Note on the Histochemical Localization of Glycogen and Pentosepolynucleotides in the Visual Cells of the Chick (Gallus gallus)

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With one plate (fig. 1)

SUMMARY

1. Glycogen was studied in retinas of the chick (Gallus gallus), fixed in Gendre's fluid and stained by the periodic acid/Schiff method. In agreement with some of the earlier data on the subject, obtained with less suitable methods, glycogen was found in the paraboloid of accessory cones and in the characteristically shaped paraboloid of the rods.

2. Basophilia, observable by means of the gallocyanin-chromalum stain and removable by ribonuclease or perchloric acid, was found in the inner segment of both chief and accessory cones, with the exception of the ellipsoid region.

INTRODUCTION

FEW data are to be found in the literature concerning the histochemical localization of glycogen in the visual cells of birds. The first reference to the subject seems to be that of Luna (1912), on Columba livia. This author described glycogen localization in the paraboloid of cells which seem to be accessory cones and rods in the sense used in the present paper. The methods used by Luna (Langhans and Vastarini-Cresi), however, are not specific (Lison, 1936), and no adequate controls were reported. Brammertz (1914), working on the same material, with Best's method after Carnoy's fixation, reported the presence of glycogen in the rod and cone layer, but gave no cytological details. Salivary digestion suggested that the staining was due to glycogen. Using absolute alcohol fixation and Best's carmine method, Schmitz-Moormann (1927) described the localization of glycogen in the myoid of cones of the pigeon's retina; rods did not stain. Uchiyama (1930), using the same method on the retina of the chick but apparently ignoring previous work on the subject, described staining of the paraboloid of the 'cones', rods being unstained. In a paper of which only an abstract could be obtained, Yoneyama (1932) observed in the same material the presence of glycogen in the ellipsoids of rods and cones. Fontana (1933), using the method of Fischer (considered

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to be unspecific by Lison (1936)) mentioned the presence of glycogen-containing granules in the visual cell layer of the retina of the pigeon and chick. Cytological details were not reported.

There is, therefore, no agreement in the literature as to the localization of glycogen in the rods and cones of the retina of birds. No reference could be found concerning the presence of pentosepolynucleotides in the visual cells of birds, although brief mention was made of their existence in the rod and cone layer of the retina of the rabbit by de Vicentiis (1949).

In this paper we present data on the localization of glycogen and pentosepolynucleotides in the visual cells of the retina of *Gallus gallus domesticus*, L., obtained by more adequate methods than those previously employed.

**Material and Methods**

Fifteen chicks of the Leghorn breed, 2–4 months old and weighing 200–400 gm. each, all light-adapted, were killed by decapitation. For the localization of glycogen the posterior poles of the eyes were immersed overnight at 4°C in Gendre's (1937) fixative. The periodic acid/Schiff (P.A.S.) method according to Gomori (1952) was performed on 7μ paraffin sections, with occasional counter-staining with Harris's haematoxylin. Control sections were digested with saliva before staining. Pentosepolynucleotides were revealed by applying the gallo-cyanin-chromalum technique (Einarson, 1952) to paraffin sections of material fixed in Gendre's or Helly's fluid. Ribonuclease digestion was performed during one hour at 56°C in a 0.1 mg./ml. crystalline ribonuclease solution in McIlvaine's citrate-phosphate buffer at pH 7.0. Control sections were incubated in the buffer alone. Extraction by perchloric acid was performed at 4°C in a 3N aqueous solution for 12–16 hours. This higher concentration than that used by Erickson and others (1949) was necessary in order to remove all cytoplasmic basiphilic substance. Control sections were incubated in water in the same conditions. Slides stained with phosphotungstic acid haematoxylin were particularly favourable for the demonstration of the ellipsoids of the visual cells. The nomenclature of the visual cell organoids used in this paper is that employed by Walls (1942).

**Results**

The structure of the visual cells in our material agrees with the description given by van Genderen Stort (1887) and Schmitz-Moormann (1927) for the pigeon, although simple cones could not be identified with certainty. Uchiyama's (1930) description of only one type of cone in the retina of the chick is therefore not confirmed.

**Rods.** These are easily recognizable in sections stained by phosphotungstic acid haematoxylin by their cylindrical and transversely striated outer segments. No negative image of either the oil globule or the paraboloid is found in our material. On the other hand, the periodic acid/Schiff reaction brings forth an intense staining of a localized region in the outer half of the inner segment
of rods, which probably corresponds to the paraboloid (compare our fig. 1, A with Wall's figure 193b (1942, p. 660) of the rods of Passer domesticus). This region has frequently a conical shape, with its base pointing to the outer segment of the rods (fig. 1, A and B). Its height corresponds approximately to one-third of the height of the inner segment. This staining of the rod paraboloid does not occur after digestion by saliva (fig. 1, C). On the other hand, a distinct saliva-resisting P.A.S. reaction is found in the outer segment of the rods, in the form of alternatively stained and unstained disks (fig. 1, A, B, C). The positive reaction of the acromeres in mammal retinas to P.A.S. has been described by Day (1950) and by Lillie (1952), and was considered by the latter author as being probably due to a galactolipo-protein complex.

