Preliminary Notes

Formation of helical strands by sodium deoxycholate as revealed by electron microscopy

In experiments in which vaccinia virus was exposed to 0.5–1.0 % sodium deoxycholate at pH 7, and then dialyzed for 2–3 h against distilled water, subsequent electron micrographs revealed long fibers surrounding the few virus particles which survive such treatment. These fibers were not seen when the virus–deoxycholate mixture was dialyzed for 10–15 h, or when virus was inactivated by urea or guanidine-hydrochloride[1].

Typical electron micrographs of purified vaccinia virus treated with 0.9 % sodium deoxycholate and dialyzed for 2–3 h are shown in Figs. 1a and 1b. Samples of the dialyzed suspensions were mounted directly on Formvar-coated grids, air-dried in the presence of osmium vapor for 1–2 h, shadowed with chromium, and examined in a RCA-3B electron microscope. The majority of virus particles surviving deoxycholate treatment appear grossly normal, as will be described in detail elsewhere. It will be seen, however, that the virus particles are surrounded by masses of fibers of indefinite length and of remarkably uniform helical structure (Figs. 1a, 1b). The smallest fiber diameter is approximately 40 Å (Fig. 1b); larger fibers (diameter approximately 100 Å) frequently appear in parallel sheets (Fig. 1a). These latter fibers are possibly composed of two or more strands of 40 Å diameter fibers, and in other micrographs two fine strands were seen "unwinding" from the ends of thicker helical fibers.

The addition of deoxyribonuclease together with 0.005 *M* $MgCl_2$ to the virus-deoxycholate suspension caused a flocculent precipitate which carried down most of the fibers. It was then observed that deoxycholate alone formed a similar precipitate on the addition of traces of $MgCl_2$. Solutions of sodium deoxycholate (0.5 % in distilled water) were therefore placed directly on grids, exposed to osmium vapor, shadowed with chromium, and examined in the usual way. Long helically-wound fibers of similar dimensions were again seen (Fig. 1c). Identical structures were obtained from two samples of commercially purified deoxycholate*. In general, the detailed helical structure was most clearly revealed in preparations that also contained virus, and micrographs of deoxycholate alone seldom showed the helices as clearly as in Fig. 1c.

Electron micrographs illustrating the fibrous helical structure of the steroid have not been previously reported in the literature. RICH AND BLOW[2] carried out X-ray diffraction studies on fibers drawn from viscous complexes of sodium deoxycholate and glycylglycine, and concluded that the complex has the form of a helix; the *a* axis of the helix varied from 36.2–49.1 Å, depending on the mole ratio of glycylglycine to deoxycholate. Further detailed studies by these authors on deoxy-

* Fisher Scientific Co., New York, and Nutritional Biochemical Corp., Cleveland.

