<table>
<thead>
<tr>
<th>Title</th>
<th>Chemical Physiology of the Compound Eye, I: On the Retinal PAS-Positive Substances (With 1 Text-figure and 4 Plates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>SUZUKI, Kenji</td>
</tr>
<tr>
<td>Citation</td>
<td>北海道大学理学部紀要 = JOURNAL OF THE FACULTY OF SCIENCE HOKKAIDO UNIVERSITY Series VI. ZOOLOGY, 15(1): 137-147</td>
</tr>
<tr>
<td>Issue Date</td>
<td>1962-12</td>
</tr>
<tr>
<td>Doc URL</td>
<td><a href="http://hdl.handle.net/2115/27359">http://hdl.handle.net/2115/27359</a></td>
</tr>
<tr>
<td>Type</td>
<td>bulletin</td>
</tr>
</tbody>
</table>

File Information: 15(1)_P137-147.pdf
Chemical Physiology of the Compound Eye, I. On the Retinal PAS-Positive Substances

By

Kenji Suzuki

Zoological Institute, Hokkaido University

(With 1 Text-figure and 4 Plates)

It is well known from behaviour research that the mode of perception of the compound eye for colour, size, form and movement of objects differs fairly from that of the camera eye (Roeder, 1953; Buddenbrock, 1952; Wigglesworth, 1950). These facts, of course, depend closely upon the morphological structure of the compound eye and the transmission mechanism of stimulus-excitation within it, but, on the other hand, they are considered to be a reflection of the specificity of chemical components making up the compound eye. Recently, knowledge on the mode of transmission of stimulation within the compound eye has rapidly been accumulated by the remarkable progress of electrophysiological technique. As regards the chemical analysis of the compound eye, contributions mainly to photosensitive and -insensitive visual pigments have recently been made (Goldsmith, 1958; Butenandt, 1957; Wald and Hubbard, 1957; Kampa, 1955). An attempt to treat a visual process of the compound eye from the standpoint of metabolism of its fundamental chemical components has been made by Langer (1959, 1960).

This paper is concerned with the distribution of periodic acid-Schiff (PAS) positive substances in the retina of the compound eye and the dynamic state of these substances in relation to the change of photic environment.

Material and Methods

The material used chiefly in this study was females of the commonest house-fly, Musca vicina Macquart which was reared in laboratory on a mixed medium containing sawdust, bean refuse (Okara) and entrails of fishes. Emerged adults were given 5% sucrose solution as a food. For the fixation of PAS positive substances, Carnoy's (abs. alcohol: acetic acid = 30: 10 in volume), Pasteels et Léonard's (dioxane saturated with picric acid: formalin: acetic acid = 85: 10: 5), and Gendre's (90% alcohol saturated with picric acid: formalin: acetic acid = 80: 15: 5) solutions were employed; microscopic images of retinal PAS positive substances were confirmed to be considerably different according to these three fixatives. In order to help the permeation of the fixatives, the compound eye was cut in two along the central vertical axis of head under a binocular microscope, and the two half-eyes were immersed into the fixative solutions for a definite time (1.5 hrs. in Carnoy's; 2.5 hrs.

Contribution No. 572 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.


137
After dehydration with alcohol series and embedding by the usual paraffin method, the eyes were cut at thickness of 5μ. PAS reaction was detected by the McManus method. In connection with PAS reaction, chrom- and argentaffin reactions of rhabdome were investigated. The fixative agents for chromaffin test were each Orth’s solution (Henle’s modified method), and a mixture solution of 10% formalin with added potassium iodate up to 5% (Lison’s method): Orth’s solution was prepared by mixing 10 ml formalin into 100 ml distilled water containing both 2.5 g K₂Cr₂O₇ and 1 g Na₂SO₄. Argentaffin reaction was detected by Masson’s silver method. After fixation of the compound eye with Carnoy’s fluid, the microscopic preparations were treated with Fontana’s ammoniacal silver solution for 36 hrs. The preparation of Fontana’s solution is as follows: concentrated ammonia aq. is poured into 5% silver nitrate solution until brown deposits disappear and thus, the obtained clear mixture is caused to become slightly turbid again by further addition of silver nitrate solution. The supernatant of this solution was used.