No staining of the rods could be observed by means of the gallocyanin-chromalum technique.

Cone. Both chief and accessory cones are found in our material (fig. 1, A). As was mentioned above, simple cones are not found in our sections. Chief cones do not stain with the P.A.S. method. Accessory cones show in the basal half of their outer segment a homogeneously stained, sharply limited, oval region (fig. 1, A, B), which we identify with the paraboloid. As this region does not stain in sections subjected to digestion by saliva (fig. 1, C), it is concluded that it contains glycogen.

Basiphilia removable by ribonuclease or perchloric acid is present in both the chief and the accessory cones in the inner segment, with the exception of the ellipsoid region (fig. 1, D, E, F). No staining of the outer segment is found. The paraboloid of accessory cones stains faintly and inconstantly (fig. 1, D, F). In oblique sections it can be clearly seen (fig. 1, F) that the distal part of the inner segment of the cones, up to but not including the ellipsoid, stains most intensely.

Discussion

The localization of glycogen in the paraboloid of cones as here described confirms with more adequate methods the earlier data of Luna (1912) and Uchiyama (1930). The results of Schmitz-Moormann (1927) in the pigeon, and Yoneyama (1932) in the chick, pointing to the localization of glycogen respectively in the 'myoids' and 'ellipsoids' of visual cells are difficult to interpret. At least in the case of the former author the disagreement with our description is not due to difference in the nomenclature used, as Schmitz-Moormann recognizes the existence of a paraboloid in the visual cells as distinct from the myoid. The localization of glycogen in the paraboloid of rods possibly agrees with the data of Luna (1912) for the pigeon, but disagrees with those of Schmitz-Moormann (1927) on the same material and Uchiyama (1930) on the chick, as these authors did not find glycogen in the rods. The characteristic staining of the rod outer segments and the form and position of the rod paraboloids as visualized by the P.A.S. technique (fig. 1, A, B), suggest that it could be used in surveys of the distribution and numbers of rods in the bird retina.
No difference could be found by us in the glycogen distribution in the visual cells of 3 chicks that were dark-adapted for 4 hours and then killed under a faint red light when compared with 3 light-adapted chicks killed under daylight. It is interesting to observe that glycogen appears in the chick cones during the 18th day of incubation (Yoneyama, 1932), at a period when anaerobic glycolysis increases (Tamya, 1927), iodopsin can first be demonstrated (Bliss, 1946), and electric phenomena due to light-stimulation can be brought out (Hasama, 1941). This may suggest some relationship of glycogen to the function of the cones.

Our results pointing to the presence of pentosepolynucleotides in the cones of the chick retina should be checked by means of ultraviolet microspectrography. Nothing can be added at present on their physiological significance.

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Fig. 1 (plate). The photomicrographs represent sections of chick retinas after Gendre’s fixation, stained by the periodic acid/Schiff (P.A.S.) technique with haematoxylin counterstaining (A, B, C), and by the gallocyanin-chromalum method (D, E, F). The scales represent 10 μ.

A. Note two double cones and one rod. Double cone 1 consists of a chief cone (above) and an accessory cone (below). Double cone 2 presents these cells in reverse order. In the chief cones can be seen the negative images of the oil globules and there is no staining by the P.A.S. method. The accessory cones present an ovoid glycogen-containing paraboloid. In the labelled cells there is some artificial retraction of the glycogen-containing substance. The labelled rod 3 and others that are found in the field, present a transversely striated outer segment, stained by P.A.S., and a conical glycogen-containing paraboloid in the inner segment.

B. Medium power view showing glycogen-containing paraboloids of accessory cones and of rods. The arrow points to one of the rod paraboloids, which are typically found in a more distal position than those of the accessory cones. The outer segments of the rods are stained by the P.A.S. method.

C. Section adjacent to the one shown in B, stained by the P.A.S. method after digestion with saliva (30 min. at 37°C). The paraboloids of the rods and accessory cones no longer stain, but staining of rod outer segments is unaffected. Basiphilic regions are found distal to the unstained paraboloids (lower part of the photomicrograph).

D. High-power view showing cones stained by gallocyanin-chromalum. Observe the basiphilia of the inner segments of the chief and accessory cones. The arrow points to a double cone, with the chief component above and the accessory one below. Note the negative images of the oil globules and unstained ellipsoids. The section was incubated overnight in distilled water at 4°C.

E. Similar section extracted with 3N perchloric acid overnight at 4°C before the gallocyanin-chromalum staining. The basiphilia of the inner segments of the cones no longer appears. Similar results were obtained with ribonuclease digestion.

F. Oblique section of a chick retina showing, diagonally, from the top left corner to the opposite one: nuclei of the rods and cones; paraboloids of accessory cones transversely sectioned and faintly stained; cytoplasm surrounding the paraboloids is moderately stained; stained chief cones (transversely cut) are also shown in this region. The next region belongs to a plane just proximal to the ellipsoids. The right-hand upper corner shows the region of the ellipsoids, oil globules, and outer segments.
FIG. 1
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