STUDIES ON THE PHOTOSENSITIVE SUBSTANCES IN THE KAMULCHI (SNAKE HEAD) RETINA

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가물치 網膜中의 視覺物質에 관한 硏究

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(Received April 30, 1959)

Since Kottogen and Abelsdorfl) first found visual violet in the retinae of certain fresh water fishes, having the wave-length of maximum light absorption at 535-540mμ, many works have accumulated on the new photosensitive pigments of the fish retinae. Briefly summarizing these works, Bayliss, Lythgoe and Tansley[2] described a substance with a maximum absorption at 520mμ from the bastard retina (*"Paralichthys Olivaceus"*) and also did another with a maximum of 540mμ from the trout (*"Onchorhynchus Mason"*), and the visual violet substance of the Chelidonichthip with 545mμ. Wald[3],[4] gave the maximum spectra of the visual pigments possessed by Calico bars as 522$\pm$2mμ. Saito[5] isolated the visual violet having its maximum absotption at 530mμ from the oriental species of carp fishes (*"Cyprinus"*). Dartnall[6] isolated the visual violet having its maximum absorption at 533mμ from the tench, pike and bleak Crescitelli and Dartnall[7] also isolated the scotopic pigments from the carp retina and measured this substance with a maximum absorption at 523mμ. Lee[8] has recently found the same visual violet pigment from the Formosan species of carp fish (*"Chanodichthys macrops* GUNTHER") with its maximum spectrum of 530mμ.

Out of these works of particular interest is the finding of new pigments from the tench retina by Dr. Dartnall[9] which were named as visual yellow 2(407$\pm$5mμ) and visual red (475$\pm$5mμ). He further suggested several other pigments awaiting discovery with hypothetical approximation of the frequencies of maximum absorption for the different pigments, in accordance with the quantum theory.

When the author had observed photosensitive pigments of several species of Korean fresh water fishes, the author had often met new substances which were not described in the previous literature. Repeated observations gave evidence that this new substance exactly corresponds to the proposed pigment awaiting discovery by Dr. Dartnall[9]. This paper hereby describes the photometric indentification of new retinal photosensitive substances from the Korean Kamulchi (Snake head).

EXPERIMENT

The fishes employed here were two-year old Korean snake head fishes (*"Ophicephalus argus* CANTOR", Known as Kamulchi in Korea and Japan), weighing an average of 500 gr and the body-length being 35cm. The average weight of each eye was 0.5mg and the size of the eye is relatively small compared with the body-size; the eye-colour is rather yellowish. The fishes were reared in a glassware-aquarium 40cm$\times$30cm$\times$20cm in size. Dark adaptation was made by placing the fishes in the dark for 20 hours after the fishes had been exposed to a light of 200 watts, 120 volts for two hours. The dark-adapted fishes were decapitated and the eyeballs were enuclated from the orbits. All the work was carried out by a dim red light of wavelength longer than 650mμ. With a pair of fine scissors the optic nerve was cut off quite close to the bulb and a single cut was made right across the equator. Each hind half of the eye-ball was placed in a vessel containing 0.65% NaCl solution and the retina was removed by a soft stroke with a crook tipped glass rod. After the removal, the retinae were put into a pool. Several pieces of them out of this pool were sandwhiched between two slide glasses and rubbed against each other to crush the retinae.

The ground retinal tissues from two eyes were placed into a glass tube and 5c.c. of 2% digitonin solution (pH 7.4) was added. They were then extracted for 24 hours. The extracts were again poured into a centrifuge tube and centrifuged at 7000 r.p.m. for 20 minutes. The contents had become quite clear. This extract was again placed into another glass tube and mixed with 10c.c. of 2% digitonin solution. Thus the prepared stock solutions were used for photometric analysis with a DU-Beckmann Spectrophotometer.