Results

I. Histological images of the compound eye without treatment for PAS reaction

   a) Dark adapted eye: The eyes used for this observation were cut off under yellow light passing through a safe filter No. 1* from flies placed overnight in a dark room, and after fixation with Carnoy’s solution for 1.5hrs., or with 10% formalin for 3 days, the eyes were dehydrated, sectioned and deparaffinized as usual. The following description is based mainly on the eyes fixed with Carnoy’s solution.

   Cornea, lens and retinal cells were stained a fairly dense yellow. In the eyes of formalin fixation, these structures showed a more concentrated colour than in the eyes treated with Carnoy’s reagent. Rhabdome was lighter in colour than retinal cells. Crystalline cones were colourless. Iris pigment cells took a dark red: retinal pigment cells, a reddish purple. In the eyes immersed in formalin, both iris and retinal pigment cells were stained a dark yellowish red and were difficult to distinguish from each other in colour (Fig. 2).

   Morphologically, iris pigment cells were moved just under lens, enclosing there densely crystalline cones. The proximal ends of iris pigment cells were extended to introduce the apexes of retinal cells into the iris part. Retinal pigment cells were migrated toward the border of the neighbouring ommatidia, and were scarcely found in the surface of retinal cells (Fig. 1A).

   b) Light adapted eye: For this experiment, living flies accomodated overnight in a dark room were irradiated by means of high pressure mercury lamp through a water vat for 5-6 hrs., and after immersion into 10% formalin or Carnoy’s solution, they were treated with the same procedure as in the dark adapted specimens. In general, retinal cells, rhabdome and basement membrane were clearly lighter in colour than in the dark adapted eye. Especially, cornea and lens of eyes fixed with formalin remarkably developed this tendency (Fig. 3).

* The filter No. 1 is sold by the Alma Optics Inst., Co. Ltd., Tōkyō; transmitting wave lengths are 530-680 mμ.
Morphological observation indicated that iris pigment cells were concentrated thickly about crystalline cones to enclose them and the proximal ends of iris pigment cells became slender in the contact part of crystalline cones with the apexes of retinal cells. Retinular pigment cells were scattered all over the retinal cell surface; therefore, it was seen in the border of the adjacent ommatidia that retinular pigment cells form a narrower vertical band of pigment than in the dark adapted eye (Fig. 1B).

II. Histological images of the compound eye immersed in Schiff’s reagent without periodic acid treatment

Compound eyes were fixed with Carnoy’s solution immediately after having been subjected to the same procedure for light and dark adaptation as described in the previous section.
Colours of iris, retinular and basement pigment cells of the dark adapted eye were rather more purplish than in the dark adapted eye which was deparaffinized without receiving the Schiff staining. It was, therefore, presumed that these pigment cells have an affinity to some degree for a reduced basic fuchsin itself. Cornea, lens, crystalline cones, retinal cells and rhabdome were all not stained. Also in the light adapted eye, the attitude of each structure toward the reduced fuchsin staining resembled closely that of the case of dark adaptation: accordingly, it was very hard to discriminate the light and dark adapted eye in the fuchsin staining alone (Figs. 4, 5, 6).

III. Distribution of PAS-positive substances in the compound eye

Three kinds of fixatives (Carnoy’s, Pasteels et Réonard’s and Géndre’s) were employed: staining was carried out by McManus’ method for PAS reaction.

a) Dark adapted eye fixed with Carnoy’s fluid: Dense purplish clumps were deposited thickly all over the eye except on the cornea and lens. Particularly, in the retinular part, this precipitation was conspicuous. Rhabdome became bold and was dyed a concentrated colour, but cornea and lens took a light colour (Figs. 7, 10).

b) Light adapted eye: This preparation was made from the eyes of flies irradiated for 2 hrs. in a long glass tube (diam. 2.5 cm, length 14 cm), in which they flew constantly toward the light during the irradiation. In comparison with the dark adapted eye, it is noteworthy that PAS substances completely disappeared from the retinular part, but in rhabdome and basement membrane, a considerably dense reaction was still remained (Figs. 8, 9).

