Note on the Chemical Constitution of the Mesoglœa of Alcyonium digitatum.

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The main organic constituent of the mesoglœa in Alcyonium digitatum would appear to belong to that class of bodies described by Kruckenberg[1] as hyalogens, so widely found among Invertebrate skeletal structures. Hyalogen is characterised by its insolubility, and its conversion by various reagents (e. g. 5 per cent. solution of caustic soda) into a soluble substance, hyalin. Hyalin is practically a mucin, yielding on decomposition a proteid-like body and a carbohydrate.

I. The following experiments show that mucin is readily obtainable from the mesoglœa; whether, in life, any of it is present as such, or whether it is derived from a hyalogen by the necessary preliminary treatment, it is, of course, difficult to say.

Fresh specimens, freed as far as possible from cellular layers, were washed well with distilled water and minced. They were then extracted with a 5 per cent. salt solution to remove any globulins, and left under lime water for seventy-two hours. The filtrate was then treated with acetic acid. A white precipitate was obtained, which, on standing for some hours, settled; it was then filtered off, and washed with water acidulated with acetic acid. It was again dissolved in lime water and re-precipitated. The collected precipitate was boiled with 2 per cent. sulphuric

[1] Kruckenberg, 'Zeit. f. Biol.,' xxii.

acid for about twenty minutes; on neutralisation a reducing sugar and a peptone-like body were obtained. A portion of the original colony, boiled direct with sulphuric acid, yielded a reducing sugar.

Throughout the investigation the endeavour was made to confirm and locate the results obtained from chemical analysis in bulk by the application of micro-chemical tests. For the micro-chemical detection of mucin, Waymouth Reid recommends thionin, as used by Hoyer,[1] which he considers to be thoroughly diagnostic. It appears probable, however, that mucin gives the ruddy purple reaction with this dye in common with other basophilic substances. Nevertheless this reaction serves to locate the position of the mucin or hyalin which had been extracted. Sections of specimens hardened in corrosive sublimate were cut and stained as recommended by Reid. The endoderm appeared blue, the mesoglœa a ruddy purple. This latter coloration was also seen in cover-slip films prepared from the precipitated substance extracted as above.

Hence we conclude that the mesoglœa yields a substance which resembles mucin—

(i) In its solubilities.
(ii) In its decomposition products.
(iii) In its micro-chemical reaction.

II. After treatment of the mesoglœa with dilute acids, however, lime water or baryta water is capable of dissolving a much larger proportion. Prolonged boiling or treatment with superheated water (120° C.) will effect the same change. Lime water or baryta water then leaves but a small granular residue, whereas the main mass of the mesoglœa is left by these reagents when repeatedly applied to the fresh material. Treatment for a few days with comparatively dilute (e. g. 50 per cent.) alcohol greatly impairs the solubility.

The filtrate from this lime water extract will on decomposition yield a reducing sugar and a proteid substance.

This recalls the condition of affairs met with in the edible

[1] Waymouth Reid, 'Journal of Physiology,' vol. xiv. Hoyer, 'Arch. f. Mikr. Anat.,' Bd. xxxvi.

bird's-nest which Kruckenberg (op. cit.) regards as composed of a hyalogen-neossidin, giving rise on solution to a hyalin-neossin. The mesoglœa further resembles the reactions of the edible bird's-nest as described by Green[1] in the absence of a cellulose reaction, and in the fact that on boiling with dilute sulphuric acid a pinkish coloration, which subsequently darkens, is seen.

Another feature frequently shown by hyalins, in which that under consideration agrees, is the absence of Millon's reaction. An application of this test micro-chemically to mesoglœa shows us that the cells alone stain red. This indicates that the proteid extracted by 5 per cent. salt solution (which presents all the characteristic features of a globulin) is located in the cells, and not in the mesoglœa; it need not then detain us.

The micro-chemistry of hyalogen presents a slight difference from that of mucin. Pieces of mesoglœa which yielded no further mucin to lime water were decalcified, and cut with the freezing microtome. Stained with thionin by Reid's method, the purple coloration of the mesoglœa only appears under artificial light, recalling the conditions of the basophilic reaction with methylene blue. The endoderm-cells appear blue both with natural and artificial light.

