## Isolation of N-acetylsialic acid from normal liver

As early as 1905 MANDEL AND LEVENE[1] showed the presence of a substance in the liver that gave a red-violet colour with orcinol-hydrochloric acid. This colour was later proved to be given by sialic acid[2] (*synonym* neuraminic acid[3]). It was therefore astonishing that KLENK AND FAILLARD[4] found a negative orcinol-hydrochloric acid reaction in normal liver tissue. On the other hand, they isolated rather large amounts of the methyl glycoside of sialic acid (1.6% calculated on dried defatted material) in amyloid degeneration of liver. They postulated that sialic acid was a component characteristic of the pathological protein occurring in amyloid degeneration. As all proteins of globulin nature hitherto investigated contain sialic acids, and a large part of the liver proteins are globulins, we applied to normal livers the recently described method[5] for the isolation of N-acetylsialic acid.

Normal human livers (checked by microscopical investigation) were removed as soon as possible after death (12–24 h) with intact blood vessels. The livers were perfused with physiological saline through the hepatic vein until the perfusion solution contained no visible amounts of blood cells or pigments. This was necessary because plasma proteins contain large amounts of sialic acid (about 600 mg N-acetylsialic acid/litre serum). After removal of blood, the livers were homogenized in a Turmix blendor and extracted twice with 4 vol. ethanol. The defatted livers were then treated with 0.01 *N* $H_2SO_4$ at 0° C to remove cations and other low molecular substances. N-Acetyl sialic acid was liberated with 0.05 *N* $H_2SO_4$ at 80° C and the hydrolysates were chromatographed on Dowex-2 in formate form[5]. The fractions containing the sialic acid were lyophilized. The crystallization was performed by dissolving 200 mg of dried material in 1 ml of distilled water, and adding 10 vol. methanol–ethanol (2:1, v/v) and 20 vol. ethyl ether. A small amount of amorphous material was removed by filtration. To the filtrate, light petroleum (b.p. 30–40° C) was added to a faint opalescence and the flask was left for 24 h. The crystals were filtered off and additional ethyl ether-light petroleum was added to the filtrate, giving another crop of crystals. The two fractions were recrystallized from methanol-water by addition of ethyl ether.

The isolated sialic acid was compared with an authentic sample of N-acetylsialic acid ($C_{11}H_{19}NO_9$) isolated from human serum. X-ray diffraction pattern, optical rotation, $R_F$-values in paper chromatography[5] and molar absorbancy indices in colorimetric methods[6] were identical.

In one case a quantitative isolation of N-acetylsialic acid was performed. The whole liver (1850 g) contained 220 mg N-acetylsialic acid measured by the resorcinol method, *i.e.* 12 mg % protein-bound N-acetylsialic acid, calculated on fresh weight. The yield of recrystallized N-acetylsialic acid was 152 mg or 69%.

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## The growth of cellulose microfibrils from *Acetobacter xylinum**

Extracellular production of cellulose microfibrils in cultures of *Acetobacter xylinum* is well established[1,2] but the mode of their formation is obscure. An electron microscope study by MÜHLETHALER suggested that the microfibrils crystallize from a high-polymeric, amorphous precursor concentrated in a homogeneous region large compared to a bacterium[2]. The preparations examined by MÜHLETHALER were pseudoreplicas of cellulose films withdrawn at intervals from active cultures grown in beer. Interpretation of the appearance of such preparations, however, may easily be complicated by debris from the culture, since insufficient washing of the films may leave residues from the beer, while too much washing may damage the specimen and remove pertinent material. The manner of formation of cellulose microfibrils has therefore been re-investigated in the electron microscope by incubating cultures in a synthetic medium of low molecular weight under conditions which minimized contamination and mechanical disturbance.