c) Dark adapted eye fixed with Géndre’s fluid: Precipitated state of PAS substances was conspicuously different from the case of preparations made with Carnoy’s fixative. PAS substances in the retinular part formed innumerable brownish yellow granules and never lumped as in Carnoy’s fixation. Cornea, lens and rhabdome showed a strong reaction. Clear positive reaction was recognized in the central flute made by contact of four crystalline cones. This fact was not observable in the eye of Carnoy’s fixation (Figs. 11, 12).

d) Dark adapted eye subjected to saliva test after Géndre’s fixation: This preparation was subjected to saliva test without formation of celloidin membrane on it in a covered glass receptacle (diam. 20 cm, depth 3 cm) including absorbent cotton saturated with water for half an hour at 37°C. Cornea, lens and central flute of crystalline cones were transparent. Yellow granules on the retinal part entirely disappeared. Iris and retinular pigment cells became lighter in colour than in the eye which did not receive saliva treatment. On the other hand, reactions of rhabdome and basement membrane remained almost unchanged (Fig. 13).

e) Dark adapted eye fixed with Pasteels et Léonard’s fluid: Rhabdome and basement membrane both were brightly stained a purplish red. In the retinal part, PAS substances were distributed in a granular form as in the eye of Géndre’s
fixation. Cornea and lens were light coloured (Fig. 14). Saliva test brought on the result that PAS granules were almost entirely absent from the retina as seen in the eye of Géndre’s fixation. But in the next point, this test produced a different result from Géndre’s fixation: cornea and central flute of crystalline cones still kept a positive PAS reaction after the execution of the test. Also in rhabdome and basement membrane, the reaction was still remained; the most concentrated colouration was found in the part where seven rhabdomeres enter in a bundle into the flute of crystalline cones (Fig. 15).

IV. Distribution of substances positive to Bauer’s reaction in the eye

a) Dark adapted eye fixed with Géndre’s fluid: The flies kept in a dark room for 4 hours were used for this experiment. Bauer’s reaction was examined in the ordinary manner with use of 4% chromic acid. A strong response was found in the neighbourhood of iris pigment cells and the central split of crystalline cones. In the retinular part, positive granules lay scattered and the rhabdome was clearly stained though the response was weak. Cornea and lens both were transparent in colour (Fig. 16).

After saliva treatment, iris pigment cells and retinular part showed a negative reaction to Bauer’s staining, while rhabdome, a positive reaction (Fig. 17).

b) Dark adapted eye fixed with Pasteels et Léonard’s fluid: Large and small purplish granules were deposited all over the eye. Rhabdome, basement membrane and central split of crystalline cones were all strong positively stained (Fig. 18).

Looking back upon the results of the observations described above in sections III and IV, it seems possible to make some generalizations. PAS and Bauer’s positive substances seen in the retinular part are probably a chemically identical substance i.e., a carbohydrate, since both are completely digested with saliva. On the contrary, the reactions of rhabdome and basement membrane to PAS and Bauer’s stainings are almost unchanged after saliva test. In the eyes of Géndre’s and Pasteels et Léonard’s fixation, substances existing in the flute of the crystalline cones are clearly positive to PAS and Bauer’s stainings, but in case of Carnoy’s fixation, they never show positive reaction to these two stainings.

V. Dynamic state of PAS-positive substances of retina in relation to the change of photic environment

In the prosecution of this experiment, flies were fed sufficiently with 5% sugar solution and brought into a glass container covered with tin foil to spend overnight in a dark room. Fixation and staining of eyes were carried out with Carnoy’s fixing argent and by means of McManus’ method respectively.

Experiment 1. After being removed to a long glass tube, the flies were irradiated through a water vat by a mercury lamp for 30 minutes in a dark room. They
flew about in the glass tube during the irradiation. In the preparation, PAS granules were not seen at all in the retinular part: lens, cornea and basement membrane were dyed a light purple and rhabdome, a dense purple (Fig. 19).

Experiment 2. After their wings and legs had been plastered with cellophane not to move freely, flies were irradiated as usual for 1 hr. PAS granules of this retina were clearly much more abundant than in Exp. 1, and there were not so great differences in their deposited state from that of a dark adapted eye. A strong positive reaction was left in cornea, lens, basement membrane and rhabdome (Fig. 20).

