Flagellar Adhesion of *Crithidia fasciculata* to Millipore Filters

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Summary

Millipore filters composed of mixed cellulose esters have been used to investigate the adhesion of flagella of *Crithidia fasciculata* to a non-living substrate. After 4 days on the surface of a culture of the flagellate, one side of the filter was covered with a monolayer of cells. In most cases the flagella penetrated the pores of the filter and at one or more points along their length presumed sites of adhesion were marked by the presence of hemidesmosomes, characterized by a thickened inner leaflet of the flagellar membrane and the presence of large numbers of fine filaments. If the interstitial space of the filter was sufficiently large, the hemidesmosomes occurred at the apex of an evagination of the flagellar membrane. These evaginations are believed to arise by movement of the flagellum relative to the point of adhesion. The addition of distilled water causes de-adhesion of the flagellum and its withdrawal from the filter. The hypothesis that cells adhere to one another and to non-living substrates by different mechanisms is discussed in the light of the results obtained here.

1. Introduction

When *Crithidia fasciculata* is grown in *in vitro* culture, a large proportion of the cells become attached by their flagella to other flagellates to form balls of cells called rosettes. These cell aggregates are maintained by flagellum-flagellum adhesions and by the adhesion of flagella to central particles of debris. In most cases, this debris can be identified as cell membrane and is probably derived from the disintegration of dead flagellates in the culture. In addition, it is known that the flagellum of *C. fasciculata* is able to adhere to non-living substrates such as glass (Wallace 1943). Coman (1961) and Berwick and Coman (1962) have suggested that the mechanism by which cells adhere to one another differs from the mechanism by which they adhere to non-living substrates. The present study was therefore initiated to examine the morphology of flagellar adhesions to such a substrate and to compare this with the flagellum-flagellum adhesions previously described from rosettes (Brooker 1970). Millipore filters composed of mixed cellulose esters have
been found to provide a suitable biologically inert substrate to which flagellates readily adhere.

2. Materials and Methods

The strain of *Crithidia fasciculata* used was that isolated by Noguchi and Tilden (1926) from the mosquito *Anopheles quadrimaculatus*. The flagellate was cultured in 3 inch Petri dishes containing 5 ml of 10% brain-heart infusion (Oxoid) and 0.1 ml of defibrinated horse blood. A sterile Millipore filter (pore size 0.22 μm) composed of mixed cellulose esters was carefully floated on the surface of the culture at the time of inoculation with the flagellate. After 4 days at 25 °C, the Millipore filter was removed from the culture and placed in 0.2 M cacodylate-buffered 5% glutaraldehyde (pH 7.4) for 15 minutes at room temperature. The fixed material was given several 1 hour washes in 0.2 M cacodylate buffer before post-fixation in 0.2 M cacodylate buffered 0.5% osmium tetroxide (pH 7.4) for 5 minutes. Before dehydration, the filter was treated with 1% uranyl acetate in 25% alcohol for 30 minutes. After dehydration in ethanol-water mixtures and absolute alcohol, the filter was placed in a 1 : 1 mixture of Araldite resin and hardener for 2 hours and then embedded in the final Araldite mixture. Sections were cut using a Porter-Blum MT 2 ultramicrotome and a diamond knife (Dupont) and were stained using lead citrate prior to examination in an EM 6 B electron microscope.

For light microscopy, thick sections of embedded material were stained with toluidine blue and examined with a Leitz Ortholux microscope fitted with an Orthomat automatic camera. To investigate the effect of distilled water on flagellar adhesion, a Millipore filter was removed from the culture medium, washed several times and then mounted in distilled water. Light microscope observations were made over a period of 3 hours.

3. Results

3.1. Light Microscopy

After 4 days on the surface of the culture, one side of the Millipore filter is found to be completely covered with a monolayer of flagellates (Fig. 1). Most of these are haptomonads, *i.e.*, possess the characteristic ovoid body and shortened flagellum. Although some cells are attached to the filter only by the tip of the flagellum, most have their flagella firmly embedded in the filter with the anterior end of the body lying very close to its surface. The dia-