Dr. C. G. Harris of Hercules Powder Company, Wilmington, USA, for the sample of *iso*dextropimaric acid.

1. Bruun, H. H. *Acta Chem. Scand.* **9** (1955) 342.
2. Bruun, H. H. *Acta Acad. Aboensis, Math. et Phys.* **19** (1954) No. 3, p. 87.

Received March 24, 1955.

Sialic Acid in Pseudomyxomatous Gels

LARS ODIN

Institute of Medical Chemistry, University of Uppsala, Uppsala, Sweden

It is well known that glycoproteins occur in considerable amounts in the contents of certain types of ovarian cysts. Little recent work, however, has been published on the nature of these glycoproteins, except for the extensively studied blood-group substances, which were shown to occur in pseudomucinous cyst fluids by Morgan and van Heyningen [1]. The carbohydrate component of the blood-group substances has been shown to consist of glucosamine, galactosamine, galactose, and fucose [2-4]. Hiyama [5] has isolated the hexosamines and galactose from the gelatinous 'paramucin' of an ovarian cyst, noting that the material gave the 'direct Ehrlich' reaction, *i. e.* it gave a violet colour when heated with Ehrlich's *p*-dimethylaminobenzaldehyde reagent without previous treatment with alkali. Jensen [6] has isolated a product from a few pseudomucinous cyst fluids thought to be hyaluronic acid. (I have not been able to find this substance in the same type of cysts.)

In a study on the protein and glycoprotein components of a great number of ovarian cyst contents and their relation to the histological type of the cysts, I have found that pseudomyxomatous gels differ not only physically but also chemically from ordinary pseudomucin. An obvious difference is the much higher content of sialic acid in the gels (about 10 % compared with 1–2 %, as determined colorimetrically). Sialic acid has now been isolated in crystalline form from this material, and by chemical analysis and X-ray powder diagrams is shown to be identical with the sialic acid prepared by Blix *et al.*[7] from the submaxillary mucin of sheep.

The pseudomyxomatous material, which forms a water-insoluble gel, was washed with water and broken up in a Turmix blendor. After several days in ethanol, when the gel had shrunk considerably, it was ground in a mortar and after further treatment with ethanol and ether, dried in a desiccator.

The results of analysis of a typical preparation from a cyst-gel are given in Table 1.

Table 1.

Nitrogen (Micro-Kjeldahl)	10.2 %
Glucosamine-HCl *	5.8 »
Galactosamine-HCl *	5.7 »
Galactose **	3.6 »
Mannose **	0.6 »
Fucose **	2.1 »
Sialic acid ***	11.8 »
Ester-sulphate	0.1 »
Ash	3.7 »

* Chromatographic separation of the hexosamines by Gardell's method [8].

** Vasseur's modification of the Tillmanns-Philippi orcinol reaction [9] and a quantitative paper chromatographic method.

*** Colour reactions with Bial's and Ehrlich's reagents [10].

From this material, thoroughly freed from inorganic matter by treatment with very dilute hydrochloric acid, sialic acid was prepared according to the principles set out by Blix [11].

10 g of the dry powder was suspended in 200 ml of water, and heated for one hour on a boiling water-bath. The suspension was centrifuged, and the supernatant filtered and freeze-dried. The small amount of material obtained was extracted with methanol, and the solvent

Fig. 1. X-ray powder diagram of sialic acid from an ovarian cyst-gel.

evaporated *in vacuo*. The residue was dissolved in methanol and a little water. Ether was added, and the amorphous precipitates, which formed regularly, were discarded. Crystalline deposits were obtained in a few days. These were purified by recrystallization from a water-methanol mixture.

Some analytical data of the crystalline material are given in Table 2.

Table 2.

Nitrogen	4.38 %
Acetyl	14.96 »
Methoxyl	0
Decomposition-point	185—187° (uncorr.)
Optical rotation	$[\alpha]_D^{23} = -32° \pm 2°$

The X-ray diffraction pattern is seen in Fig. 1. It is identical with that of sialic acid isolated from sheep's submaxillary mucin.

The same sialic acid was isolated from the pseudomyxomatous contents of two other cysts, and from the peritoneal gel of a pseudomyxoma peritonei also present in one of the cases. (The contents of a mucocele appendicis in the last case had the same composition as the other gels. Isolation of sialic acid was not attempted because of lack of material.)

A detailed report of this work will be published elsewhere.

1. Morgan, W. T. J. and van Heyningen, R. *Brit. J. Exptl. Pathol.* **25** (1944) 5.
2. Bray, H. G., Henry, H. and Stacey, M. *Biochem. J. (London)* **40** (1946) 124.
3. Bendich, A., Kabat, E. A. and Bezer, A. E. *J. Am. Chem. Soc.* **69** (1947) 2163.
4. Aminoff, D. and Morgan, W. T. J. *Nature* **162** (1948) 579.
5. Hiyama, N. *Tohoku J. Exptl. Med.* **51** (1949) 327.
6. Jensen, C. E. *Acta Pharmacol. Toxicol.* **10** (1954) 83.
7. Blix, G., Lindberg, E., Odin, L. and Werner, I. *Nature* **175** (1955) 340.
8. Gardell, S. *Acta Chem. Scand.* **7** (1953) 207.
9. Vasseur, E. *Acta Chem. Scand.* **2** (1948) 693.
10. Werner, I. and Odin, L. *Acta Soc. Med. Upsaliensis* **57** (1952) 230.
11. Blix, G. *Hoppe-Seylers Z. physiol. Chem.* **240** (1936) 43.

Received March 26, 1955.

A New Method for Peroxide Determination

SVEN ARRHENIUS

Kemiska Avdelningen, Kunglig Veterinärhögskolan, Stockholm, Sweden

In a previous paper [1] vanillin dissolved in 70 % sulphuric acid was shown to be a qualitative reagent on peroxides. In trying to apply this test for quantitative analysis we found that with regard to colour production, hydrogen peroxide differs from the tertiary butyl peroxides $(H_3C)_3C-OOH$ and $(H_3C)_3C-OO-C(CH_3)_3$. Hydrogen peroxide (Fig. 1) gives a green colour, only slightly dependent on the added amount of peroxide. Thus this colour reaction can not be used as a quantitative reagent. Moreover, the colour is not sufficiently different from that of aged solutions of protocathecuic aldehyde (demethylated vanillin) or piperonal (vanillin -2 H) in 70 % sulphuric acid.

The tertiary butyl peroxides also colours vanillin—sulphuric acid green, but after a few hours the colours shift over violet into deep blue (Fig. 1). The blue reaction products (called "vanillin-blue" in this paper) precipitate on diluting the sulphuric acid solution with ice water, preferably with at least five volumes. The precipitate dissolves completely without impairement of the colour in concentrated acids, like sulphuric, phosphoric, acetic or formic. Hydrochloric acid should be avoided as evolution of chlorine was observed. Organic bases and alkali destroy vanillin-blue. The precipitate was washed with ice water until the pH of the water was 5, and dried *in vacuo* over $CaCl_2$. In standing at room temperature, vanillin-blue changes colour and solubility.

The formation of vanillin-blue increases with temperature. As the peroxides and vanillin-blue itself, however, decompose on heating, it will be an optimum for the reaction. According to preliminary experiments, this optimum is about 37° C. There are also many practical reasons to use this temperature, and all data given here are obtained by heating the samples at 37° C. The formation of vanillin-blue is slower at higher concentrations. For practical reasons the reaction was stopped after 20 hours. Consequently, the dosage curves will not be straight lines but curved downwards.