Experiment 3. Only heads were irradiated for 3 hrs. in a physiological salt solution* immediately after having been cut off from dark adapted living flies. PAS granules were deposited in a large amount within retina and their distribution was not greatly different from that of heads placed in the physiological solution in darkness for 3 hrs. (Figs. 21, 22).

Experiment 4. Retina of a dark adapted eye was detached as clearly as possible from optic lobes, brain and various glands under a binocular microscope. This operation was performed in a physiological salt solution. The retina was transferred to another receptacle containing a fresh physiological solution and was irradiated for 2 hrs. PAS granules of this irradiated retina were distinctly less numerous than in the dark adapted retina immersed into the physiological solution for similar hours (Figs. 23, 24).

Experiment 5. Before this experiment was undertaken, flies were sufficiently (4 hrs.) irradiated in a glass tube in order to cause PAS granules to be consumed perfectly from their retinas: thus, the flies subjected to such a treatment for light adaptation were divided into four experimental groups.

a) The flies were injected with 4.5% saccharose (ca. 0.02ml) almost isotonic to their body fluid into abdomen and were placed in darkness for 5 hrs. A considerably quantity of PAS granules appeared throughout the retina (Fig. 25).

b) Lapping with their labellae 4.5% saccharose sufficiently, the flies were removed to darkness for 5 hrs. PAS granules were distinctly recognized in the retinular part though the amount was not so much as in the flies which were injected with saccharose (Fig. 26).

c) Into the abdomen of these flies was injected distilled water and they were placed in darkness as usual. PAS granules were imperceptible in the retinas. Rhabdome seemed to present an outer appearance swollen with water and the lightest colour among these four experimental groups (Fig. 27).

d) The flies were left to darkness for 5 hrs. after the treatment stated above for light adaptation. PAS granules were not yet accumulated in the retinas, but rhabdome was stained densely in colour (Fig. 28).

* The composition of the physiological salt solution was M/7.4 NaCl 100, M/7.4 KCl 3.7, M/11 CaCl₂ 2.3 in volume in addition to 0.002% NaHCO₃.
Examining the above four experimental results, it seems that there is obvious difference between them for the accumulation of PAS positive substances: in this experiment, needless to say, in order to minimize the influences of their free movement, the wings and legs of the flies were stuck with cello tape before accommodation into darkness. Mass appearance of retinal PAS granules is, therefore, considered to mean a migration of saccharose together with body fluid from abdomen to eye, and consequently an effect of saccharose injection upon the accumulation of retinal PAS granules.

**Experiment 6.** This experiment was designed to examine further a hypothesis of carbohydrate migration. Flies were left overnight in a dark room after sufficiently lapping 1% saccharose, and on the next morning, were divided into three groups for this experiment.

a) The flies were tied tightly from the joint of head to thorax with a slender silk thread under an insensitive yellow light and were placed in darkness for 9 hrs. Upon examination, the retina was found to be covered with a mass of PAS substances and to show a typical microscopic image for dark adaptation (Fig. 29).

b) Similarly, after having been bound at the joint of head, the flies were irradiated for 9 hrs. Retinal PAS granules showed a quantitatively remarkable decrease, though some deposits were as yet remained (Fig. 30).

c) After wings and legs had been stuck with cellotape, the flies were irradiated for 9 hrs. without treatment for tying the joint of head. The retina entirely lost PAS granules but in the rhabdome there was left an obvious reaction for PAS staining (Fig. 31).

Through experiment 6, flies were all ascertained to be living when put into the fixing agent. Two important facts may be pointed out from the results of the experiment viz., PAS granules were consumed in retina by irradiation for a long term (cf. Exps. a and b), and moreover, they disappeared from the eye of flies without binding of the joint of head more quickly than in the eye of head-bound flies (cf. Exps. b and c). Perhaps the latter fact was caused by migration of retinal PAS granules to some other parts of the body in addition to their consumption in retina itself by light stimulation. These facts are, thus, considered to offer additional powerful evidence supporting the hypothesis of carbohydrate circulation within fly's body.

