Chitin in Pogonophora

POGONOPHORA secrete and inhabit tough, horny tubes. Recently, Hyman[1] quoted unpublished results indicating that the tubes of Pogonophora were composed of cellulose. In view of the presumed phylogenetic relationship of Pogonophora with lower chordates this might have been expected, since the tunicate test is rich in cellulose (tunicin). However, on examining three species of *Siboglinum* (*S. atlanticum* Southward and Southward, *S. inermis* Southward and Southward[2], and *S. caulleryi* Ivanov) kindly given to us by Dr. A. J. Southward, we found the tubes to be lacking cellulose, but rich in chitin. Later, through the kindness of Academician Ivanov, we were given specimens of the tubes of *Zenkevitchiana longissima* Ivanov[3], and the same conclusion about their composition was reached.

Tubes of the four species available withstood treatment for 20 min. in saturated aqueous potassium hydroxide at 150° C. The alkali-treated tubes, after washing, were coloured brown by iodine in potassium iodide solution, becoming violet when this was replaced by dilute sulphuric acid[4]. Alkali-treated tubes were soluble in mineral and acetic acids. Both cellulose and chitin are relatively stable in alkali, under the conditions specified; but the above colour reactions, and solubility in acids, are properties shown only by chitin[5]. The tubes resisted solution in Schweitzer's cuprammonium reagent, in which cellulose dissolves; and the same result was obtained if tubes were pretreated in strong alkali (as above) or in a solution of chlorine dioxide in acetic acid ('diaphanol').

Chitin, being considered as a polymer of 2-acetamido-2-deoxy-α-D-glucopyranose (N-acetyl-D-glucosamine), on acid hydrolysis yields D-glucosamine; and, on enzymic degradation, N-acetyl-D-glucosamine[6]. The pogonophoran tubes showed these reactions.

Tubes of the four species were hydrolysed in sealed vessels with 6 *N* hydrochloric acid at 100° C. for 6 hr. After drying the hydrolysate over phosphorus pentoxide and potassium hydroxide, the residue was taken up in water and run on partition paper chromatograms against D-glucose and D-glucosamine hydrochloride, using eight different solvent mixtures. The chromatograms were sprayed with aniline hydrogen phthalate, silver nitrate, or the Elson and Morgan reagents. A substance (evidently an amino-sugar) behaving like glucosamine (or galactosamine) was present in large amounts; but no glucose was detectable. On conversion to the corresponding pentose by Stoffyn and Jeanloz[7] procedure *B*, the acid hydrolysate of *S. atlanticum* yielded arabinose, confirming the presence of glucosamine (as opposed to galactosamine).

Tubes of *S. atlanticum* were prepared for enzymic hydrolysis by heating with 4 *N* aqueous potassium hydroxide at 100° C. for 3 hr. (to remove the substances other than chitin mentioned below), and the residual chitin was dispersed by brief treatment in 12 *N* hydrochloric acid; after washing, the suspension was incubated at 37° C. with an enzyme preparation from the puff ball *Lycoperdon pyriforme*[5], buffered at *p*H 5·0, having chitinase and cellulase activity. Samples taken after 18, 42 and 66 hr. were concentrated *in vacuo* and chromatographed in butanol/ethanol/water (4 : 1 : 5). N-acetyl-glucosamine was shown to be present with the Elson and Morgan reagent, and no glucose was detectable with aniline hydrogen phthalate.

Proteins invariably accompany chitin, and a number of amino-acids were found in the acid hydrolysate of the tubes. These amino-acids must have been derived from a very stable protein in so far as it withstands environmental dissolution; but it is not yet known whether stabilization is derived from disulphide links (being a keratin), or quinonoid links (being a sclerotin).

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Production of Experimental Scurvy in a Bird Species

IT has been generally supposed that all species of animals except the primates and the guinea pig are able to synthesize ascorbic acid according to their requirements. In a previous communication[1], using our technique of incubating liver and kidney tissues of different species of animals with D-glucuronolactone as substrate in presence of cyanide, it was shown that in some species the kidney could effect the synthesis and in some the liver. In the evolutionary ascent the mechanism for the synthesis of ascorbic acid appeared to pass from the kidney to the liver and then to disappear altogether. It was also unexpectedly observed that neither the liver nor the kidney tissue of the red-vented bulbul (*Pycnonotus cafer*, Linn.) and of the Indian fruit-bat (*Pteropus medius*) could effect the synthesis. Thus at least one avian species, the red-vented bulbul, and one mammalian species, the Indian fruit-bat, were apparently incapable of synthesizing ascorbic acid. It was suggested, therefore, that the cyanide technique might offer a simple means for determining the capacity of different species for the synthesis of ascorbic acid. This supposition has now been proved to be correct by actually placing some red-vented bulbuls on a scorbutic diet and producing in them ascorbic acid deficiency symptoms, which could be cured by ascorbic acid.

Nine red-vented bulbuls were put on a diet of parched gram powder for a fortnight to get them used to the diet. Then they were placed on the scorbutic diet composed as follows: 80 parts of parched gram powder (autoclaved as an aqueous paste at 18 lb. per sq. in. pressure for 45 min.), 12 parts of casein, 5 parts of yeast powder, 1 part of Steenbock's salt mixture No. 40 and 2 parts of cod-liver oil. The average weight of the birds fell gradually from 35 gm. to 28 gm. in 15 days and they all showed deficiency symptoms. These were loss of feathers and particularly the tail, drooping of the head, sluggishness and peevishness. On the sixteenth and seventeenth days, two birds dropped steeply in