

N-Acetyl-Neuramic Acid in the Sea Urchin Jelly Coat

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The jelly coat surrounding the sea urchin egg has been subject to a number of investigations. Runnström *et al.*¹ showed that the jelly in solution has a strongly acid character and that it is rich in carbohydrate. Analytical ultracentrifugation showed one strong and a weaker component. These represent highly asymmetrical macromolecules with high sedimentation constant. Vasseur² showed that about 80 % of the jelly substance consisted of sulfated polysaccharides and 20 % of peptides or proteins. He determined the character of the monoses in a number of species. Fucose is the most commonly found monose, but it may be replaced by galactose or may be admixed with either galactose or glucose. The chemical and physical properties of the jelly of different species has been further elucidated by work of Tyler,³ and Nakano and Ohashi.⁴ It is still an open question as to whether the jelly contains glucosamine (see Vasseur,² Krauss,^{5,6} Tyler³).

In the analyses of the contents of the jelly a residue of 10–15 % always remained unresolved. This residue may still contain components of interest.

This paper will demonstrate that *N*-acetyl-neuramic acid is a component of the jelly at least in some Mediterranean sea urchin species.

Material and methods. The jelly was prepared from eggs of *Arbacia lixula*, *Paracentrotus lividus* and *Sphaerechinus granularis* from the Naples region. The jelly was removed from the suspended eggs by sucking them repeatedly through bolting silk of appropriate mesh. The eggs were then allowed to sediment or centrifuged in ordinary sea water. The supernatant contained the jelly solution. This was dialyzed against running tap and distilled water, and concentrated to reduced volume by means of polyethylene glycol (Carbowax A 6000). The jelly substance was precipitated: (a) with 0.1 % cetylpyridinium chloride, (b) with 75 % ethanol, or (c) analyzed directly from concentrated solution in distilled water.

N-Acetyl-neuramic acid was assayed by the direct Ehrlich's reaction according to Werner *et al.*⁷ and identified by the spectrometric curve obtained in the wave-length range 450–650 m μ . The reference-curve was prepared with *N*-acetyl-neuramic acid supplied from Sigma, Chemical Company.

The jelly substance was hydrolyzed with hydrochloric acid which was finally evaporated in desiccator under vacuum. The residue was dissolved in distilled water and chromatographed on Whatman filter paper No. 1. Two-dimensional ascending chromatography was employed using butanol-acetic acid-water (4:1:1) as the mobile phase in one direction and

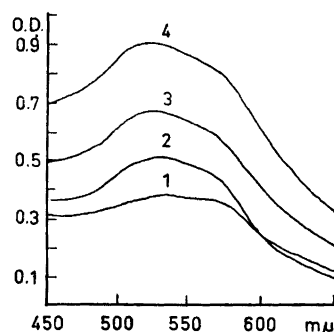


Fig. 1. A spectrophotogram of *Sphaerechinus* jelly tested with directly applied Ehrlich's reagent on *N*-acetyl-neuramic acid. Curves: 1. jelly solution in distilled water before precipitation, 2. reference-curve of *N*-acetyl-neuramic acid, 3. 0.27 % suspension of cetylpyridinium chloride precipitate, 4. 0.16 % suspension of ethanolic precipitate.

methanol-ammonia (conc.)-water (90:2:8) in the other. The position of sugars on the chromatogram was detected by Tollens silver reagent as used by Immers⁸ and amino acids or aminosugars with 0.5 % ninhydrin in acetone.

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Results. Fig. 1 refers to a spectrophotogram of the reaction product of a com-

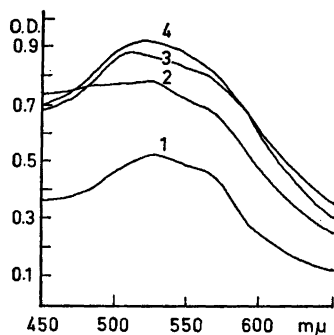


Fig. 2. A spectrophotogram of different sea urchin jellies precipitated with cetylpyridinium chloride and tested with Ehrlich's reagent on *N*-acetyl-neuramic acid. Curves: 1. reference-curve of *N*-acetyl-neuramic acid, 2. *Arbacia lixula*, 3. *Paracentrotus lividus*, 4. *Sphaerechinus granularis*.

ponent of *Sphaerechinus* jelly and the directly applied Ehrlich's reagent. The curves 1, 3, and 4 show the result when the jelly solution was a) concentrated in distilled water, b) precipitated with 0.1 % cetylpyridinium chloride, or c) precipitated with 75 % ethanol. The course of the curves

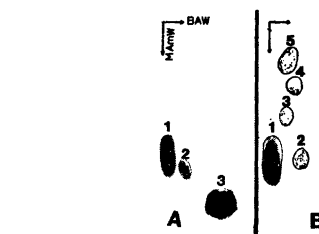


Fig. 3. Two ascending chromatograms (A and B) which were carried out under identical conditions. Hydrolysate of *Paracentrotus* jelly precipitated with cetylpyridinium chloride: BAW, butanol-acetic acid water. MAmW methanol-ammonia-water. Chromatogram A developed with Tollens' silver reagent gave spots: 1, neuramic acid; 2, glucose; 3, fucose. Chromatogram B developed with ninhydrin gave spots: 1, neuramic acid; 2, alanine; 3, glycine; 4, glutamic acid; 5, aspartic acid.

is identical with that of the reference curve showing the spectrophotogram of *N*-acetyl-neuramic acid after direct reaction with Ehrlich's reagent. As shown in Fig. 2, similar results were obtained with the jellies of *Arbacia* and *Paracentrotus*.