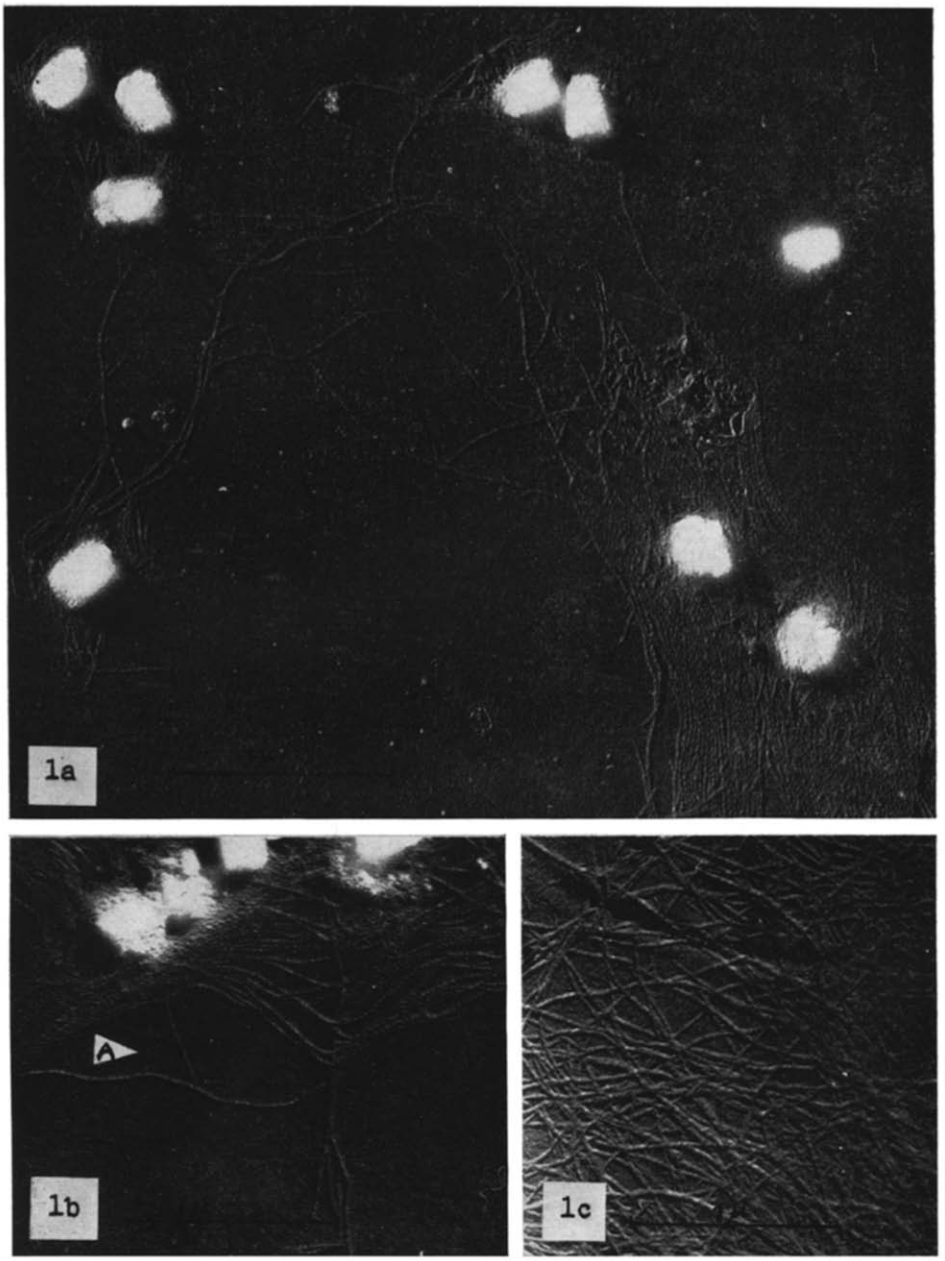

Fig. 1. Electron micrographs of vaccinia virus-deoxycholate mixtures and of pure deoxycholate. (a) Typical appearance of virus-deoxycholate suspension after dialysis for 3 h. (b) Similar preparation showing a fine fiber (A). (c) Sodium deoxycholate alone.

cholate and other steroids have shown that gel formation is determined by various factors including pH, ionic strength, and type of ion in the buffer[3]. The diameter of the smaller fibers seen in our electron micrographs is closely similar to that calcu-

lated from X-ray diffraction patterns[2], and the helical structure predicted from X-ray diffraction is strikingly revealed in the electron microscope.

Deoxycholate has been widely used to prepare deoxyribonucleic acid from bacteria[4] and infectious "ribonucleic acid" from animal viruses[5]. Since the steroid may form long helical fibers under certain conditions, and hence dialyzes slowly, electron micrographs taken after using it should be interpreted cautiously.

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Type of attachment of sialic acid in ox-brain mucolipid

Sialic acid is easily liberated from brain mucolipid preparations under mildly acid conditions[1,2]; it may thus partly be attached terminally to the rest of the glycolipid polymer in a comparatively labile glycosidic linkage. Since structural models proposed heretofore for these interesting and complex substances are entirely hypothetical, we have undertaken a study of the various linkage types supporting the architecture of the polymer. We limit ourselves here to a consideration of sialic acid and to the behavior of a purified ox-brain mucolipid, both intact and after hydrolytic removal of sialic acid, towards oxidation by periodate (Fig. 1).

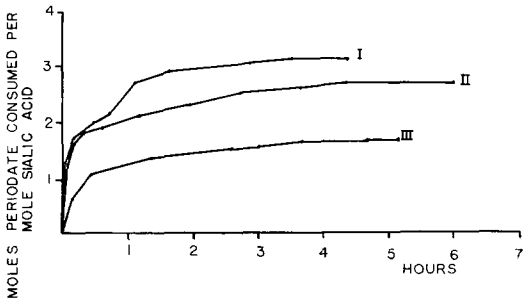

Fig. 1. Oxidation with metaperiodate at pH 4. I, free sialic acid; II, mucolipid after treatment with 0.1 *N* H_2SO_4 at 80° for 2 h; III, intact mucolipid.

The preparation of the mucolipid and, from it, of crystalline ovine sialic acid (5-N-acetylneuraminic acid) were described previously[2]. The reactions were carried out in 5 m*M* aqueous solutions of sodium metaperiodate around pH 4. Oxidant