

## THE ORGANIC CONSTITUENTS OF THE SCALES OF FISH.

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Mörner<sup>1</sup> has shown that the scales of many species of fish contain, in addition to mineral matter and collagen, a peculiar albuminoid. To this albuminoid he gave the name *ichthylepidin*. Previous observers had overlooked this substance and had considered that the organic matter in fish scales consisted wholly of collagen. The present study was undertaken to determine how widely ichthylepidin is distributed in the scales of the common American fishes.

Mörner prepared his ichthylepidin in the following way: The clean scales were digested at room temperature with a large excess of 0.5 per cent hydrochloric acid, 0.05 per cent caustic potash, and 0.01 per cent acetic acid. Each digestion extended over several days. This treatment removed soluble proteids, most of the guanin, the chondroitin-sulphuric acid, and the inorganic matter. The residual scales were then digested with 0.1 per cent hydrochloric acid at 40° C. The residue thus freed from collagen was washed with alcohol and ether, and dried. The substance so obtained (pure ichthylepidin) was insoluble in boiling water, in cold dilute acids, and in alkalies; but it was soluble in hot solutions both of dilute acids and alkalies, and in the cold concentrated solutions of the same. It gave a strong Millon's reaction and contained much loosely combined sulphur (as shown by the blackening of the substance when boiled with an alkaline solution of lead acetate). By the two latter reactions the presence of ichthylepidin may, according to Mörner, be determined in fish scales.<sup>2</sup>

Mörner examined the scales of 12 species of fish, all of which showed the presence of ichthylepidin; in the ganoid scales of the American gar-pike (*Lepisosteus osseus*), however, he found no trace of it.

In the course of our work we have studied 40 species of American fish representing 25 families. In every case the scales were taken from fresh, healthy fish, and,

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<sup>1</sup>Zeit. f. phys. Chemie, 1898, vol. 24, p. 125.

<sup>2</sup>Mörner, op. cit., p. 136: "Wenn man untersuchen will, ob die Schuppen einer Fischart ichthylepidinhaltig sind oder nicht, genügt es, sie nach vorgängiger Entkalkung durch Kochen, theils mit Millonschen Reagens, theils mit alkalischer Bleilösung zu prüfen; wenn dabei tief dunkelrothe Färbung, resp. Schwarzfärbung ausbleibt, ist die Gegenwart des ichthylepidins ausgeschlossen."

after being carefully freed from adhering tissue, were thoroughly scrubbed with water and washed by decantation until the water remained perfectly clear. The clean scales were then twice extracted with a large excess of 0.5 per cent hydrochloric acid (real HCl), each extraction lasting 12 hours. The scales were then washed with water until free from chlorides and finally tested for ichthylepidin as mentioned above. In some cases, where the scales could not be removed mechanically, the integument was dissolved by dilute (5 per cent) sodium hydroxide, and the scales so separated were then treated as above described. Mörner found ichthylepidin to be unchanged after several days' exposure to 5 per cent sodium hydroxide. We confirmed this fact by treating some menhaden scales with the alkali for three days, after which the presence of ichthylepidin was detected as readily as before. It was found necessary to treat the integument of all the elasmobranchs, the sun-fish (*Mola mola*), the puffer (*Spheroides maculatus*), and the remora (*Echeneis naucrates*) with 5 per cent alkali.

The scales of the following fish gave positive reactions for ichthylepidin when treated as described above:

