hens.

Cells with glycogen-containing nuclei (%)	Numbers of the glandular systems
0~ 9.9	6
10~19.9	15
20~29.9	15
30~39.9	5
40~49.9	6
50~59.9	2
60~69.9	1
Total	50
Average	25.0%

As seen in the Table 1, the percentages of the glycogen-containing nuclei showed a considerable variation, ranging from 0 to 70 per cent, the average of the 50 glandular systems being 25.0 per cent. Thirty out of the 50 systems

had percentages from 10 to 30 per cent. The results revealed that the cells with the glycogen-containing nuclei occupied approximately one-fourth of the total glands cells, though the percentage was high remarkably in some systems. The reason why such variation in the percentages took place was not determined in the present study.

No glycogen-containing nuclei was observed in the anterior lingual glands of the cocks (Fig. 4).

### Discussion

The present authors agree with the opinion that the anterior lingual glands in the chicken may be regarded as the mucous gland, since they do not contain the zymogen granules. However, the fact that the serous-type like cells in the glands contained only a small amount of the mucin suggested that they might have some different activities other than the mucin secretion. Further study on the possible functional significance of the anterior lingual glands in the chicken is planned by the present authors.

Modern reseaches on the chemical organization of the nucleus have indicated that this structure contains a basic protein, a nonhistone protein of higher order, the nucleic acids, lipids and some inorganic compounds (2). No glycogen or polysaccharides has been demonstrated in the nucleus, neither by the biochemical nor by the histochemical techniques. It is surprising that, therefore, a prominent deposition of the glycogen takes place in the nucleus of the anterior lingual glands of the laying hens. The fact that all birds were in good health and that the intra-nuclear glycogen was demonstrated only in the hens surely eliminated the possibilities that this deposition might be regarded as pathological. The present authors are inclined to believe that the deposition may have some important significance connected with the laying activities in the hen, since the anterior lingual glands in the non-laying hens seem to have only a few gland cells with the glycogen-containing nuclei (3). This will be fully discussed at another opportunity.

### Summary

The lingual salivary glands were histologically studied in nine cocks and seven laying hens. It was concluded that the anterior lingual glands are the mucous gland, though they appeared somewhat different from the posterior glands which were typical mucous glands. A prominent deposition of the glycogen and vacuolation in the nuclei were observed in the anterior gland cells of the laying hen. The present authors are inclined to believe that this deposition of the glycogen in the nuclei may have some important significance in the laying activities in the hen. The remaining part of the karyoplasm of the nuclei containing glycogen had a small amount of glycogen, and was

faintly PAS reactive. PAS reactive substance was found in the Golgi apparatus of the anterior gland cells.

