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ΠΡΟΕΔΡΙΑ ΜΑΞ. Κ. ΜΗΤΣΟΠΟΥΛΟΥ

ΑΝΑΚΟΙΝΩΣΕΙΣ ΜΗ ΜΕΛΩΝ

ΒΙΟΧΗΜΕΙΑ.— **A comparative chromatographic studie about the presence of pterins in the eyes of invertebrates and of lower and higher vertebrates** *, *by Anastasios A. Christomanos and Chrysanthi Pavlopulu* **. Ἀνεκοινώθη ὑπὸ τοῦ Ἀκαδημαϊκοῦ κ. Γ. Ἰωακείμογλου.

It was reported some years before that the skin and various organs of the body of different classes of animals contained a serie of Pterinic compounds.

In the last time many papers have been published concerning this field of research, mainly by M. Viscontini and his school (1, 3).

Hadorn and alias (2), could detect the presence of Pterins in the eyes of Drossophila Melanogaster. Hadorn and Mitchell made extensive use of the chromatographic technique for the determination and the identification of the Pterinic compounds on mutants of the fly Dros. Melanogaster. The differences they could detect were striking and they showed relation to the age, the sex and the mutant type. Nevertheless it is to point out that in each time the identity of the observed Pterins by different authors is not quite completely established. Furthemore in many cases

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* ΑΝΑΣΤΑΣΙΟΥ Α. ΧΡΗΣΤΟΜΑΝΟΥ καὶ ΧΡΥΣΑΝΘΗΣ ΠΑΥΛΟΠΟΥΛΟΥ, Συγκριτικὴ χρωματογραφικὴ μελέτη περὶ τῆς παρουσίας πτερινῶν εἰς τοὺς ὀφθαλμοὺς διαφόρων τάξεων ζώων.

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the Pterins, mainly those of the skin, are mixed with other coloured pigments of non Pterinic character.

Our knowledge of the Biochemistry of the Pterins of various animals is unhappily still yet very restricted and the only way to proceed comparatively is the two dimensional chromatography by comparison of the Rf values, the fluorescence in the U. V. light and the spectrophotometric absorption curves.

We thought subsequently, that it would be from great interest to study the precence of Pterins by comparative chromatographie of the eyes of lower and higher vertebrates as it was also reported by Euler and Adler (4), Polonowski and Busnel (5) that Pterins could be detected besides the various organs of fishes, also it their eyes (6, 7).

Experimental technique

The retina, the choroid and the vitreous humor of the eyes of the various animals were, after plucking out the eyes, crushed on an end of a filter paper strip and dried by a stream of air of 40°. The free end of the strip was placed in a little glass jar containing a mixture of Methanol / Pyridin / Butanol / Water (80 : 34 : 30 : 50) in a way like to the descendent chromatography, so that the above solvents, passed through the spots were the eyes have been crushed. Substances that are soluble in the mixture of the solvents moved downwards whith the solvent which dropped and was collected in a small glass beaker. The whole system was put under a good closing cylinder in the darkness, about 24 - 48 hours. After this time the collected solvent, which showed a brownish-yellow color, was concentrated by the rotatory vacuum pump by 37°, to 2 - 3 ml, from whom 0.5 ml. were used each time for a two dimensional chromatography. The first run took place with a solvent mixture of Methanol : Pyridin : Water (160 : 8 : 40). The second run was carried out with a mixture of Butanol : Pyridin : Water (60 : 60 : 60). After the first and the second run the filter paper was examined by the U. V. lamp, and the fluorescent spots were located and marked by a pencil. After cuting them out they were extracted by the Butanol : Pyridin : Water mixture, as previous described. The extract was then examined

spectrophotometricaly for its absorption, by a Beckman automatic spectrophotometer.

The various animals from which the eyes were examined were:

A. Mollusks, (Cephalopoda).
Squid, (Sepia Officinalis).

B. Amphibians.
Frog, (Rana Ridibunda).

C. Fishes, (Pisces).
a. Bass, (Diplodus Vulgaris).
b. Spanish Sea Bream, (Pagellus Erythrinus).
c. Goldfish, (Carassius Auratus).
d. Gildfish, (Sparus Auratus).
e. Red Mullet, (Mullus Surmuletus).
f. Carp, (Cyprinus Carpio).
g. Eel, (Anguilla Anguilla).

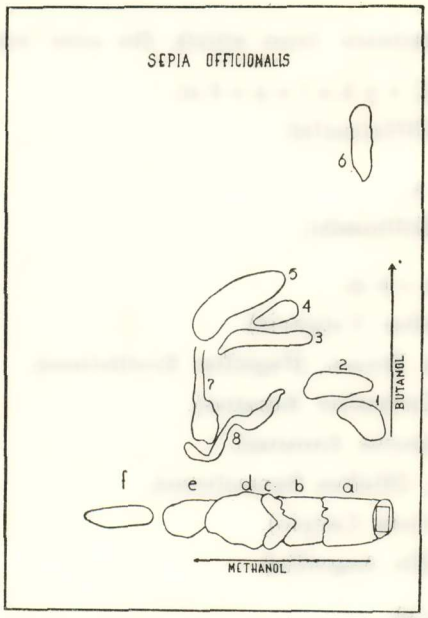
D. Birds, (Aves).
Hen, (Gallus Domesticus).

E. Mammals, (Mammifera).
a. Cat, (Felis Domestica).
b. Sheep, (Ovis Aries).
c. Ox, (Bovis).
d. Man, (Homo).

Results

The results of our researches are depicted by the following pictures 1 - 14. Because of the very small concentration of the extracted spots, in most cases the absorption curves were not clear enough for an exact identification, but anyhow the absorption maxima of the range 350 mμ and 230 mμ, plead for the Pterinic character of the greater number of the fluorescent spots. Theire identity was confirmed by comparing their chromatographic behaviour, with that of authentic samples, in the same solvent systems. Nevertheless, in many cases the identity could not be elucidated, by the fact that spots of the same fluorescence showed very different Rf values.

A. *Sepia Officinalis, Squid.*



Pict. 1.— Bidimensional chromatography of the extract of the eyes of Sepia Officinalis.

A. Color of spots by U. V. after the fist run.

a = orange, b = violet, c = blue, d = greenish, e = violet, f = violet.