EXPERIMENT I:

At first the optical density of the unbleached stock solution was measured, then two photometric cells, one of which contained the unbleached solution, the other contained the blank of 2% digitonin solution, were both exposed to an electric light bulb, 200 watts, 120 volts at a distance of 9cm. for 20 minutes. The heat filters used here were 3% $CuSO_4$ solutions in a colorimetric cell of the Klett-Summerson-Photo-electric Apparatus (5cm in width and 2cm in depth). The temperature of the cell contents was increased 2°C by 20 minutes illumination. The measurement of light transmittance of the copper sulphate solution alone is given in Fig. I. The sample solutions were measured for the absorption spectrum after this illumination.

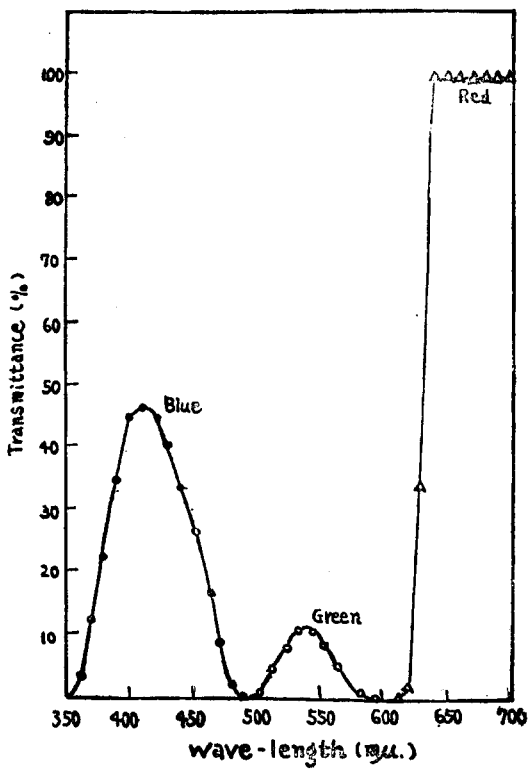

Fig. 2. The light transmittance of the three different light filters.
-·-·-·-, Blue filter. -o-o-o-, Green filter. -△-△-△-, Red filter.

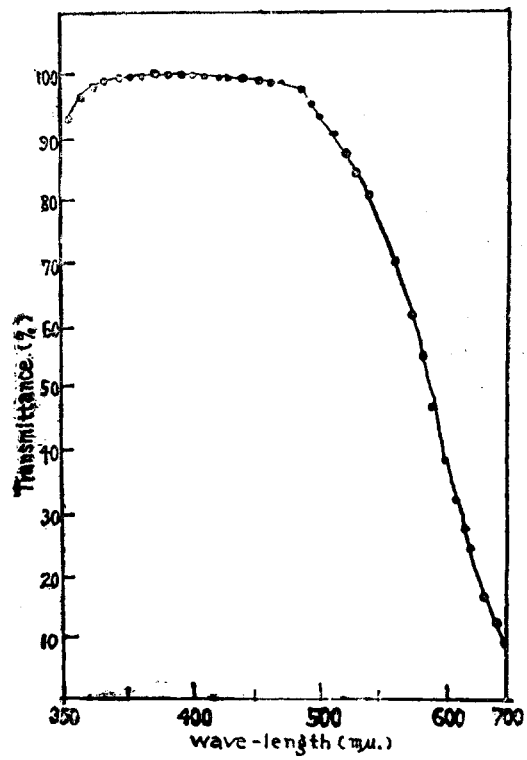

Fig. 1. The light transmittance of 3% $CuSO_4$ Solution.

EXPERIMENT 2:

In order to clarify the photosensitivity of the extracted samples, to the three primary colour lights, three different light filters for blue, green and red were applied to the bleaching of solutions instead of copper sulphate solutions in experiment I. The wave-lengths of maximum light absorp-

tion for the pigment substances were measured before and after the illumination with these filters. Each measurement was of course compared with 2% digitonin blank solution. The solution flashed with a red filter for 20 minutes showed a temperature increase of 5°C and the room temperature was kept at about 15°C during the whole procedure. The light transmittance of the three filters is given in Fig. 2.

