Contributions from the Biological Laboratory of the U. S. Fish Commission,
Woods Hole, Massachusetts.

THE CHEMICAL COMPOSITION OF THE SUB-DERMAL CONNECTIVE TISSUE OF THE OCEAN SUN-FISH.

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The tissue under examination was obtained from a sun-fish* taken near the laboratory of the United States Fish Commission at Woods Hole, and at the time the study was undertaken had been preserved in alcohol for nearly a year. The preserved fragments were of a creamy white color, homogeneous, very tough and inelastic, and under the microscope were found to consist almost entirely of elastic-like fibers. As no reference to the chemical composition of this substance could be found, the following analyses were undertaken to determine (1) whether the tissue was composed wholly of elastin, and (2) what was the essential constituent if the tissue was found not to be composed of elastin.

The analyses were conducted as follows: A few grams of the alcoholic tissue were cut into small fragments, rinsed in water, and dried at 110° C. When dry the fragments were of yellow color, semitransparent, hard, and brittle. They were insoluble in hot or cold 10 per cent sodium carbonate, but cold 50 per cent acetic acid caused them to swell enormously, although they did not dissolve. The dry fragments were soluble in strong potassic hydrate, in hot 1 per cent potassic hydrate after one hour, in cold 7 per cent potassic hydrate after two hours, and in the hot 7 per cent potassic hydrate were dissolved in a few minutes. Deflagrated with soda and niter they showed the presence of sulphur and of a trace of phosphorus.

The alcoholic tissue was insoluble in ammonia (sp. gr. 0.955), in lime water, in cold 1 per cent hydrochloric acid, and in hot or cold 10 per cent sodium carbonate. It was slowly soluble in boiling water, cold 10 per cent hydrochloric acid, cold 1 per cent potassic hydrate, and glacial acetic acid. It was readily soluble in strong hot hydrochloric acid, and nitric acid, in cold potassic hydrate (14 per cent), and in hot 5 per cent potassic hydrate.

A characteristic of collogen is its property of gelatinization. To test this property in the tissue under examination, the following experiments were made: About 50

*The sun-fish, *Mola mola* (Linnæus), is found on the Atlantic coast, in summer as far north as the Newfoundland Banks. It occurs in such numbers that ten or more may be seen in a single day, the large black dorsal fins elevated above the surface of the water betraying the presence of the animals as they drift leisurely along. Their total unfitness for food and their pelagic life have rendered them safe from the persecutions of the fishermen, and not only are they abundant, but the individuals attain to enormous size. Specimens 7 or 8 feet in length weigh several hundred pounds. A very large percentage of the weight of each individual is made up of a firm tissue superficially resembling blubber, but not oleaginous. By the following chemical analyses this tissue, heretofore considered worthless, is shown to yield collogen, an albuminoid, which is the basis of all glues and gelatins. The fish are thus shown to have considerable commercial value.—H. C. Bumpus.

grams of the finely divided and well-washed tissue were boiled for 6 hours in half a liter of distilled water, the water being renewed as it evaporated. The tissue almost completely dissolved, and the yellowish solution on cooling became somewhat viscid, but it did not gelatinize, even when evaporated to a volume of only 100 c. c. The concentrated solution was diluted and filtered. It was neutral to litmus, was precipitated by phosphotungstic acid, and by phosphomolybdic acid; the precipitates were insoluble on heating, but were soluble in potassic hydrate. When precipitated by Millon's reagent the precipitate was soluble in excess of reagent. When precipitated by tannic acid, by metaphosphoric acid, absolute alcohol, and mercuric chloride in presence of free hydrochloric acid the precipitates were insoluble.

The solution gave no precipitate with acetic acid and potassium ferrocyanide, neutral or basic acetate of lead, alum, cupric sulphate or acetate, ferric chloride, sodium orthophosphate, or mercuric potassium iodide. It was not blackened when boiled with lead acetate and potassic hydrate. It gave a strong biuret reaction, and on boiling did not reduce copper. Hot concentrated sulphuric acid and glacial acetic acid produced no violet coloration. Hot strong nitric acid gave no precipitate or coloration, but subsequent addition of ammonia caused a deep orange tint. Saturated with ammonium sulphate, the solution gave a copious precipitate, which floated on the surface of the liquid, forming a coagulum-like layer. Picric acid in saturated solution produced a light-yellow precipitate, insoluble in excess of the reagent, soluble in hot, insoluble in cold water. On shaking, a stiff coagulum formed above the subnatant clear liquid, so that the test tube could be inverted without loss of its contents.[1]

To a few centimeters of the original solution, several drops of formaldehyde (40 per cent solution in water) were added, and the whole evaporated to dryness. The residue was insoluble in hot or cold water. It had become apparently formogelatin.

The reactions given above show that true proteids, also chondrin, are absent, or present in quantities too small for detection. All the phenomena, especially the peculiar and characteristic reactions given by picric acid, ammonium sulphate, and formaldehyde, point to the presence of gelatin, notwithstanding the fact that the solution did not gelatinize and that the original tissue was soluble in dilute potassic hydrate. Elastin can be present only in traces, if at all, since it is insoluble in boiling water, even after 96 hours' boiling,[2] and insoluble also in 1 per cent potassic hydrate,[3] which dissolves the tissue under consideration.

The following experiments were made to determine whether other substances were present with the collogen-like albuminoid.