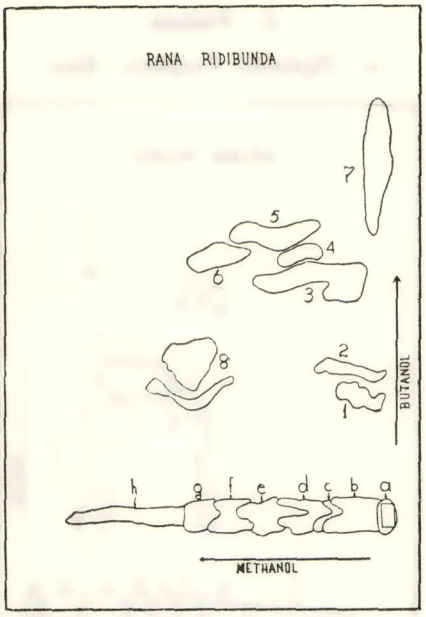
B. Color of spots and Rf after the second run.

1 = orange, Rf Methanol 0.05, Butanol 0.24. Absorpt. Max. 465, 289mμ.
2 = violet, Rf Methanol 0.13, Butanol 0.32
3 = blue, Rf Methanol 0.13, Butanol 0.50
4 = greenish-blue, Rf Methanol 0.27, Butanol 0.50. Absorpt. Max. 350, 289 mμ and Minim. ad 280 mμ.
5 = yellow-greenish, Rf Methanol 0.38, Butanol 0.53. Absorpt. Max. 450, 360 and 290 mμ.
6 = Not fluorescent rose spot, Rf Methanol 0.06, Butanol 0.92
7 and 8 = Spots with white fluorescence, giving a positive reaction for Arginine and Histidine.

Comments: Spot 1 belongs probably to compound relative to Flavinadeninnucleotid. Spot 2 coincides with the Rf of 2-amino-6-hydroxypteridin-8-carbonic acid. Spot 3 and spot 4 represent Isoxanthopterin (8) and Xanthopterin derivatives (9), while spot 5 belongs to Riboflavin, or some relative compound. The nature of spot 6 is unknown. Spots 7 and 8 represent Peptides.

B. Amphibians.

Frog, Rana Ridibunda.



Pict. 2.— Bidimensional chromatography of the extract of the eyes of Rana Ridibunda.

A. Color of spots by U. V. after the first run.

a = orange-yellowish, b = braunish yellow, c = blue, d = violet, e = yellowish white, f = blue, g = violet blue, h = faint rose.

B. Color of spots and Rf after the second run.

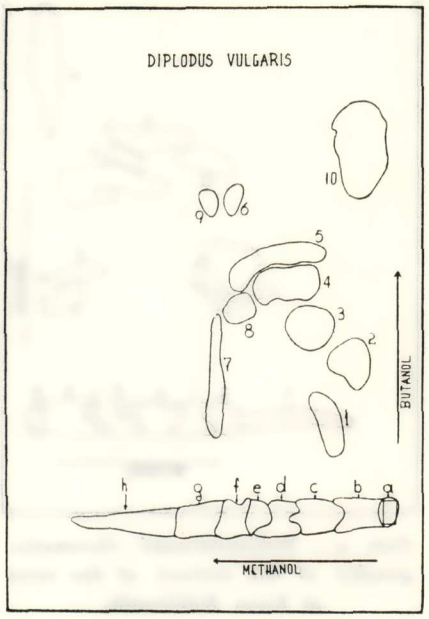
1 = greenish-blue, Rf Methanol 0.07, Butanol 0.27
2 = Pale blue, Rf Methanol 0.1, Butanol 0.35. Absorpt. Max. 290 mμ.
3 = Violet, Rf Methanol 0.17, Butanol 0.57. Absorpt. Max. 340, 260 mμ.
4 = Blue, Rf Methanol 0.2, Butanol 0.63. Absorpt. Max. 285 mμ.
5 = Yellow, Rf Methanol 0.25, Butanol 0.67. Absorpt. Max. 455, 360. 290 mμ.
6 = Blue, Rf Methanol 0.39, Butanol 0.61. Absorpt. Max. 350, 290 mμ.
7 = Not fluorescent braunish red spot, Rf Methanol 0.02, Butanol 0.84
8 = Faint rose fluorescence, spot giving positive reaction for Arginine and Histidine.

Comments: The Rf of spot 1 and the fluorescence coincides with Flavinadeninnucleotide. Spot 2 coincides with 2-amino-6-hydroxypteridin-8-carbonic acid. Spot 3 coincides with Ranachrom 4 or Isoxanthopterin (10), while spot 4 belongs probably to Ranachrom 1 or Pyrrolchrom (11, 12, 13). Spot 5 represents because of his high Rf value

rather Bufo Yellow 2, isolated from toads by Hama and Obika (14), than Riboflavin. Spot 6 represents Ichtyopterin or a relative compound (4, 6a).

C. Fishes.

a. Diplodus Vulgaris, Bass.



Pict. 3.— Bidimensional chromatography of the extract of the eyes of Diplodus Vulgaris.

A. Color of spots by U. V. after the first run.

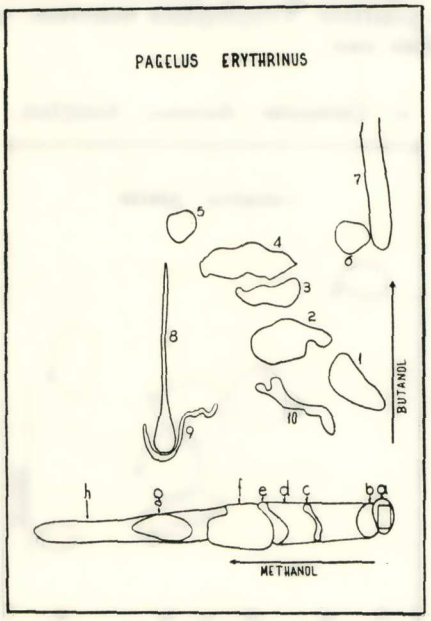
a = orange-rose, b = yellowish-white, c = faint blue, d = faint blue, e = braunish-yellow, f = white, g = yellowish-white, h = pale rose.

B. Color of spots and Rf after the second run.

1 = orange, Rf Methanol 0.14, Butanol 0.21
2 = faint blue, Rf Methanol 0.18, Butanol 0.36
3 = Blue-green, Rf Methanol 0.18, Butanol 0.45. Absorpt. Max. 340, 250 mμ.
4 = Blue, Rf Methanol 0.24, Butanol 0.56. Absorpt. Max. 340, 255 mμ.
5 = Yellow, Rf Methanol 0.28, Butanol 0.62. Absorpt. Max. 465, 370, 290 mμ.
6 = Blue, Rf Methanol 0.37, Butanol 0.76. Absorpt. Max. 360, 255 mμ.
7 = White, Rf Methanol 0.41, Butanol 0.34
8 = Blue, Rf Methanol 0.36, Butanol 0.49
9 = White, Rf Methanol 0.43, Butanol 0.75
10 = Not fluorescent braunish spot.