RESULTS AND DISCUSSION

The result of experiment 1 were shown in Fig. 3. As shown in Fig. 3, curve I, the spectra of the substances before illumination showed their maximum absorption at 350mμ,, 420mμ, 550mμ, and 590mμ, and curve III, the difference spectrum between curves I and II, indicated its maxima at 390mμ, 427mμ, 550mμ and 595mμ. Based on these results an inference no doubt can be made that the Kamulchi fish may possess almost cone cells in the retina, because of no substances observable at the wavelengths of 520-540mμ, in which the visual violet of other species of fresh water fishes exists. Concerning the photosenitive substances contained in the retinal cone cells, V. Sudnitz[10] first found the cone substances from the dark-abapted tortoises *"Testudo graeca"*, and next to the discovery by Wald[11] of iodopsin (570mμ), Hosoya et al.[12] have succeeded in isolating the similar substances from the Formosan tortoises *"Ocadia sinensis"*, lying at the wave-length of 460mμ, 570mμ and 670-700mμ. Following the recent success of the separation of cone cells by means of the sucrose float method by a group of Japanese workers, Kimura and Hosoya[13], they have discovered that the retinal cells of the Japanese tortoises *"Geoclemys reevesii"* entirely consist of cone cells alone. The pigment substances of these cone cells have been found at the maximum absorptions of 480mμ, 560mμ, and 650mμ. At the same time, Tanabe[14] has described five other maximum absorptions at 460mμ, 510mμ (visual purple), 540mμ, 570mμ and 630mμ from the cone substances of the chicken retinae. Recently Wald[15] has synthesized a new sensitive substance, called cyanopsin by marking a combination of retinen 2 and chicken conopsin.