#### **Acknowledgement**

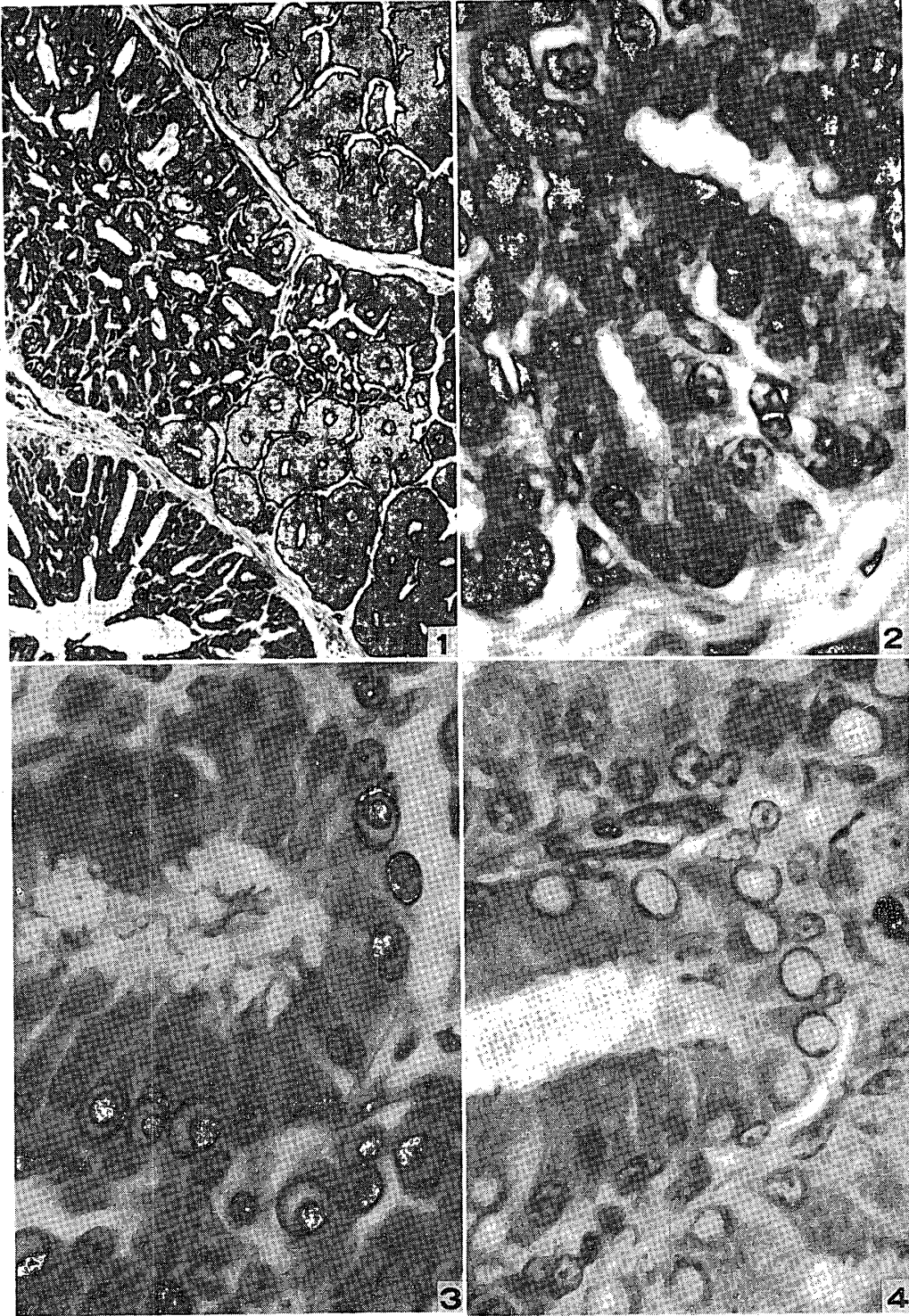
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**Plate 1****Explanation of Figures**

- Fig. 1. The lingual glands of the laying-hen. PAS-hematoxylin stain.  $\times 100$ .  
The posterior lingual glands (at right) contained a large amount of the mucin, but the anterior glands (at left) only a little.
- Fig. 2. Anterior lingual gland of the cock PAS-hematoxylin stain.  $\times 1000$ .  
No glycogen was present in the gland cell nuclei.
- Fig. 3. Anterior lingual glands of the laying hen. PAS-hematoxylin stain.  $\times 1000$ .  
Remarkable deposition of the glycogen was found in the gland cell nuclei.
- Fig. 4. Anterior lingual glands of the laying-hen. PAS-hematoxylin stain after saliva digestion.  $\times 1000$ .  
The intra-nuclear glycogen disappeared.





**Plate 2****Explanation of Figures**

Fig. 5. Successive stages of the glycogen deposition in the nuclei of the anterior lingual gland cells. PAS-hematoxylin stain.  $\times 1000$  (Drawing).

PAS-positive substance is present in the cytoplasm (mucin and Golgi apparatus) and in the nuclei (glycogen).

The Karyoplasm of D~J is faintly PAS reactive (glycogen).

A The cells with the glycogen-free nuclei ("normal" form).

B~D The cells with the glycogen-free but vacuole-containing nuclei.

E~J The cells with the nuclei in increscent stages of the glycogen deposition.