Comments: Spot 1 belongs to substances relatives to FAD or FMN because their very low Rf, like spot 1 of Sepia Officinalis. Spot 2 coincides with 2-amino-6-hydroxypteridin-8-carbonic acid, while spot 3 represents Isoxanthopterin carbonic acid. Spot 4 belongs probably to Xanthopterin. Spot 5 is represented by Riboflavin or a very relative compound. Spot 6 represents probably Ichtyopterin. Spots 7 and 8 are Peptides which showed positives reactions of Tryptophan and Tyrosine. Spots 9 and 10 have an unknown constitution, and probably are oxidation products of the retinal pigments.

b. Pagellus Erythrinus, Spanish Sea Bream.



Pict. 4.— Bidimensional chromatography of the extract of the eyes of Pagellus Erythrinus.

A. Color of spots by U. V. after the first run.

a = orange-rose, b = blue-greenish, c = yellowish, d = white, e = blue, f = yellow, g = pale violet, h = pale rose-white.

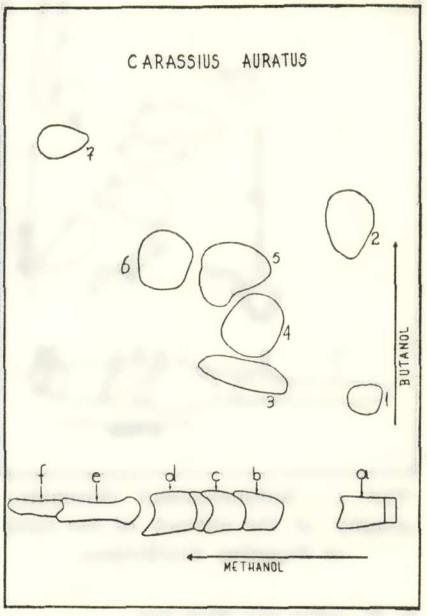
B. Color of spots and Rf after the second run.

1 = Blue-green, Rf Methanol 0.05, Butanol 0.31. Absorpt. Max. 430, 350, 290 mμ.
2 = Blue, Rf Methanol 0.22, Butanol 0.41. Absorpt. Max. 350, 290 mμ.
3 = Blue, Rf Methanol 0.24, Butanol 0.52. Absorpt. Max. 340, 288 mμ.
4 = Yellow, Rf Methanol 0.28, Butanol 0.61. Absorpt. Max. 450, 355, 290 mμ.
5 = Blue, Rf Methanol 0.47, Butanol 0.67. Absorpt. Max. 340, 255 mμ.

6 = Blue, Rf Methanol 0.05, Butanol 0.82
7 = Not fluorescent braunish spot, Rf Methanol 0.02, Butanol 0.82
8 = Rose, Rf Methanol 0.50, Butanol 0.14
9 = rose, Rf Methanol 0.50, Butanol 0.14
10 = Yellowish-white, Rf Methanol 0.19, Butanol 0.26.

Comments: Spot 1 is of unknown constitution, probably a Flavin derivative. Spot 2 belongs to an Isoxanthopterin derivative, whereas spot 3 represents Isoxanthopterin carbonic acid. Spot 4 is represented by Riboflavin. Spot 5 belongs to Ichtyopterin. Spots 6 and 7 are of unknown constitution, the last is not of Pterinic character. Spots 8, 9 and 10 are Peptides giving positive reaction with Ninhydrin. Spot 8 gives a positive Tryptophan reaction, while spot 10 gives a positive Arginine one.

c. Carassius Auratus, Goldfish.



Pict. 5.— Bidimensional chromatography of the extract of the eyes of Carassius Auratus.

A. Color of spots by U. V. after the first run.

a = yellow-greenish, b = blue-greenish, c = violet, d = violet, e = faint violet, f = pale orange.

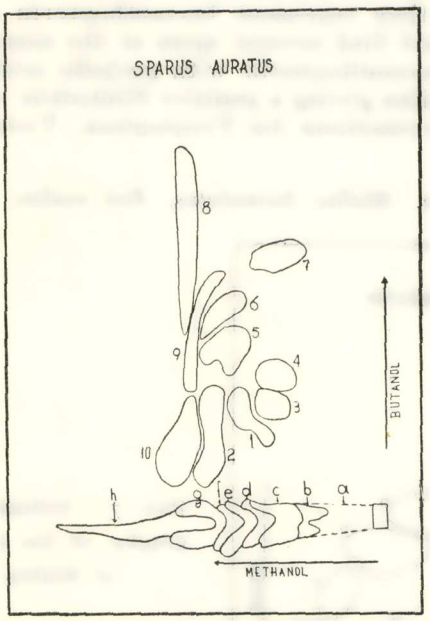
B. Color of spots and Rf after the second run.

1 = Yellow-greenish, Rf Methanol 0.05, Butanol 0.27
2 = Yellow-greenish, Rf Methanol 0.09, Butanol 0.70

3 = Blue pale, Rf Methanol 0.34, Butanol 0.33
4 = Greenish-blue, Rf Methanol 0.32, Butanol 0.46. Absorpt. Max. 410, 290 with a Minim. ad 280 mμ.
5 = Violet, Rf Methanol 0.38, Butanol 0.58. Absorpt. Max. 355, 295 mμ.
6 = Violet, Rf Methanol 0.54, Butanol 0.61. Absorpt. Max. 298 mμ.
7 = Pale Violet, Rf Methanol 0.80, Butanol 0.90. Absorpt. Max. 345, 285 mμ.

Comments: Spots 1 and 2 are of unknown constitution, perhaps they represent Flavin compounds. Spot 3 is represented by Isoxanthopterin, while spot 4 belongs to Isoxanthopterin carbonic acid. Spot 5 is of unknown constitution. Spot 6 represents Ichtyopterin, whereas spot 7 is of unknown constitution.

d. Sparus Auratus, Gildfish.



Pict. 6.— Bidimensional chromatography of the extract of the eyes of Sparus Auratus.

A. Color of spots by U. V. after the first run.

a = Faint yellow, b = pale blue, c = faint blue, d = yellowish, e = faint blue, f = violet, g = yellow, h = faint rose-yellow.

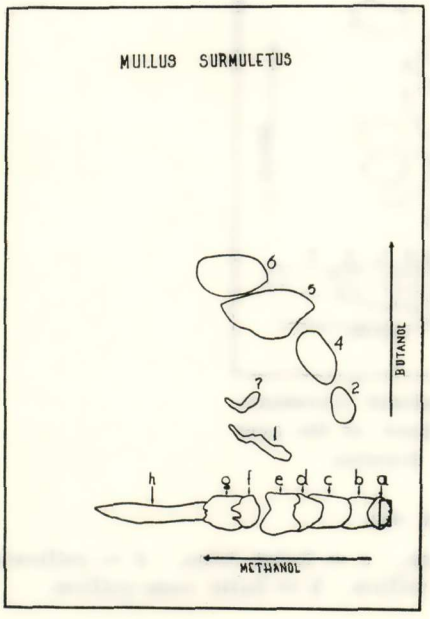
B. Color of spots and Rf after the second run.