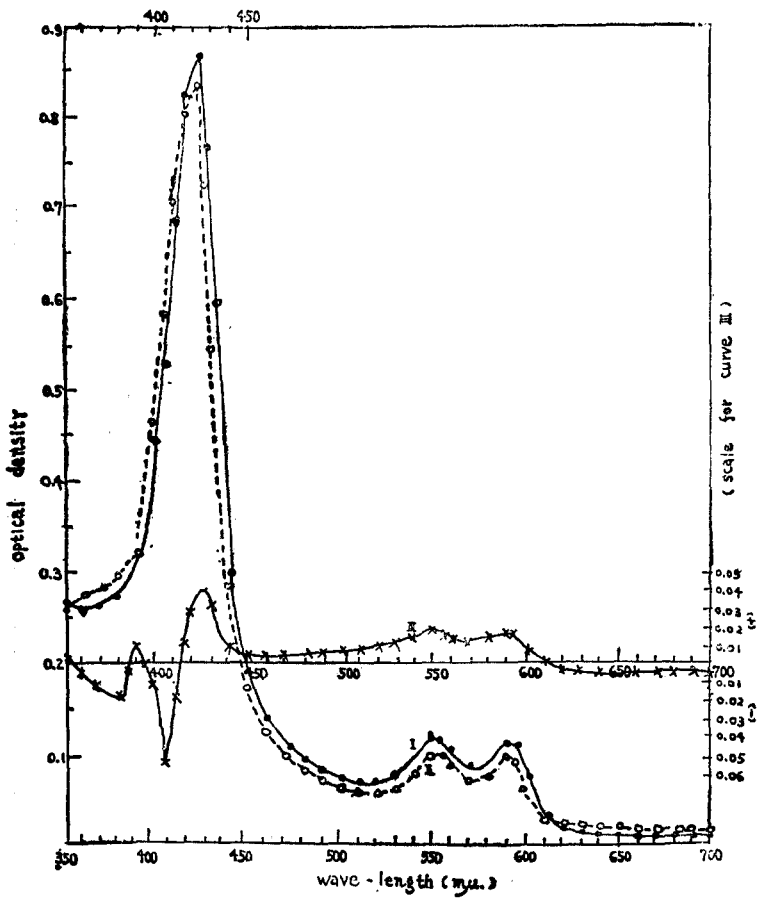

Fig. 3. The absorption spectrum of the extract with 2% digitonin solution from kamulchi retinae.
curve I, the absorption spectrum of the extract.
curve II, the same after 20 minutes exposure to white lamp 200 watts 120 volts.
curve III, absorption difference caused by bleaching.

Discussing my results from the kamulchi fish, it is very interesting

to note that one of the substance at 550mμ is very much in accord with one of the substance found by V. Studnitz[16] from the snake *"Tropidonotus natrix"*, eyes which were isolated with a method of digitonin and other extraction, having three maximum spectra at 468mμ, 550mμ and 655mμ. It is also very interesting to find the same substance in which maximum absorption at 550mμ is present in both animals, the snake and the kamulchi, since both have a very great similarity with each other in skin spots and other ecological points.

The other substance found at the maximum spectrum of 590mμ from the kamulchi was believed photometically to be the same one as that discovered at the same spectrum by Hanstrom[17] from the monkey retinae, which contains several pigments at 460mμ and 590mμ.

In the case of the two other substances which exist at the wave-lengths of 427$\pm$2mμ and 390mμ, they are believed to be new ones which have never been reported before in literature to the best of my knowledge in reviewing the past papers. One may recall the works of Dr. Dartnall[a] and Hartridge[18] about 10 years ago, that is to say, Dr. Dartnall[a] proposed several photosensitive substances to be later found at certain maximum spectra, 427mμ, 450 mμ, 609mμ and 655mμ. Hartridge[18] also made out a tentative table for scotopic substances by adding the following pigments to the table in order to make this complete: visual green (657mμ), visual orange (450mμ), visual yellow No. 1 (427mμ), visual yellow No. 3 (389mμ), etc. It is of particular interest to know that my newly discovered substances of 427mμ and 389mμ are both already suggested as ones awaiting discovery by Dr. Dartnall and Hartridge.

The data obtained by the experiment 2 illustrated in Fig. 4. curve III, the difference spectrum between curves I and II, curve IV, the same between curve I and the exposure to red light. curve V, the same between curve I and the exposure to green light.

The sequence of photosensitivity of the pigment substance found at 390 mμ to the three primary colours was observed to be in an order blue light $>$ red light = green light $>$ white light, while the substance of 427$\pm$2mμ