**VI. Histochemistry of rhabdome**

Up to the present, the chemical compositions of rhabdome has been almost unknown, although it has been expected to include some chemical substances participating in the photochemical reaction of compound eye. As stated previously, the present writer observed that rhabdome is tinged a purplish colour by PAS and Bauer's reactions, and that the colour is not lost after saliva treatment. This observation suggests that the responses of rhabdome do not always originate
in only the existence of a carbohydrate. It is well known that PAS reaction is not specific for carbohydrate, and also that it is seen for chromaffin substances. In order to confirm the chromaffinity of rhabdome, heads of flies were immersed into Orth's solution for 4 hours. The rhabdome was positively stained a light yellow. According to Lison (1953), this reaction results from adsorption of bichromate on cell surface (pseudo-chromaffinity). For the distinction of true- from pseudo-chromaffinity, newly obtained compound eyes were put into a neutral 10% formalin containing 5% potassium iodate for 3 days (Lison's method); rhabdome was tinted a dark brown colour and thus, it became clear that it has a true chromaffinity. In relation to the chromaffin reaction, argentaffinity of rhabdome was investigated. After having been fixed with Carnoy's solution and sectioned at 5μ, the preparations were placed in Fontana's ammoniacal silver solution for 36 hrs. in a dark room (Masson's method). This reaction was found to differ between dark and light adapted eyes. The rhabdome of dark-adapted eye was dark reddish brown; while in light-adapted eye, rhabdome took a fine light yellow undistinguishable from the colour of retinal cells (Figs. 32, 33). The comparison of the reaction was made in the preparations of light and dark adapted eyes which were always paired and received the same treatment for argentaffin reaction. The tendency of the dark-adapted eye toward having a stronger response than the light-adapted eye was distinctly observed also in a true chromaffin reaction (Figs. 34, 35). But in eyes fixed with Orth's solution (pseudo-chromaffinity), it was fairly difficult to find response difference of rhabdome between light and dark adapted eyes (Figs. 36, 37).

**Discussion**

There are two kinds of PAS-positive substances in the compound eye: one of them is easily digested by saliva but the other is not digested. The former is, of course, carbohydrate; it is distributed in all parts within the compound eye. Carbohydrate is accumulated in the eye in darkness and is gradually consumed there by irradiation of light as probably an energy source for chemical reaction (cf. Exps. 3, 4 and 6c). Also in the eyes of guinea pig, Shimizu and Maeda (1953) histochemically observed that the dark adapted retina contains abundant amounts of glycogen as compared with the light adapted retina. On the other hand, there are some evidences that retinal carbohydrate is not consumed only in the compound eye. Retinal carbohydrate of flies moving freely within a tube vanished more swiftly than in flies with wings and legs plastered with cellopate under light irradiation (cf. Exps. 1 and 2). This may mean that a part of retinal carbohydrate was used as an energy supply for body movement. Furthermore, under irradiation, retinal carbohydrate of flies tied from the joint of head to thorax was distinctly much more than in flies not subjected to such a tying treatment (cf. Exp. 6). This fact is interpreted to indicate that excessive retinal carbohydrate is
caused to migrate from eye to other parts of body as a result of a light stimulation.

In his biochemical study on *Calliphora* compound eye, Langer (1959) points out repeatedly that in darkness all retinal sugar is supplied from other parts of head. Treherne (1958) reports that in the locust *Stictocera gregaria*, excess glucose was accumulated in the haemolymph as trehalose. The present writer observed in the injection experiment that carbohydrate of compound eye is probably stored by migration of body fluid including sugar from abdomen to eye (Exp. 5). It may be concluded as a generalization from the considerations stated above that carbohydrate of the compound eye is supplied in darkness together with the migration of body fluid from abdomen and head, and as a result of irradiation of light, it is not only expended in the retina itself but also sent out from eye to other parts of the body.