1 = Greenish-yellow, Rf Methanol 0.37, Butanol 0.25
2 = Faint blue, Rf Methanol 0.42, Butanol 0.21. Absorpt. Max. 410, 330, 290 mμ.

3 = Violet, Rf Methanol 0.28, Butanol 0.28
4 = Violet, Rf Methanol 0.27, Butanol 0.38
5 = Yellow-greenish, Rf Methanol 0.38, Butanol 0.42
6 = Blue, Rf Methanol 0.39, Butanol 0.51. Absorpt. Max. 290 mμ.
7 = Greenish-yellow, Rf Methanol 0.26, Butanol 0.64. Absorpt. Max. 295 mμ.
8 = Not fluorescent red-yellow spot giving positive Ninhydrin and Tryptophan reaction, Rf Methanol 0.47, Butanol 0.70.
9 = Yellowish not fluoresc. spot giving positive reaction of Ninhydrin and Tyrosine, Rf Methanol 0.46, Butanol 0.47.
10 = Violet, giving positive Ninhydrin and Arginine reaction.

Comments: The identification of the spots was very difficult, because the spots did move very close together, a fact which was probably due to the presence to a large quantity of Peptides. Spot 1, 5 and 7 have the same greenish yellow fluoresence but very different Rf. We think that they represent Isoxanthopterin oxidation products, because we could find several spots of the same fluorescence after oxydation of Isoxanthopterin with perjodic acid. Spots 8, 9 and 10 belongs to Peptides giving a positive Ninhydrin reaction and respectively positives reactions for Tryptophan, Tyrosine and Arginine.

e. Mullus Surmuletus, Red mullet.



Pict. 7.— Bidimensional chromatography of the extract of the eyes of Mullus Surmuletus.

A. Color of spots by U. V. after the first run.

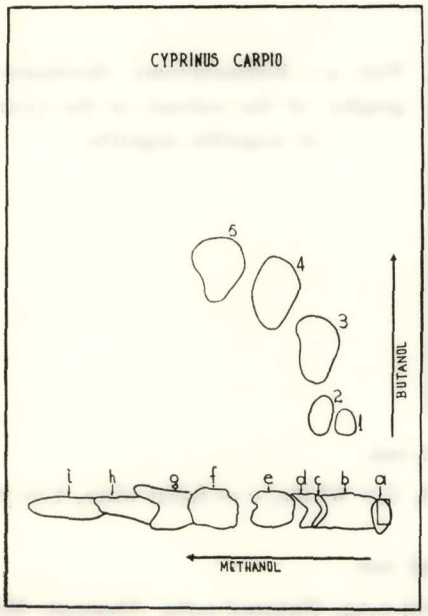
a = orange, b = faint yellowish, c = greenish-blue, d = violet, e = faint violet.

B. Color of spots and Rf after the second run.

1 = Orange, Rf Methanol 0.27, Butanol 0.15. Absorpt. Max. 340, 260 mμ.
2 = Blue, Rf Methanol 0.09, Butanol 0.24.
3 = Blue, Rf Methanol 0.27, Butanol 0.46. Absorpt. Max. 340, 235, 220 mμ.
4 = Yellow, Rf Methanol 0.27, Butanol 0.46. Absorpt. Max. 450 365, 290 mμ.
5 = Blue, Rf Methanol 0.33, Butanol 0.55. Absorpt. Max. 370, 280 mμ.
6 = White, Rf Methanol 0.41, Butanol 0.32.

Comments: Because the Rf values of spot 1 were very unstable, it is uncertain if the orange spot should be regardet as a degradation product of retinal pigments, or as a substance relative to Xanthopterin, according also to the different Absorpt. Spectrum. Spot 2 coincides with 2-amino-6-hydroxypteridin carbonic acid. Spot 3 could be determined as Isoxanthopterin compound according to his Absorpt. Spectrum. Spot 4 shows a very similar Absorption with Riboflavin, or to Xanthopterin B, first isolated by Nawa and Taira (8). Spot 5 is an unknown Pterin which shows very similar Rf to Ichtyopterin. Spot 6 belongs to a Peptide giving a positive Ninhydrin reaction.

f. Cyprinus Carpio, Carp.



Pict. 8.— Bidimensional chromatography of the extract of the eyes of Cyprinus Carpio.

A. Color of spots by U. V. after the first run.

a = orange, b = braunish-yellow, c = faint yellow, d = braunish-

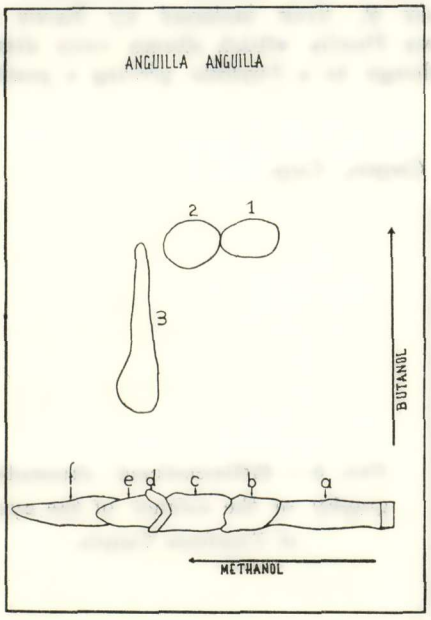
yellow, e = blue, f = violet, g = faint yellow-rose, h = faint violet, i = white.

B. Color of spots and Rf after the second run.

1 = greenish-yellow, Rf Methanol 0.09, Butanol 0.21.
2 = faint blue, Rf Methanol 0.14, Butanol 0.21. Absorpt. Max. 289 mμ.
3 = greenish-yellow, Rf Methanol 0.17, Butanol 0.39. Absorpt. Max. 295 mμ.
4 = Blue, Rf Methanol 0.27, Butanol 0.50. Absorpt. Max. 340, 225 mμ.
5 = Blue, Rf Methanol 0.40, Butanol 0.57.

Comments: The greenish yellow spot 1 is due to FMD. Spot 2 to 2-amino-6-hydroxypteridin-8-carbonic acid, whereas spot 3 represents an Isoxanthopterin oxidation product. Spot 4 is of unknown constitution, whereas spot 5 represents Ichtyopterin (15, 16).

g. Anguilla anguilla, Eel.