We shall not err greatly, then, if we describe the organic constituent of the mesoglœa as mainly a hyalogen, probably mixed with some amount of mucin. The hyalin obtained from the hyalogen is precipitated on saturation with ammonium sulphate as a gummy mass.

III. As stated above, after extraction of previously acidified mesoglœa with lime or baryta water a small amount of a somewhat granular residue is left. Examined under the microscope, it is seen to consist of clumps of globules which yield the xanthoproteic reaction. They recall in some measure the description given by Mall[2] of the appearances of elastin acted upon by acids or alkalies. The substance, however, is not elastin, as is seen by its having no affinity for Victoria blue or

[1] J. R. Green, 'Journal of Physiology,' vol. vi.

[2] Mall, 'Anat. Anzeig.,' iii Jahrg.

safranin, and in its resistance to prolonged tryptic action. It swells up, however, under the action of this enzyme. It is markedly insoluble, showing no diminution in bulk on addition of weak or strong acids and alkalies, cold or boiling. The only exception to this is concentrated sulphuric acid, which causes it to swell up, and after boiling for a short time to dissolve with an accompanying pink coloration, soon passing to a light brown. No leucin, peptone, or sugar could be demonstrated as a product of this action.

These reactions remind us somewhat of the insoluble residue described by Schäfer[1] as obtained from the entostemite of Limulus. The total amount, however, is but slight.

An artificial pancreatic digestion of mesoglœa yields the results which might be anticipated from the foregoing. The residue was composed of antialbumid (soluble in 1 per cent. caustic soda) calcareous matter (readily soluble in dilute acetic acid) and the granular substance just referred to. The filtrate yielded albumoses, peptones, a reducing sugar, crystals of leucin and of tyrosin—the last presumably from the cells, since in them alone is Millon's reaction successful.

IV. In investigating a material like the mesoglœa two questions naturally suggest themselves: does it contain gelatine? and does it contain nucleo-albumen? (*a*) Does the mesoglœa contain gelatine? In some experiments on this point, thin slices of mesoglœa were treated with distilled water under pressure at 120° C. for over two hours; in others, portions of it were minced and boiled with distilled water for 1½ hours. The subsequent procedure was that described by Young[2] for retiform tissue. The extract was filtered, while boiling, through a hot filter into alcohol. The precipitate which formed was collected after standing for some hours, dissolved in distilled water, boiled, and filtered. The filtrate was concentrated to a very small bulk. In all cases this did not "jelly" perceptibly. Hence we conclude—

[1] Schäfer, foot-note to Ray Lankester on "Skeleto-trophic Tissues," 'Q. J. M. S.,' vol. xxiv.

[2] R. A. Young, 'Journal of Physiology,' vol. xiii.

The mesoglœa does not contain gelatine. Hence, also, it cannot contain chondrin-like bodies. The popular term "jelly" applied to this substance appears to have no basis in chemical fact.

(*b*) Does the mesoglœa contain nucleo-albumen? The recognition of this body in several non-cellular tissues suggests its presence here. To test this point a colony was stripped of its external layers and treated by Halliburton's method for extracting nucleo-albumen.[1] None could be detected.

Thanks to the method recently introduced by Lilienfeld and Monti,[2] the examination of this question micro-chemically is also feasible. Specimens hardened in osmic acid were cut with the freezing microtome, then washed thoroughly and placed in a solution of ammonium molybdate. After being washed for a few seconds in a mixture of ether (9 parts) and water (1 part), they were put into a 20 per cent. ethereal solution of pyrogallic acid. The cells in such specimens were seen under the microscope to be stained black, but the mesoglœa was not. Hence we conclude—

The mesoglœa does not contain nucleo-albumen.

V. Conclusions:

(i) The mesoglœa of Alcyonium digitatum is chiefly composed of a hyalogen.

(ii) Prior to the conversion of the hyalogen into hyalin the mesoglœa will yield a mucin.

(iii) It also contains a small amount of an insoluble albuminoid body, whose nature was not determined.

(iv) It does not contain gelatine or nucleo-albumen.

[1] Halliburton, 'Brit. Assoc. Reports,' 1888; 'Journal of Physiology,' vol. xiii.

[2] Lilienfeld and Monti, 'Zeit. f. physiol. Chem.,' Bd. xvii. See also Gourlay, 'Journal of Physiology,' vol. xvi.