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|--|--|
| 1. Winter flounder ( <i>Pseudopleuronectes americanus</i> ). | 17. Squeteague ( <i>Cynoscion regalis</i> ).           |
| 2. Alewife ( <i>Pomolobus pseudoharengus</i> ).              | 18. Bonito ( <i>Sarda sarda</i> ).                     |
| 3. Blue-fish ( <i>Pomatomus saltatrix</i> ).                 | 19. Scup ( <i>Stenotomus chrysops</i> ).               |
| 4. Hickory shad ( <i>Pomolobus mediocris</i> ).              | 20. Hake ( <i>Phycis chuss</i> ).                      |
| 5. Chogset ( <i>Tautoglabrus adspersus</i> ).                | 21. Sea bass ( <i>Centropristes striatus</i> ).        |
| 6. Butter-fish ( <i>Rhombus triacanthus</i> ).               | 22. Silverside ( <i>Menidia notata</i> ).              |
| 7. Tarpon ( <i>Tarpon atlanticus</i> ).                      | 23. Striped minnow ( <i>Fundulus majalis</i> ).        |
| 8. Mackerel scad ( <i>Decapterus macarellus</i> ).           | 24. Tautog ( <i>Tautoga onitis</i> ).                  |
| 9. Sea robin ( <i>Prionotus carolinus</i> ).                 | 25. Mummichog ( <i>Fundulus heteroclitus</i> ).        |
| 10. Salmon ( <i>Salmo salar</i> ).                           | 26. Remora ( <i>Echeneis naucrates</i> ).              |
| 11. Cod ( <i>Gadus callarias</i> ).                          | 27. Rudder-fish ( <i>Palinurichthys perciformis</i> ). |
| 12. Haddock ( <i>Melanogrammus aeglefinus</i> ).             | 28. Common shad ( <i>Alosa sapidissima</i> ).          |
| 13. Crevallé ( <i>Caranx crysos</i> ).                       | 29. Sturgeon ( <i>Acipenser sturio</i> ).              |
| 14. Summer flounder ( <i>Paralichthys dentatus</i> ).        | 30. Perch ( <i>Morone americanus</i> ).                |
| 15. Menhaden ( <i>Brevoortia tyrannus</i> ).                 | 31. Jumping mullet ( <i>Mugil cephalus</i> ).          |
| 16. Tile-fish ( <i>Lopholatilus chamaeleonticeps</i> ).      | 32. King-fish ( <i>Menticirrhus saxatilis</i> ).       |
|  | 33. Broad minnow ( <i>Cyprinodon variegatus</i> ).     |

In all the above cases the reactions given by Millon's reagent were very marked, the red coloration being brilliant. The darkening caused by warming with the lead-alkali solution was very variable in intensity. This reaction was strong in Nos. 3, 11, 20, 21, 22, and 29 of the above list, very faint in Nos. 1, 14, 15, 27, and 28, and in the other cases ranged between the two extremes.

It is interesting to note that the ganoid scales of the sturgeon (*Acipenser sturio*) gave strong reactions with both Millon's reagent and with the lead-alkali solution, although Mörner, as above noted, found the ganoid scales of the gar-pike (*Lepisosteus osseus*) to give negative results with these reagents. In our experiments the scales were taken from the median portion of the "lateral line." They were extracted with 5 per cent HCl for 5 days, washed free from acid, and extracted with 0.05 per cent caustic potash in excess and again washed. After reextraction with 0.5 per cent HCl for 2 days, the scales were given a final washing and submitted to the tests for ichthylepidin.

In the remora (No. 26) both the scales and "sucking disk" were examined. The scales were obtained by digesting the integument with 5 per cent caustic soda for 36 hours (in the cold). The scales so removed were variable in size and shape; they were cycloidal, usually long and narrow, and were mostly pointed at one end and

rounded at the other. Intermixed were other more circular scales somewhat smaller and not pointed. They gave, after decalcification, positive reactions for ichthylepidin. The "sucking disk" of the remora, when removed and treated in the same way as the integument, also gave strong positive reactions for ichthylepidin. This fact indicates that the substance may be present not only in scales, but also in highly specialized modifications of the fins.