Pict. 9.— Bidimensional chromatography of the extract of the eyes of Anguilla anguilla.

A. Color of spots by U. V. after the first run.

a = white, b = yellow, c = violet, d = white, e = faint blue, f = yellowish-white.

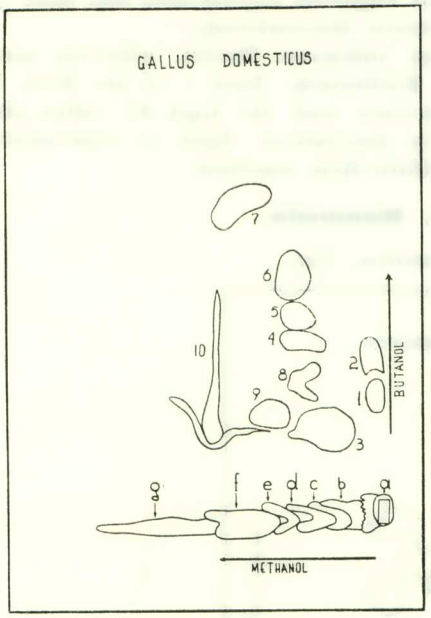
B. Color of spots and Rf after the second run.

1 = Yellow-greenish, Rf Methanol 0.52, Butanol 0.63. Absorpt. Max. 410, 340, 280 mμ.
2 = Violet, Rf Methanol 0.54, Butanol 0.62. Absorpt. Max. 295 mμ.
3 = Violet, Rf Methanol 0.57, Butanol 0.42.

Comments: The poorness of the number of the fluorescent spots is very impressive. The Yellow-greenish spot 1 has a very similar absorpt. Spectrum to Riboflavin. Spot 2 coincides under the same circumstances with Ichtyopterin, isolated from fish scales. Nevertheless it should be pointed out that the isolated Ichtyopterin show very variable Rf. The fluorescent spot 3 is due to the simultaneous presence of Tryptophan and Tyrosine, giving a positive Ninhydrin reaction. So it can be regardet as a Peptide.

D. Aves, Birds.

Gallus domesticus, Hen.



Pict. 10.— Bidimensional chromatography of the extract of the eyes of Gallus domesticus.

A. Color of spots by U. V. after the first run.

a = rose, b = yellow, c = blue, d = violet, e = faint violet, f = greenish yellow, g = faint violet.

B. Color of spots and Rf after the second run.

1 = Yellow, Rf Methanol 0.02, Butanol 0.30
2 = Yellow, Rf Methanol 0.03, Butanol 0.40
3 = Blue, Rf Methanol 0.14, Butanol 0.23
4 = very pale blue, Rf Methanol 0.19, Butanol 0.45
5 = Violet, Rf Methanol 0.20, Butanol 0.51
6 = Blue, Rf Methanol 0.21, Butanol 0.60
7 = Yellow-greenish, Rf 0.32, Butanol 0.80. Absorpt. Max. 350, 285 mμ.
8 = White, Rf Methanol 0.20, Butanol 0.34
9 = Blue, Rf Methanol 0.27, Butanol 0.27
10 = Not fluorescent redish spot, Rf Methanol 0.40, Butanol 0.31.

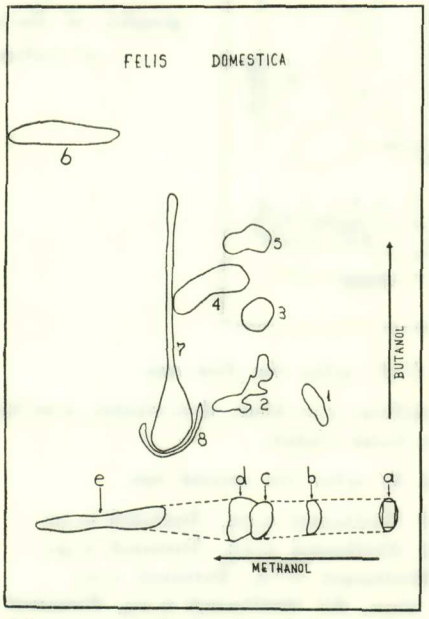
Comments: From the first sight it was non unexpected, that the Rf of the spots of the extracts of the eyes of the Hen were very different in relation to those of the fishes. That a great number of spots was of Pterinic character, was proved by their Absorption which showed Maxima between 350 - 240 mμ. We like to state that only some of the fluorescent spots had nearly the same fluorescent color and at the same time the same Rf like certain spots of the fish eyes, and that without any presupposition of their identity.

For exemple : Spot 1 of the Hen coincides nearly with spot 1 of Pagellus Erythrinus and 1 of Carassius Auratus, spot 2 of the Hen with spot 2 of Cyprinus Carpio. Spot 4 of the Hen coincides with spot 3 of Diplodus Vulgaris (probably Isoxanthopterinic carbonic acid) and further with spot 2 of Pagellus Erythrinus and spot 3 of Mullus Surmuletus (Isoxanthopterin derivatives).

Spot 5 of the Hen represents an unknown Pterin, whereas spot 6 coincides with spot 4 of Rana Ridibunda. Spot 7 of the Hen, because for his yellowish fluorescence and the high Rf value plead for a Riboflavin or Sepiapterin derivative. Spot 10 represents a Peptide which shows positive Ninhydrin reaction.

E. Mammals, Mammalia.

a. Felis Domestica, Cat.



Pict. 11.— Bidimensional chromatography of the extract of the eyes of Felis domestica.

A. Color of spots by U. V. after the first run.

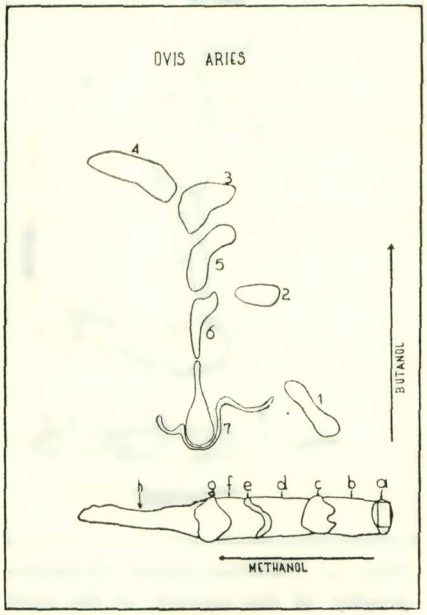
a = faint rose, b = faint yellowish-white, c = faint yellowish-white, d = pale blue-white, e = violet.