A hydrolysate of *Paracentrotus* jelly precipitated with cetylpyridinium chloride

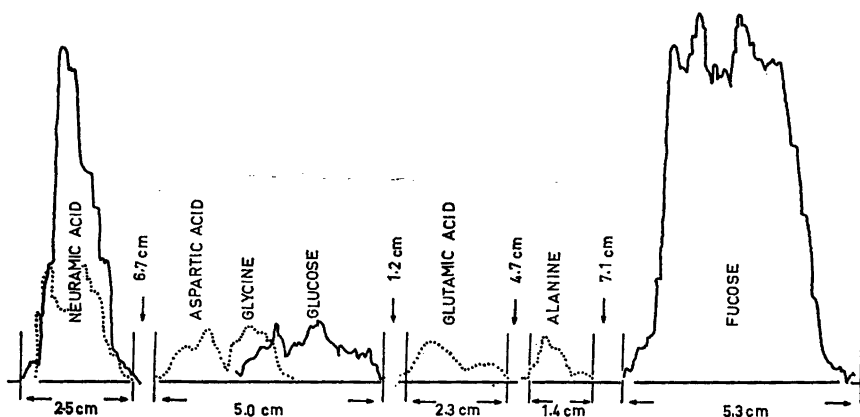


Fig. 4. The content of monosaccharides, neuramic acid, and amino acids in jelly of *Paracentrotus* according to microdensitometric readings of two parallel one-dimensional descending paper chromatograms from hydrolysate of the jelly precipitated with cetylpyridinium chloride. Solid line presents the results obtained when using Tollens' silver reaction; hatched line, that obtained when using ninhydrin. Solution: butanol-acetic acid-water (4:1:1). The numbers in cm indicate the position of the spots on the chromatogram.

gave three spots indicating mono-saccharides (Fig. 3A and its legend) and five spots indicating amino acids (Fig. 3B). After careful testing, the spot No. 1 of Figs. 3A and 3B was identified as neuramic acid in its keto or pyranose form.

The cetylpyridinium chloride precipitated *Paracentrotus* jelly which was subjected to hydrolysis contained as dominating component fucose but also in minor quantity glucose. Moreover neuramic acid is present as an important component, see Figs. 3 and 4. In natural or precipitated but not hydrolyzed jelly this last component is present in the form of *N*-acetylneuramic acid. Figs. 3B and 4 show furthermore the presence in the hydrolysate of four amino acids (alanine, aspartic acid, glutamic acid, glycine). In addition, the hydrolysate contains sulfate and calcium.

The presence of neuramic acid was detected also in the jelly substance precipitated with cetylpyridinium chloride or ethanol of *Arbacia* and *Sphaerechinus*. It was also found as component in acid mucopolysaccharides which were isolated from eggs and embryos of the three sea urchin species studied.

Discussion. The results reported above raise the question as to whether *N*-acetylneuramic acid constitutes an integrated part of the jelly coat substance or whether its presence is of a more fortuitous nature caused by its diffusion from the egg surface. A dual origin of components of the jelly coat is made probable through the immunological studies carried out by Baxandall, Perlmann and Afzelius^{8,10} and by Baxandall.¹¹ Moreover the question arises as to whether the presence of *N*-acetylneuramic acid contributes to the species specific agglutination of spermatozoon exhibited in many species by the jelly coat substance. These questions are subject to further work.

1. Runnström, J., Tiselius, A. and Vasseur, E. *Arkiv Kemi, Mineral. Geol.* **A 15** (1942) No. 16.
2. Vasseur, E. *The chemistry and physiology of the jelly coat of the sea urchin egg*. Stockholm 1952.
3. Tyler, A. *Am. Naturalist* **83** (1949) 195.
4. Nakano, E. and Ohashi, S. *Embryologia* **2** (1954) 81.
5. Krauss, M. *Biol. Bull.* **96** (1949) 74.
6. Krauss, M. *Science* **112** (1950) 759.
7. Werner, I. and Odin, L. *Acta Soc. Med. Upsalien.* **57** (1952) 230.

8. Immers, J. *Arkiv Kemi* **16** (1960) 63.
9. Baxandall, J., Perlmann, P. and Afzelius, B. A. *J. Cell Biol.* **23** (1964) 609.
10. Baxandall, J., Perlmann, P. and Afzelius, B. A. *J. Cell Biol.* **23** (1964) 629.
11. Baxandall, J. J. *Ultrastruct. Res.* **16** (1966) 158.

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Pyrimidines from Malonyl Chloride and Nitriles

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In 1962 Davis, Elvidge and Foster¹ described a reaction between malonyl chloride and α -methylene nitriles leading to 3-substituted 2-chloro-4,6-dihydropyridines, considered to exist in the α -pyridone form. Attempted extension of this reaction to α -halogenonitriles unexpectedly afforded 2-substituted 4-chloro-6-pyrimidones.^{2,3} Thus fluoroacetonitrile gave (I), substantiated by UV, IR, NMR, and mass spectra, and by hydrogenolysis to the known 2-methyl-4-pyrimidone (II).⁴ As reported,² bromoacetonitrile and α -bromopropionitrile likewise gave (III) and (IV), respectively, but chloroacetonitrile gave a mixture of pyridine (IX) and pyrimidine (V) products. Reinvestigation of the earlier work confirmed the claims¹ with one exception:^{2,3} this was that acetonitrile yielded the pyrimidone (VI) and not a pyridine as had been thought from the m.p. and Cl analysis. Part of the new identification of (VI) involved hydrogenolysis to (II). In amplification of the reaction between propionitrile and malonyl chloride,¹ it was observed^{2,3} that the main, pyridine product (X) was accompanied by traces of the pyrimidine (VII).

Meanwhile, Bernatek and Stensrud encountered the malonyl chloride-nitrile reaction during some other work and found⁵ that the product (m.p. 233°) from acetonitrile must be a pyrimidine (VI).