PAS reaction of rhabdome is not caused to disappear by saliva test and moreover, rhabdome itself has chrom- and argentaffinity. According to Lillie (cited from Okamoto, Ueda and Maeda, 1958), chromaffin substances are positive also in respect to PAS reaction. Positive PAS reaction in this case is considered to be based upon the existence of adrenaline and hydroxyalkylamino radical (Lison, 1953). It is established today that chromaffin reaction is specific to orthophenol compounds, i.e., catechol radical. Argentaffin reaction is seen for phenolic compounds and other reducing substances like aldehydes, melanin, ascorbic acid, etc. Therefore, it may be sure that rhabdome includes at least orthophenol compounds having a strong reducing action. Perhaps these compounds may include catechol amines such as adrenaline and noradrenaline.

Summary

In the compound eye of a fly, *Musca vicina* Macquart, there are two kinds of PAS-positive substances, one digestable by saliva and the other not digestable. The former was deposited in all parts within the eye while the latter was restricted in distribution mainly to rhabdome and basement membrane. The digestable PAS substances are a carbohydrate, i.e., glycogen. From the experimental results of tying the joint of head to thorax, and of injecting sugar solution to abdomen, it was indicated that carbohydrate of compound eye is accumulated from abdomen in darkness, while by irradiation of light, it is not only consumed but also caused to migrate from the eye to other parts of body. Rhabdome is positive to PAS, chrom- and argentaffin reactions and seems to contain in itself catechol amines like noradrenaline and adrenaline.

Finally, the writer extends his hearty thanks to Prof. Mayumi Yamada for his kindness shown in the course of the experiments.
**Literature cited**


**Explanation of Plates I-IV**

**Plate 1**

Figs. 2 and 3. Sections of dark and light adapted eyes deparaffinized after fixation of Carnoy's solution.

Figs. 4 and 5. Longitudinal and the transversal sections of dark adapted eye stained with a basic fuchsin without putting into periodic acid.

Fig. 6. Section of light adapted eye similarly treated.

Figs. 7 and 8. Longitudinal sections of dark and light adapted eyes subjected to PAS reaction by McManus' method after Carnoy's fixation.

Figs. 9 and 10. Transversal sections of light and dark adapted eyes subjected to the similar PAS staining.
Plate 2

Figs. 11 and 12. Transversal and longitudinal sections of dark adapted eye subjected to PAS reaction after fixation with Gendre's solution.

Fig. 13. Section showing PAS reaction of dark adapted eye subjected to saliva treatment after Gendre's fixation.

Figs. 14 and 15. Sections showing PAS reaction of dark adapted eye not subjected and subjected to saliva test after fixation of Pasteels et Leonard's solution.

Figs. 16 and 17. Sections showing Bauer's reaction of dark adapted eye not subjected and subjected to saliva test after fixation with Gendre's solution.

Fig. 18. Section of dark adapted eye subjected to Bauer's reaction after fixation with Pasteels et Leonard's solution.

Fig. 19. Section showing PAS reaction of fly freely moving during the irradiation of light for 30 min.

Fig. 20. Section showing PAS reaction of fly with wings and legs stuck with cellotape during light irradiation for 1 hr.

Plate 3

Figs. 21 and 22. Sections showing PAS reaction of eyes which were taken from each of heads exposed to darkness and light for 3 hrs.

Figs. 23 and 24. Sections showing PAS reaction of retinas exposed to the light and darkness for 3 hrs.

Figs. 25, 26, 27 and 28. Sections showing PAS reaction of flies, each of which was injected, fed with saccharose, injected with distilled water, and left in darkness without such treatments for 5 hrs.

Figs. 29, 30 and 31. Sections showing PAS reaction of flies, each of which was exposed to darkness and light irradiation for 9 hrs. after being tied at the joint of head to thorax, and was placed in darkness for 9 hrs. without such a tying treatment.

Plate 4

Figs. 32 and 33. Sections showing an argentaffin reaction of dark and light adapted eyes.

Figs. 34 and 35. Sections showing a chromaffin reaction of dark and light adapted eyes by means of potassium iodate.

Figs. 36 and 37. Sections showing a chromaffin reaction of dark and light adapted eyes by means of potassium bichromate.
K. Suzuki: Retinal PAS-Positive Substances in the Compound Eye
K. Suzuki: Retinal PAS-Positive Substances in the Compound Eye
K. Suzuki: Retinal PAS-Positive Substances in the Compound Eye
K. Suzuki: Retinal PAS-Positive Substances in the Compound Eye