B. Color of spots and Rf after the second run.

1 = very pale blue, Rf Methanol 0.17, Butanol 0.26
2 = pale white, Rf Methanol 0.34, Butanol 0.30
3 = Yellowish, Rf Methanol 0.32, Butanol 0.49
4 = very pale blue, Rf Methanol 0.44, Butanol 0.55
5 = faint yellowish-white, Rf Methanol 0.33, Butanol 0.67
6 = Violet, Rf Methanol 0.80, Butanol 0.93
7 = White, Rf Methanol 0.48, Butanol 0.49
8 = White, Rf Methanol 0.48, Butanol 0.26.

Comments: The fluorescence color and the Rf of spot 1 of the Cat coincides with spot 3 of the Hen (Isoxanthopterinic carbonic acid). The constitution of the other spots is of unknown character, with very different and very high Rf values. Spot 8 and 7 coincide with Guanin. Spot 4 moves and shows the same Rf as Riboflavin. Spot 6 is probably a Pterinic compound of unknown constitution.

b. Ovis Aries, Sheep.



Pict. 12.— Bidimensional chromatography of the extract of the eyes of Ovis aries.

A. Color of spots by U. V. after the first run.

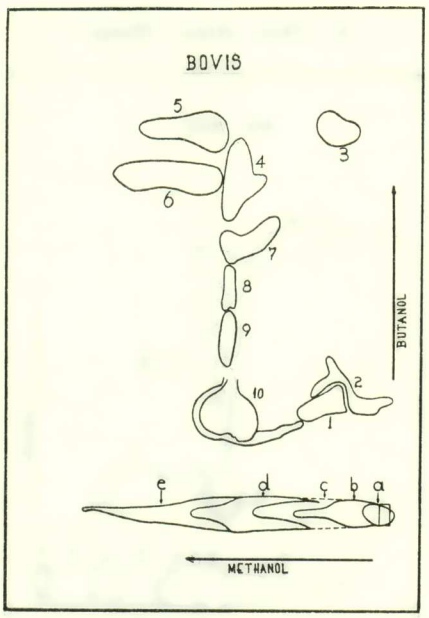
a = very faint rose, b = braunish, c = faint blue, d = braunish. e = white, f = violet, g and h = very faint violet.

B. Color of spots and Rf after the second run.

1 = pale blue, Rf Methanol 0.18, Butanol 0.23
2 = White, Rf Methanol 0.30, Butanol 0.54
3 = Yellowish-greenish, Rf Methanol 0.49, Butanol 0.81
4 = Pale violet, Rf Methanol 0.69, Butanol 0.80
5 = very faint violet, Rf Methanol 0.48, Butanol 0.67
6 = Jellowish-white, Rf Methanol 0.54, Butanol 0.35
7 = White-rose, Rf Methanol 0.45-0.40, Butanol 0.16

Comments: Spot 1 coincides with the Rf of Isoxanthopterinic carbonic acid. Spot 2 is probably a Peptide giving positive Ninhydrin reaction. Spot 3 is represented by Riboflavin, whereas spot 4 with the absorption maxim. at 345 and 280 mμ is probably a Pterinic compound of unknown constitution. Spots 5, 6 and 7 represent Peptides. They give positives reactions with Ninhydrin, especially spot 6 gives a positive Tyrosine and Tryptophan reaction.

c. Bovis, Ox.



Pict. 13.— Bidimensional chromatography of the extract of the eyes of Bovis.

A. Color of spots by U. V. after the first run.

a = yellowish-braunish, b = yellowish-white, c = white, d = violet.

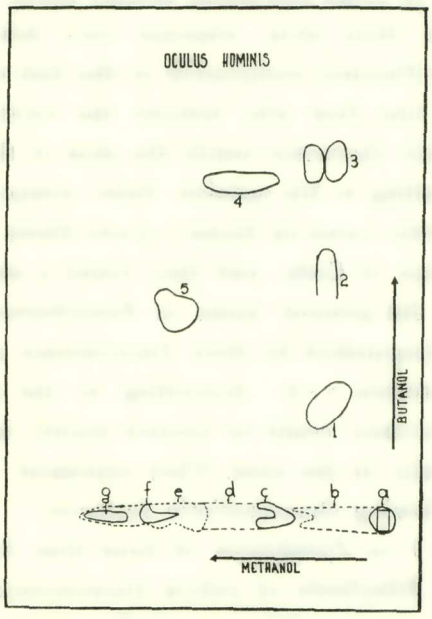
B. Color of spots and Rf after the second run.

1 = faint blue, Rf Methanol 0.16, Butanol 0.25
2 = Orange-yellowish, Rf Methanol 0.09, Butanol 0.27

3 = Yellow-greenish, Rf Methanol 0.15, Butanol 0.91
4 = Yellow, Rf Methanol 0.38, Butanol 0.81
5 = Violet, Rf Methanol 0.5, Butanol 0.92
6 = faint violet, Rf Methanol 0.53, Butanol 0.82
7 = faint violet, Rf Methanol 0.34, Butanol 0.65
8 = Yellowish-white, Rf Methanol 0.39, Butanol 0.42
9 = very faint violet, Rf Methanol 0.39, Butanol 0.42
10 = Yellowish-red, Rf Methanol 0.39, Butanol 0.24

Comments: Spot 1 coincides with spot 1 of the Sheep, which is represented by Isoxanthopterinic carbonic acid. The orange-yellowish color of spot 2 coincides with the Rf of a Flavinadeninnucleotid. All other spots showed a very high Rf Butanol values which coincides in some way with those of the Sheep, but are of unknown constitution, probably oxidation products of Retinal pigments. Spots 8, 9 and 10 are represented by Peptides which give positive Ninhydrin reaction. It is noteworthy to mention that Euler and coworkers (6b) noticed the presence of Ichtyopterin or a very similar pigment in the retina of the ox. It is probable that spot 7 of our chromatography is represented by Ichtyopterin.

d. Oculus Hominis, Man.



Pict. 14.— Bidimensional chromatography of the extract of the eyes of Man.

A. Color of spots by U.V. after the first run.

a = faint orange, b = faint white, c = rose-white, d = white,

e = faint blue, f = black spot without fluoresence, g = black spot whithout fluorescence.

B. Color of spots and Rf after the second run.

1 = faint rose, Rf Methanol 0.2, Butanol 0.38
2 = Not fluorescent braunish spot, Rf Methanol 0.2, Butanol 0.72
3 = Pale orange, Rf Methanol 0.19, Butanol 0.95
4 = White-rose, Rf Methanol 0.4, Butanol 0.89
5 = Violet, Rf Methanol 0.55, Butanol 0.61.

Comments: The only resemblance between the fluorescent spots of the extract of the eyes of Man and the above examined mammals, could be determined between spots 3 of Man and Ox, and also between spots 5 of Man and Sheep which are both Peptides.