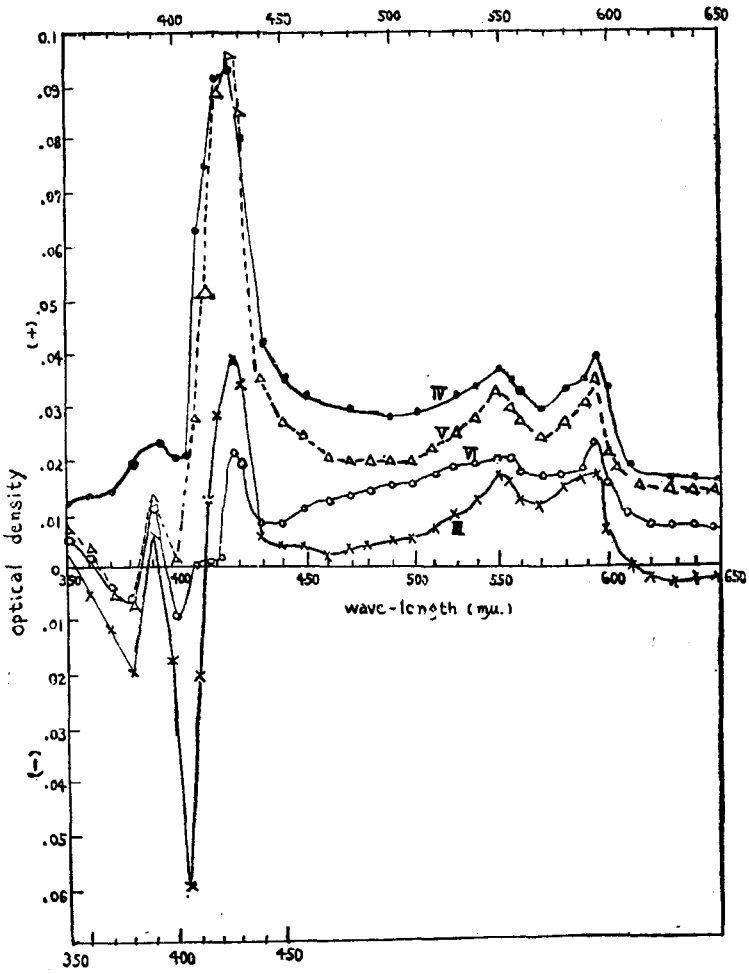

Fig. 4. The order of photosensitivity.
Curve III, the difference between curve I and curve II (Fig. 3.).
Curve IV, the differencer between curve 1 and the exposure to blue light.
Curve V, the same between curve I and the exposure to red light.
Curve VI, the sam (between curve I and the exposure to green ligh

being in a sequence of red>blue>white>green. The substances which exist at 550mμ and 595mμ were found to have a sequence of phototosensitivity as being blue>red>green> white. Of these the strongest sensitivity to light was possessed by the substance of 427mμ, the remaining ones showed more or less the similar sensitivity between each other. The maximum wave-lengths between curves I and III are almost unchangeable at 550mμ but in case of the substance at 590mμ, it appeared to show a slight from the wave-length of 590mμ to the point of 595mμ and thus 420mμ to 427mμ, 350mμ to 390mμ, respectively. These observations revealed the same results with tests for the stock solutions, either pH 6.7 or pH 7.4, and also no change was observed between the tests under dim red ray and dim blue ray of less than 480mμ. There were observed also no changeable results upon the maximum spectra, while the measurement of optical density was performed from the shorter wave-length to the longer side, or vice versa. The same results were also obtained from the two comparable samples extracted under two different temperatures, 5°C and 11°C, Another comparative test was made of two different extracts, one was extracted with 2% digitonin and the other extracted with normal distilled water, pH 6.8, the result showed some difference in the difference spectrum, that is, the maximum spectra of both extracts before the illumination were the same. The difference spectrum of the water extracted one before and after the illumination indicated an extremely short distance as compared with that of 2% digitonin, thus rendered an inapparent difference. The difference spectrum for water extracts is illustrated in Fig. 5.