All the elasmobranch fishes examined gave negative results for ichthylepidin, as did also two teleosts, the sun-fish and puffer. The species investigated, with detailed analyses, are here given:

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|---|---|
| 34. Dog-fish ( <i>Mustelus canis</i> ).           | 37. Bonnet skate ( <i>Raja erinacea</i> ).  |
| 35. Sand shark ( <i>Carcharias littoralis</i> ).  | 38. Sun-fish ( <i>Mola mola</i> ).          |
| 36. Dusky shark ( <i>Carcharhinus obscurus</i> ). | 39. Puffer ( <i>Spheroides maculatus</i> ). |

The scales of the above-named species were isolated by means of caustic potash and then decalcified and tested. In no case was there any indication of loosely combined sulphur. Millon's reagent, though it gave a slight reaction in most experiments, did not yield the characteristic rich ruby-red color given by the scales cited in the first list. The test indicates that these scales have an organic basis quite different from the others, as the following analyses will show:

*Dog-fish (Mustelus canis)*.—Portions of the integument of several specimens recently killed were freed from extraneous matter, so far as possible, then washed thoroughly and placed in 5 per cent caustic soda. After three days the connective tissue had dissolved and the minute scales were washed carefully by decantation. They were now extracted with 0.5 per cent HCl for several days, the acid being frequently renewed. The washed, decalcified scales were now tested, as usual, with Millon's reagent and lead-alkali. With the former there was a faint pink coloration; with the latter there was no blackening. About a gram of the decalcified scales was now boiled in a liter of water for some time, the water being renewed as it evaporated. After a few hours the liquid became yellowish, and gelatin was detected in solution by the usual tests; after boiling for 30 hours the scales had almost completely gone into solution, a very minute quantity only remaining undissolved. The solution had the characteristics of a solution of gelatin, although it did not gelatinize, even after concentration. This was doubtless due to the long-continued boiling, which, as is well known, converts gelatin into its ungelatinizable form, B-glutin. The dog-fish scales thus seem to have only collagen as their organic basis, and to be quite free from ichthylepidin.

*Sand shark (Carcharias littoralis)*.—The scales were isolated and treated as described above, except that the alkali extraction lasted five days. They behaved in every way like those of the dog-fish, and like them are free from ichthylepidin and have collagen as their organic basis.

*Dusky shark (Carcharhinus obscurus)*.—The isolated scales reacted in every way like those of the dog-fish and sand shark.

*Bonnet skate (Raja erinacea)*.—The skin with scales, or rather spines, attached was removed from the caudal and posterior portions of the pectoral fins and treated with 5 per cent alkali (NaOH) as before. The star-shaped spines thus isolated were washed thoroughly, extracted several days with 0.5 per cent HCl which was frequently changed. The residue was washed until the filtrate was free from chlorides. The scales gave no blackening with lead-alkali and only a faint pink color with

Millon's reagent. When boiled with water for 30 hours the scales were almost completely dissolved, and after 36 hours the undissolved residue was so small as to be negligible. The solution gave all the tests for gelatin. It gave also a strong biuret test, but not Adamkiewicz's test. Nitric acid gave no yellow color or precipitate, but subsequent addition of ammonia caused a yellow coloration. The solution responded to the alkaloidal reagents, and gave Allen and Tankard's<sup>1</sup> test for gelatin. No ichthylepidin is present. The organic matter of skate spines thus appears to be identical with that of the scales of the other elasmobranch fishes.

*Sun-fish (Mola mola).*—The integument was freed as far as possible from the subdermal collogenous tissue and treated with caustic soda, as before. The scales so isolated were washed thoroughly and extracted with HCl as usual. They gave a strong Millon's test, but no lead-alkali reaction. The decalcified and extracted scales were soluble in 5 per cent NaOH in 24 hours (differing from ichthylepidin, which remains unchanged for 5 days).<sup>2</sup> The decalcified scales were slowly but almost completely soluble in boiling water, and after 3 days only a trace remained undissolved. The solution was concentrated but did not gelatinize, although it gave all the reactions of gelatin. The scales of *Mola* are thus analogous in composition to those of the elasmobranchs above noted, and are quite different from those of the higher fish with which it is classified. It is interesting to note in this connection the studies of Milne Edwards<sup>3</sup> and of Parker<sup>4</sup> on the vascular system of this species. Milne Edwards observed that in this fish "the coronary arteries are supplied not only from the fourth gill-arch as in other teleosts, but also from the third, fifth, and sixth arches, as in elasmobranchs." Parker has confirmed this, and has shown also that the sun-fish has two coronary arteries, as is general in elasmobranchs. To quote Parker:<sup>5</sup>