Cells were prepared free of cellulose as described by HESTRIN AND SCHRAMM[1] except that they were not freeze-dried but stored as a suspension in phosphate-citrate buffer, pH 6.0, 0.01 *M* in phosphate. Drops of an appropriate dilution of the suspension in 2% glucose, 0.27% $Na_2HPO_4$

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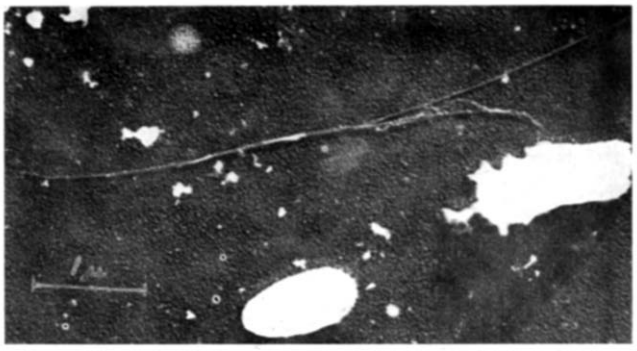

Fig. 1. Cellulose microfibrils from *A. xylinum* after 1 min incubation.

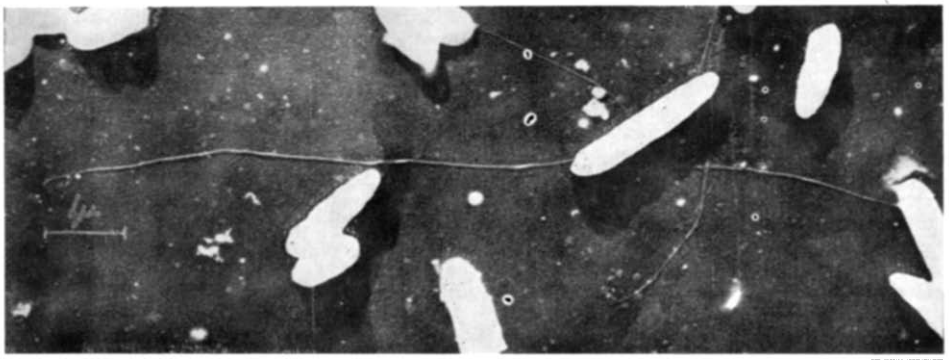

Fig. 2. Montage of three electron micrographs of successively adjacent portions of cellulose microfibrils from *A. xylinum* after 2 min incubation.

and 0.115% citric acid, pH 6.0, were placed on formvar films floating on the same medium at 30° C. Incubation of the drops was stopped at 0–10 min by pipetting 40% formaldehyde solution underneath the films. Glucose and salts were removed by repeated replacement of the solution under the films with distilled water. The films carrying the drops were then mounted on copper grids, dried, shadowed with Pd–Au and examined.

Zero-time preparations showed no microfibrils among the bacterial cells. After 1–2 min incubation, however, many discrete short microfibrils (about twice a bacterial cell length) were seen dispersed randomly among the cells (Fig. 1). The length of the microfibrils (but not their width) increased rapidly with time of incubation (Fig. 2) so that after 10 min it could not be determined accurately. Many isolated microfibrils were seen and no indication of definite association between bacterial cells and microfibrils could be detected. Even after 1 min incubation, microfibrils were often intertwined like a rope, the strands being approximately equal in length (Fig. 1). Both blunt and tapered ends of microfibrils have been observed (Fig. 2). No evidence of amorphous intermediate material was seen either near the cells or about the microfibrils in any preparation. Examination of pseudoreplicas of cellulose films from cultures in various media, including beer, also failed to reveal any amorphous material from which microfibrils might crystallize.

These observations confirm MÜHLETHALER's[2] conclusion that microfibril formation takes place remote from the bacterial cell wall. However, they are strong evidence against the suggestion of an extensive high polymeric amorphous, intermediate phase. In contrast, bacterial cellulose microfibrils appear to grow rapidly from initially short, discrete, threads by the addition of material to the ends of the threads. The molecular size of this material is apparently too low for it to be retained by the formvar film or to be resolved in the electron microscope. Quantitative studies of the rate and mechanism of this addition are now under way.

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