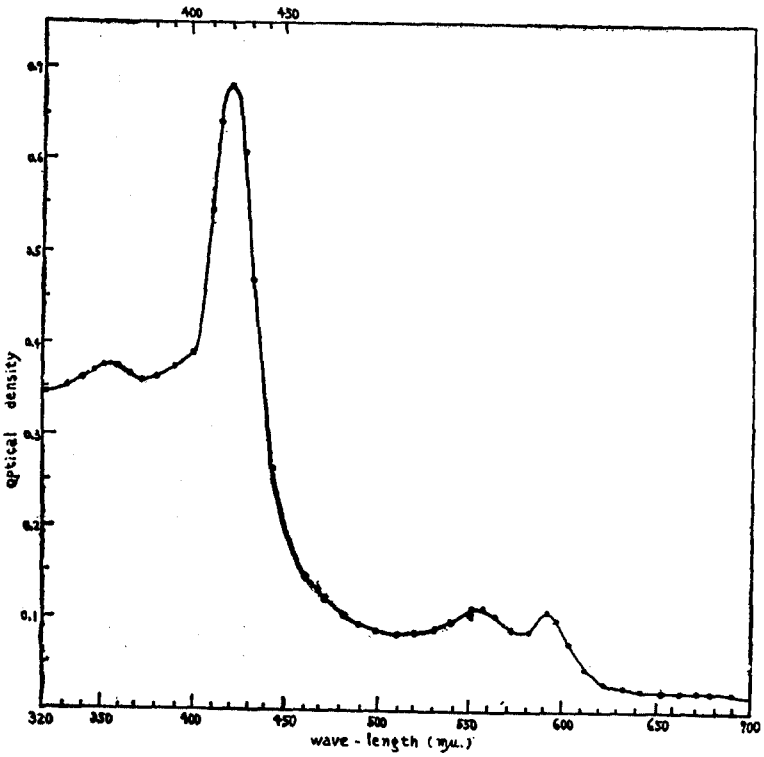

Fig. 5. The absorption sectrum of the extract with distilled water from kamulchi retinae.

SUMMARY

A spectrophotometric analysis was made to photosensitive substances extracted from the Korean kamulchi retina *"Ophicephalus argus"* and the following findings were made.

1. The wave-length of maximum absorption photosensitive substances extracted with 2% digitonin from the dark-adapted kamulchi retina are found at 350mμ. 450mμ, 550mμ and 590mμ. The maximum difference spectrum between the dark-adapted and the exposed kamulchi retinae is 390mμ, 427±2mμ, 550mμ and 595mμ.
2. The order of photosensitivity of these extracted substances to the primary colour was observed as follows:
   (a) The photosensitivity of the substance which exists at 390mμ apears in a sequence of blue light> red ligh' =green light>white light.
   (b) That of the substance at 427mμ appears in a sequence of red>blue>white>green.
   (c) That of both substances at 550mμ and 595mμ were in an order of blue> red> green>white.

3. The substances at which maximum absorptions were present 390mμ and 427mμ are the same substances which have been already proved to exist.

4. The photosubstances of the kamulchi retina can be extracted with distilled water, but the difference spectrum there of was not so distinct as that extracted with 2% digitonin.

5. Despite the fact that the kamulchi fish is one of the fresh water fishes, it does not possess visual violet like the tortoises.

The author wishes to acknowledge his gratitude to Prof. Bong Soo Park and Dr. Dong Zoon Kim for suggestions and encouragement, and also to Miss Kyoung Sook Sin for the operation of DU-Beckmann spectrophotometer.

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＜摘　要＞

가물치 (*Ophicephalus argus*)의 眼球網膜에 存在하는 視覺物質을 spectrophotometry 한 結果 다음과 같다.

(1) 暗順應된 가물치 眼球網膜에서 2% digitonin으로 視覺物質을 抽出하여 光照射前의 吸光 spectrum을 測定한 結果 吸光 maximum은 350mμ, 420mμ, 550mμ, 590mμ에 있었으며 光照射後의 吸光 spectrum과의 difference spectrum의 吸光 maximum은 390mμ, 427±2mμ, 550mμ, 595mμ에 있었다.

(2) 가물치 網膜中의 視覺物質인 390mμ, 427mμ, 550mμ, 595mμ의 三原色光에 대한 感光度는 다음과 같다.

a. 390mμ은 blue light＞red light＝green light＞white light.

b. 427mμ은 red light＞blue light＞white light＞green light.

c. 550mμ과 595mμ은 blue light＞red light＞green light＞white light.

(3) 390mμ과 427mμ에서 吸光 spectrum의 maximum을 가지는 視覺物質은 그 存在를 豫見하던 새로운 色素이다.

(4) 가물치의 視覺物質은 蒸溜水에도 溶出되나 2% digitonin 水溶液으로 抽出하였을 때보다 明確하지 못하였다.

(5) 가물치는 淡水魚임에도 불구하고 거북類와 같이 visual violet는 發見할수가 없었다.