Several grams of the alcoholic tissue were cut up, washed, and treated with cold 5 per cent potassic hydrate. After 24 hours the tissue was almost wholly dissolved; the solution was filtered, slightly acidified with acetic acid, and submitted to the following reactions. Phosphotungstic acid gave a precipitate soluble on heating, and phosphomolybdic acid, tannic acid, and metaphosphoric acid also gave precipitates. The solution was not precipitated by acetic acid and potassium ferrocyanide, nitric acid, basic or neutral lead acetate, or copper sulphate, and it gave a strong biuret reaction. There was no coloration with hot sulphuric and glacial acetic acid. The fact that the precipitate given by phosphotungstic acid is soluble by heating shows, according to the researches of Mallet,[4] that true proteid substances can not be present in the

[1] Allen and Tankard's test for gelatin. Allen's Com. Organ. Analysis, vol. IV, 1898, p. 469.
[2] Chittenden and Hart. Zeit. für Biologie, 7 Bd., s. 369—(abs.—Horbaczewski Zeit. für Phys. Chemie, Bd. 6, s. 330.)
[3] Ibid.
[4] U. S. Dept. Agric., Division of Chem., Bull. 54, 1898, pp. 20–21.

solution; for proteid substances, including gelatin and chondrin and excepting peptones, give precipitates insoluble on heating. In testing the boiling-water solution of the tissue as given before, the phosphotungstic acid precipitate was found to be insoluble on heating.

The behavior of the substance with digestive fluids was as follows: (A) Fragments of the tissue were rapidly digested at 40° C., when treated with a pepsin hydrochloric acid solution. (B) When treated with an alkaline trypsin solution, the fragments were attacked with extreme slowness. For this experiment about 40 grams of the alcoholic tissue were cut up fine, well washed to remove excess of alcohol, and then treated with a 0.5 per cent sodium carbonate solution of pancreatin, to which a few fragments of thymol were added. The temperature was kept at or near 40° C. After four days the tissue had been only slightly attacked, but at the end of eight days it was almost completely dissolved. When boiled in water (for 10 minutes) and then subjected to the action of the trypsin solution, the fragments were dissolved almost completely in 24 hours. The same result was obtained by first swelling the washed tissue fragments in dilute acetic acid and then submitting them to digestion. The behavior of the subdermal connective tissue toward digestive fluids seems to identify it with collogen, the basis of bone, cartilage, and other gelatine-yielding substances of the body. True collogens are wholly unaffected by tryptic digestion, unless they have been heated previously with water or swelled with acids.[1]

QUANTITATIVE ANALYSIS.

A preliminary analysis was made to determine the percentage of nitrogen, sulphur, and ash in the dried alcoholic tissue. No attempt was made to estimate the amount of phosphorus, since the qualitative examination showed only mere traces of this element. In this, as in the following analysis, the nitrogen was determined by the Gunning modification of the Kjeldahl method, using mercuric oxide as an oxidizing agent, and cochineal as an indicator in the back-titration. Sulphur was estimated as barium sulphate in the usual way, after fusion of the tissue with sodium carbonate and potassium nitrate. Carbon and hydrogen were determined as usual by combustion with cupric oxide, a zone of lead chromate being placed after the oxide to arrest any sulphur dioxide present.

About 50 grams of the alcoholic tissue were cut up, dried for several days at 90° C., pulverized and redried at 110° C., to constant weight. Analysis of this dry substance gave the following results:

Quantity of substance (grams).	Constituents.					
	Ash.		Nitrogen.		Sulphate of barium.	
	Grams.	Per cent.	Grams.	Per cent.	Grams.	Per cent of sulphur.
1.5040	.0221	1.47	.....	.....	.....	.....
.8162	.....	.....	.1337	16.37	.....	.....
.2829	.....	.....	.04746	16.77	.....	.....
.2414	.....	.....	.04032	16.70	.....	.....
.2065	.....	.....	.03458	16.74	.....	.....
.6498	.....	.....	.....	.....	.0245	.53
.6836	.....	.....	.....	.....	.0206	.42
Mean.	.....	1.47	.....	16.64	.....	.47

[1] Hoppe-Seyler.—Handbuch d. Phys. u. Path. Chem. Analyse, 6 Auf., s. 270.

These results shed no light on the proteid composition of the tissue, except that the percentage of nitrogen corresponds almost exactly to that given by Chittenden and Hartwell[1], for the nitrogen in elastin prepared from the neck bands of cattle. This fact has little weight, however, in view of the high percentage of ash present in the tissues under consideration.

About 50 grams of the tissue were reduced to fragments, and washed repeatedly, by decantation, with cold water. The fragments were then washed with 90 per cent alcohol for 24 hours, and allowed to stand under ether, with frequent shaking, for 36 hours. The material was then removed, freed from excess of ether, and dried in the air bath at 110° C., to constant weight. The dry fragments were tough and leathery, and could not be powdered. A portion gave with Millon's reagent a light pinkish color. Analysis of this dried substance gave the following results:

Quantity of substance (grams).	Constituents.							
	Water.		Carbon dioxide.		Nitrogen.		Ash.	
	Grams.	Per cent of hydrogen.	Grams.	Per cent of carbon.	Grams.	Per cent.	Grams.	Per cent.
.3229	.1988	6.84	.5704	48.18	.....	.....	.0013	.40
.3658	.2227	6.75	.6476	48.28	.....	.....	.....	.....
.3381	.....	.....	.....	.....	.06146	18.17	.....	.....
.4695	.....	.....	.....	.....	.08568	18.25	.....	.....
Mean..	.....	6.80	.....	48.23	.....	18.21	.....	.40

By difference, oxygen + sulphur = sum of means from 100 = 26.36 per cent.

The high percentage of nitrogen shows at once that the tissue is not composed of elastin. Both the carbon and the nitrogen determinations would indicate that the tissue is a collogenous substance, and under appropriate treatment it has yielded glue of excellent quality.

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[1] Abs.—Allen. Com. Organ. Analysis, vol. IV, 1898. [Appendix.]