This confirmation of Milne Edwards's description shows that a really remarkable condition exists in the coronary arteries of the sun-fish. The presence of dorsal as well as of ventral coronaries, and the origin of the latter from more than one pair of visceral arches, are features so universally characteristic of elasmobranchs and so generally absent from teleosts that, while the sun-fish has most of the characteristic structural features of the latter, the arteries of its heart ally it unquestionably with the elasmobranchs.

This is interesting, as the composition of the scales (exoskeleton) is also analogous to that of elasmobranch scales, and the morphological structure of the scale is also very similar.<sup>6</sup>

*Puffer (Spheroides maculatus).*—The skin of a puffer was removed, freed from adhering tissue, and treated with 5 per cent caustic soda. After 2 days the residue of star-shaped spines was washed thoroughly and decalcified with 5 per cent HCl as above. The extracted scales gave no blackening with lead-alkali; Millon's test was distinct. The decalcified scales were treated with boiling water to determine if the basis was all collagen or if ichthylepidin was present. After boiling 30 hours there was a small residue; whether this was ichthylepidin or not we were unable to decide. The solution gave all the reactions for gelatin.

<sup>1</sup> Allen, Commercial Organic Analysis (1898), vol. 4, p. 469.

<sup>2</sup> Mörner, op. cit., p. 131.

<sup>3</sup> Milne Edwards (1858), Leçons sur la physiologie et l'anatomie comparée de l'homme et des animaux. Tome 3, p. 341.

<sup>4</sup> Parker. Note on the blood vessels of the heart in the sun-fish. Anatomischer Anzeiger, vol. 17, No. 16-17.

<sup>5</sup> Parker, op. cit., p. 315.

<sup>6</sup> Cf. Turner, Natural History Review (1862), p. 185.

## SUMMARY.

The above experiments show that in the scales of the teleosts ichthylepidin is widely distributed; in the scales of elasmobranchs and of *Mola mola* and *Spheroides maculatus* it is absent. The species cited by Mörner in his paper as having ichthylepidin in their scales are here given:

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|--------------------------------|---------------------------------|--------------------------------|
| 1. <i>Abramis brama</i> .      | 5. <i>Coregonus lavaretus</i> . | 9. <i>Lucioperca sandra</i> .  |
| 2. <i>Aspius rapax</i> .       | 6. <i>Esox lucius</i> .         | 10. <i>Perca fluviatilis</i> . |
| 3. <i>Carassius vulgaris</i> . | 7. <i>Leuciscus idus</i> .      | 11. <i>Salmo salar</i> .       |
| 4. <i>Clupea harengus</i> .    | 8. <i>Leuciscus rutilus</i> .   | 12. <i>Sebastes marinus</i> .  |

In the ganoid scales of the gar-pike (*Lepisosteus osseus*) he found no ichthylepidin. Our experiments, however, show that it is detected by Mörner's reactions in the scales of the sturgeon (*Acipenser sturio*).

The scales of the sun-fish have the same general chemical composition as those of the elasmobranchs, and quite different from the scales of other higher fishes.

## PROPORTION OF COLLOGEN AND ICHTHYLEPIDIN.

Mörner found the relative amounts of collagen and ichthylepidin in the organic matter of the scales of four species of fish to be, approximately, 4 parts of the former to 1 part of the latter. The determinations were made indirectly by estimation of the total sulphur. In two cases we have found the relative amounts directly by separation of the ichthylepidin and weighing it, the collagen being calculated from the difference. The process was carried out as follows: The scales were removed, cleaned, and decalcified until no more mineral matter was removed by the 0.5 per cent HCl used. The residual scales were washed free from chlorides with water, then with alcohol and ether, and dried to constant weight at 105° C. The dry organic matter so obtained was weighed, and digested with a large excess of 0.1 per cent HCl at 40 to 45° C. for 12 days. Thymol was used to prevent putrefaction. The residual ichthylepidin was filtered off, washed thoroughly with water, alcohol, and ether, and dried at 105° C. The loss in weight which the organic matter had suffered was taken as collagen.