DISCUSSION

There are no avalaible references about the Pterinic pigments of the eyes of the squid, on the contrary several papers have been published about the pigments of frogs and toads, mainly about the fluorescent Pterinic compounds of their skin, whereas very few papers have been published about the Pterinic compounds of the fish eyes (17).

Coda (11) was the first who noticed the existence of fluorescent compounds of Pterinic character inside the skin of the frog species *Rana Nigromaculata.* According to his opinion these compounds posses an unknown biochemical role. Later on Hama (13) and Hama and Obika (14) could confirme the findings of Coda, and they found a number of fluorescent pigments to which the general name of *Ranachromes* was given. These pigments were distinguished by their fluorescence color and the different Rf, as Ranachromes 1 - 6. According to the previous mentioned authors, the Ranachromes could be located mainly in the skin, but were found also occasionaly in the eyes. They represent Pterinic compounds and exhibit the following characteristic features:

a. *Ranachrome 1* or *Pyrrolchrom* of faint blue fluorescence, Rf 0.46, b. *Ranachrome 2* or *Riboflavin* of yellow fluorescence, Rf 0.43, c. *Ranachrome 3* of blue fluoresence, Rf 0.40, d. *Ranachrome 4* which represents a pterinic compound similar or related to *Ichtyopterine* of violet fluorescence and Rf 0.33, e. *Ranachrome 4B* has a blue fluorescence and a Rf of 0.24, *Ranachrome 5* which is very probably 2-amino-6-hydroxypteridine-8-carboxylic acid, and exhibits a blue fluorescence showing a Rf of 0.22,

f. *Ranachrome 6* of yellow fluorescence and a Rf of 0.06, which is after Hama and Obika (14) Flavine-adenine-dinucleotide or Flavine-adenine-mononucleotide. It was supported by Hama (13) that the Pterinic compounds are partly free and partly combined with proteins in the skin and in the eyes. The rupture of the Pterinic-protein bond could be performed through the action of light, or by chemicals, or by oxidation, therefore this rupture could be related ad least in the eyes, to the mechanism of the Biochemistry of the sight.

Hama and Obika could confirme, experimenting on different species of frogs and toads, as Bufo Vulgaris and Bufo bufo, all previous researches, and furthermore they successed to isolate from the skin of toads, larges quantitys of Ranachrome 1 and Isoxanthopterin and they confirmed also the presence of other three different yellow fluorescent pigments, probably of Pterinic character, which were called *Bufo yellow 1 and 2*, and *Rhacophore-jaune*, which is probably the same with the yellow pigment isolated from the sepia mutant of Drossophila Melanogaster, studied the first time by Forrest and Mitchell (18), and which represents according to this authors the N^{10}-lactoyl-9-10-dihydro-2-amino-6-hydroxypteridin-8-carboxylic acid, called also *Sepiapterin* (7).

In our chromatographic studies on the frog Rana Ridibunda, we could detect the presence of Ranachrome 1 and 2, as well as of a yellow Pteridin, which because of its very high Rf, looks rather like the Bufo yellow 2. But considering that our chromatographic solvents, (Methanol: Pyridin: Butanol: Water), were of a different composition in relation to the solvents used by Hama and Obika, and since the chromatograms with pure Riboflavin showed the same Rf as we observed, we rather incline for the presence of Riboflavin in the extract of the eyes of the frog Rana Ridibunda.

Considering the presence and isolation of Pterinic compounds in fish, we have to mention that Euler reported in addition to Riboflavin, of another blue fluorescent compound in the fish eyes.

Hadjioloff (19) published in 1932 a list of different fishes which showed a blue and a green fluorescence of their skin. Hüttel and Sprengling (20) supported that the blue fluorescent spots were due to Ichtyopterin or Fluorescianin. Later it was prooved by Polonowski and Busnel (5), and also by Fontaine and his collaborators (6, 21), that the blue

and green Pterinic compounds found by Hadjioloff could be separated chromatographicaly in a greater number of different fluorescent compounds. We could confirme this findings by our experiments. Therefore the blue and violet fluorescent substances as we know today from researches of Viscontini and his coworkers (1), is due to compounds deriving mainly from Xanthopterin and Isoxanthopterin, and especially from their oxidation products. The separation of fish species on the basis of the blue or green fluorescence of their skin, according to Hadjioloff (19), can not be taken as a right one, because in most of the species which we examined, blue and green respectively green-yellow fluorescence were found at the same time. The fact the fluorescent color depends upon the Ph of the solvent, is already known, but in our case the Ph of the solvent remained the same (Ph=7), therefore the different color of the chromatographicaly detected spots was due to different Pterinic compounds, a supposition which was strengthend by the different Rf's.

As it is evident from our above results, we found in many cases, for exemple in Pagellus Erythrinus, four blue fluorescent spots with different Rf, one blue-green fluorescent, as well as another of a yellow fluorescence. Therefore we have to do with different Pterinic compounds on the same species.

In most of the examined fishes, we could detect (with increasing Rf), Flavinadeninnucleotides, 2-amino-6-hydroxypteridin-8-carbonic acid, Isoxanthopterin, Ichtyopterin, Riboflavin, Sepiapterin and a serie of other blue and green-yellowish fluorescent pigments of unknown constitution. The fact, that most of the fluorescent spots were of Pterinic character, was shown by their absorption curves, which showed Maxima from 350 to 230 mμ. In some fishes we found yellow fluorescent pigments not showing the features of Riboflavin, for exemple Sparus Auratus, Cyprinus Carpio, but we found them also in the eyes of birds, like the Hen, and in Mammals like the cat and the ox.

On the other hand it is worthy to state, that in mammals and especially in the eyes of Man the blue fluorescence becomes weaker, till to complete lack for Man. It is biologicaly very interesting that the fluorescent compounds decrease in stricte relation, as we progress from the lower to the higher vertebrates.

It is also important that the two kinds of fresh water fishes we

have examined, namely Carassius Auratus and Cyprinus Carpio, did not contain Riboflavin in their eyes.

The biological significance of the presence of Pterins in the eyes is still unknown. Possibly the Pterins having a similar composition to Folic acid, play partly combined with Protein, a role as coenzymes. Their Proteidic character was already mentioned by Fontaine (6) and Hüttel and Sprengling (20), who found Pterinic-Protein complexes in the skin of fish. It is very probable that similar complexes existe also in the retina of the eye. In our researches because of the extraction methods, and especially because by drying the extracts by an air stream of 40° bevor their chromatographic separation, it is very probable that oxidation occurs.

The presence of Pterinic compounds in the eyes as well as of Riboflavin, is not occasional but is undoubtedly related in some way to the biochemical mechanisme of the sight, especially in lower vertebrates. Blair and Graham (22) observed by chromatography of the extract of the skin of the snake Dendroaspis Viridis among other fluorescent Pteridins, not fluorescent yellow pigments. We could also find yellowish and red-braunish pigments in the extracts of the eyes, of the squid, frog, Pagellus Erythrinus, Sparus Auratus, and Gallus Domesticus.

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SUMMARY

The precence of fluorescent Pterinic compounds in the extracts of the eyes of different animals was proved by two dimensional chromatography. Between the fluorescent compounds predominate mainly the blue fluerescent Pterins. Isoxanthopterin, 2-amino-6-hydroxypteridin-8-carboxylic acid, Ichtyopterin, Biopterin, Sepiapterin and other fluorescent oxidation products of Xanthopterin and Isoxanthopterin could be identified. We detected also Pterins with a green-yellowish to orange fluorescence of unknown constitution. An intense yellow fluorescence is given from Riboflavin, which was found only in salt water fishes. The presence of fluorescent substances in the eyes is getting less, as we proceed from the lower to the higher vertebrates. There is a deficiency of Pterins in the eyes of Man. We would like to suggest that the Pterinic-Protein complex plays up to now an unknown role in the Biochemistry of the sight.

ΠΕΡΙΛΗΨΙΣ

Διὰ διδιαστάτου χρωματογραφίας τοῦ ἐκχυλίσματος τῶν ὀφθαλμῶν διαφόρων τάξεων ζῴων, διεπιστώθη ἡ παρουσία πτερινικῶν φθοριζουσῶν ἑνώσεων ἐντὸς αὐτῶν. Κυρίως ἐπικρατοῦν αἱ κυανῶς φθορίζουσαι πτερῖναι, εἰς τὰς ὁποίας συγκαταλέγονται ἡ ἰσοξανθοπτερίνη, τὸ 2-αμινο-6-υδροξυπτεριδινο-8-καρβονικὸν ὀξύ, ἡ ἰχθυοπτερίνη, ἡ βιοπτερίνη καὶ ἄλλαι πτερινικαὶ ἑνώσεις, πιθανῶς προϊόντα ὀξειδώσεως τῆς ξανθοπτερίνης καὶ ἰσοξανθοπτερίνης. Πλὴν τούτων ἐνυπάρχουν πτερῖναι πρασινοκιτρίνου μέχρι πορτοκαλεοχρόου φθορισμοῦ, ὡς καὶ τοιαῦται κιτρίνου, ὡς π. χ. ἡ σεπιαπτερίνη καὶ ἄλλαι ἔτι ἀγνώστου δομῆς. Ἔντονον κίτρινον φθορισμὸν δεικνύει ἐπίσης καὶ ἡ ριβοφλαβίνη, ἥτις ἀνευρίσκεται κυρίως εἰς τοὺς ὀφθαλμοὺς τῶν ἰχθύων τῶν ἁλμυρῶν ὑδάτων, ἀλλὰ καὶ εἰς τὸν βάτραχον, μετ' ἄλλων κιτρίνων χρωστικῶν. *Ἡ παρουσία τῶν πτερινῶν ἐντὸς τῶν ὀφθαλμῶν βαίνει ἐλαττουμένη ὡς ἀνερχόμεθα ἐκ τῶν κατωτέρων πρὸς τὰ ἀνώτερα σπονδυλωτά, ἐλλείπουσα τελείως ἀπὸ τὸν ὀφθαλμὸν τοῦ ἀνθρώπου.* Ἐκφράζεται ἐνταῦθα ἡ ἄποψις ὅτι αἱ πτερῖναι εὑρηνται ἡνωμέναι ἐντὸς τῶν ὀφθαλμῶν πρὸς πρωτεΐνην, ἀποτελοῦσαι πιθανῶς συνένζυμον ὁλοφυράματος ἔχοντος σχέσιν πρὸς τὴν βιοχημείαν τῆς ὁράσεως. Τὴν ἄποψιν ταύτην ἔχουν ὑποστηρίξει καὶ ἕτεροι ἐρευνηταί.

Ὁ Ἀκαδημαϊκὸς κ. **Ἰω. Χαραμῆς,** μετὰ τὴν ὡς ἄνω ἀνακοίνωσιν, προέβη εἰς τὰ ἑξῆς περὶ αὐτῆς σχόλια :

Εἰς ἕνια ἔντομα, ὡς ἡ Drosophila Melanogaster, παρετηρήθησαν μεταλλάξεις διακριθεῖσαι ἐκ τοῦ χρώματος τῶν ὀφθαλμῶν των.

Οἱ ἀσχολούμενοι μὲ τὴν Γενετικὴν καὶ οἱ Βιοχημικοὶ ἐν συνεργασίᾳ διεπίστωσαν, ὅτι αἱ προκαλοῦσαι τὰς χρωστικὰς ταύτας μεταλλάξεις εἶναι αἱ πτεριδῖναι, τῶν ὁποίων ἡ χημικὴ ὑπόστασις ἔχει ἤδη καθορισθῆ.

Εἰς τὰ ἔντομα αὐτὰ αἱ πτεριδῖναι ἀνευρέθησαν καὶ εἰς ἄλλους ἱστοὺς πλὴν τοῦ ὀφθαλμοῦ. Καθωρίσθη δὲ ἐν συνεχείᾳ ὅτι ἡ σύνθεσις ὁμάδος πτερινῶν εὑρίσκεται ὑπὸ ὡρισμένον γεννητικὸν ἔλεγχον.

Ἀπαρχὴν εἰς τὴν κατεύθυνσιν αὐτὴν ἔδωσεν εἰς Η.Π.Α. ὁ MITCHELL καὶ οἱ συνεργάται του, ἀπὸ τοῦ ἔτους 1950.

Παρ' ἡμῖν ὑπάρχει διεξοδικὴ ἐναίσιμος διδακτορικὴ διατριβὴ τοῦ κ. Ν. Κοκκόλη, συνεργάτου τοῦ Καθηγητοῦ κ. Πανταζῆ.

Τὰς λεπτομερείας ἀπὸ πλευρᾶς καθαρῶς χημικῆς καὶ βιολογικῆς, τῆς ὁμάδος τῶν πτεριδινῶν καὶ τῆς ἀποστολῆς αὐτῶν, ἰδίᾳ τοῦ τύπου τῶν φθοριζουσῶν ἑνώσεων, ἐρευνοῦν εἰσέτι οἱ βιολόγοι καὶ γενετικοί.

Συγχαίρομεν τὸν Καθηγητὴν κ. Χρηστομάνον καὶ τοὺς συνεργάτας του διὰ τὴν λίαν ἐνδιαφέρουσαν ἐρευνητικὴν ἐργασίαν των.