17.7950 grams of dry organic matter from menhaden scales left a residue of 4.2255 grams (ichthylepidin) when treated as described above.

8.1550 grams of dry organic matter from shad scales left a residue of 1.9570 grams.

|                     | Menhaden. | Shad.     |
|---------------------|-----------|-----------|
|                     | Per cent. | Per cent. |
| Ichthylepidin ..... | 23.74     | 24.00     |
| Collogen.....       | 76.26     | 76.00     |

The two species thus have nearly the same relative organic composition, and the numbers found are in close agreement with Mörner's estimations. It seems that the ratio between the amounts of the two albuminoids is nearly constant in various fish whose scales contain ichthylepidin.

It may be remarked that a great difference exists (apparently) in the collagen of scales containing no ichthylepidin and those containing the latter substance. In the latter case the collagen is very loosely combined (also noticed by Mörner), a large

proportion of it being removed by boiling for 2 hours and also by digestion at 40° C. for a day with 0.1 per cent HCl. In the former case, however, the collogen is very firmly combined and is dissolved only by long-continued boiling (30 to 40 hours), and is much less affected by dilute acid digestion. Whether this is due to an essential difference in the nature of the collogen itself or to a difference in the chemical combination of it with other constituents of the scale is a subject now under investigation.

#### UTILIZATION OF THE COLLOGEN.

Some experiments relating to the use of the large amount of collogen present in fish scales were made tentatively. Menhaden scales were used, as these fish are now utilized in immense quantities in fertilizer works, and the removal and use of their scales, if possible, could be made at least expense. The scales can be readily removed from menhaden—more readily, indeed, than in the case of most fish.

Analyses were made to determine the amount of the collogen in the scales, both when dried at 150° C. to constant weight and when only air-dried. Also the scales from an average-sized specimen were removed and weighed to get data for determining the amount of gelatin which large quantities of moist fish would yield.

The scales from one menhaden (one-half hour after removal from the water and weighing 155 grams) were removed, washed, and thoroughly scrubbed to remove adhering tissue and then dried in the air at 20° C.

The scales, air-dried, weighed 6.9565 grams; dried to constant weight at 105° C. they weighed 5.5215 grams. The moisture in the air-dried scales then is 20.58 per cent, and the fish thus yielded 1.26 per cent scales, air-dried, or 1.004 per cent scales dried at 105° C.; that is, an average moist menhaden yields 1 per cent of its weight in anhydrous scales. One ton of fish would yield 20 pounds of dry scales.

The scales from several specimens were removed, cleaned, and dried in the air, and then to constant weight at 105° C.; 3.8180 grams of these scales were ignited and gave 1.5679 grams of ash, or 41.07 per cent ash. The organic matter by difference was 58.93 per cent. As shown above, the dry organic matter of menhaden scales contained 23.74 per cent of ichthylepidin and 76.26 per cent of collogen. Hence the following protocol:

| Scales.                  | Air-dried.                                       | Dried at 105° C.                                 |
|--------------------------|--|--|
|                          | <i>P. ct.</i>                                    | <i>P. ct.</i>                                    |
| Moisture.....            | 20.58  | 00.00  |
| Ash.....                 | 32.61  | 41.07  |
| Organic matter.....      | 46.80 {Ichthylepidin, 11.11.<br>Collogen, 36.69. | 58.93 {Ichthylepidin, 13.99.<br>Collogen, 44.94. |
| Average fish yields..... | 1.26 scales.                                     | 1.00 scales.                                     |

Thus a ton of menhaden should yield nearly 10½ pounds of pure scale-gelatin (16 per cent moisture, as in the usual commercial article). As Mörner has shown this gelatin to be remarkably pure, containing only about 0.1 per cent ash, it should command, when properly prepared, a high commercial value.

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