Electron microscopical evidence of the evolution of corynebacteria-like microorganisms within human erythrocytes

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Summary. The corynebacteria-like microorganisms evoluting in the haemocultures take origin from electron dense granular bodies carried within the erythrocytes.

An incorporation of labelled nucleosides and amino acids has been observed in suspensions of erythrocytes[1,2] and platelets[3] from clinically normal human subjects, and attributed to the metabolic activity of bacterial L forms. The incubation of suspensions of platelets in suitable media often gave rise to the multiplication of cocci attributable to various strains of *Staphylococcus epidermidis* and taking origin from unstable L forms carried within the platelets[4–7]. Following the incubation of total blood or erythrocytes, the growth of polymorphous often branched corynebacteria-like microorganisms together with cocci has been observed.[8].

The occurrence in the circulating human blood of the L forms of a microorganism recognized as *Bacillus licheniformis* (var. *endoparasiticus,* Benedek) eventually reverting in subcultures to the sporogenous conventional form through stages resembling Listeria or a diphteroid, has been repeatedly described: see Pease[9] and Bisset[10], also for a review of the literature. Phase contrast examinations and electron microscopy (gold-palladium shadowed preparations) appeared to be indicative of the close association of the infection with the erythrocytes (Pease[11,12]).

In the course of research concerning the multiplication of microbes in cultures from samples of whole blood from patients in various pathological situations, we have recognized by means of electron microscopy on ultrathin sections the evolution of corynebacteria-like forms taking origin from electron dense granular bodies carried within the erythrocytes. This is an account of such observations.

No reference will be made to the pathological situation of the donors since the microbial strains resembling corynebacteria which have been considered in the course of the present research have not shown any difference of behaviour which might be related to a particular disease.

Materials and methods. Specimens of blood from 110 patients with various pathological situations have been utilized. The cultures were made in BBL prepared culture bottles (Division of Becton, Dickinson and Co., Cockesville, USA) containing 25 ml of trypticase soy broth with agar surface, carbon dioxide and sodium polyanethole sulfonate. With all precautions for sterility, 2.5 ml of blood were introduced into the bottles by means of the BBL blood taking units.

The bottles, never opened, were incubated at 37 °C and

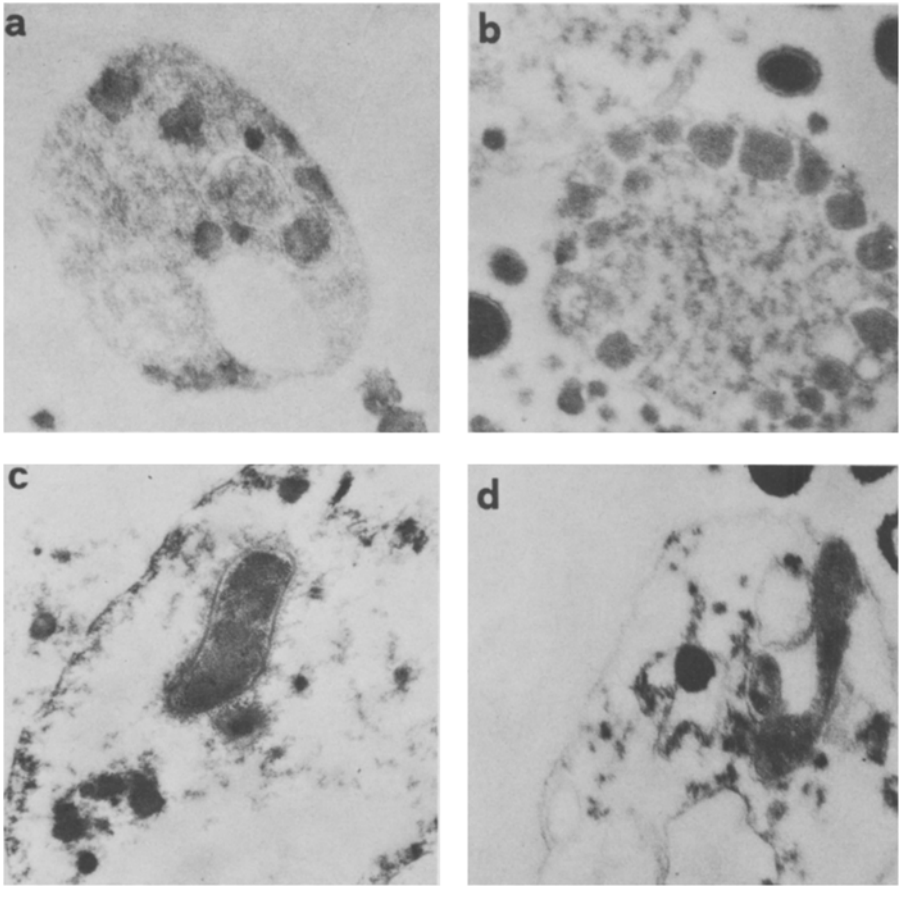

Fig. 1. Corynebacteria-like microorganisms taking origin from electron dense granular bodies may be recognized within the erythrocytes undergoing a spontaneous process of lysis. a, ×35,000; b, ×23,000; c, ×15,000; d, ×26,000.

examined at successive intervals of 3–5 days in order to recognize an eventual bacterial growth on the agar surface and/or a process of haemolysis. After 4–8 weeks' incubation the bottles were shaken, samples of the blood suspensions collected and utilized for the preparation of the subcultures and for optical and electron microscopy.

For the subcultures and the characterization of the bacteria, conventional methods were utilized[13]; a detailed account of the results will be given in a further publication. For optical microscopy smears from the primary cultures and the subcultures were stained with Giemsa, basic fucsin, Gram, Ziehl-Nielsen for acid fast recognition, and Albert for the recognition of metachromatic granules. The examination of unstained preparations with UV light was also carried out.

For electron microscopy, samples of the primary cultures showing a bacterial growth were centrifuged at 3500 × g for 10 min, the sediments prefixed in glutaraldehyde, fixed in 1% OsO_4 in 0.1 M phosphate buffer pH 7.4 and embedded in araldite. The specimens were stained with uranil acetate and lead acetate and examined with a Philips EM 201 electron microscope.

Results and discussion. In the course of the incubation of some primary cultures, a bacterial growth was observed on the agar surface of the bottles, together with a more or less pronounced process of haemolysis. In other cases, the haemolysis was fairly evident, but no bacterial growth was detectable by means of naked eye or hand lens inspection of the agar surface. At the end of the incubation, the cultures from 6 patients carrying recognized bacterial forms not considered to be in the scope of the present research (*P. mirabilis, E. coli, S. tiphi, Salmonellae sp.*) were discarded. Other cultures and subcultures, from blood repeatedly drawn (2–4 times at 2–10 days intervals) with coincident results from 38 patients, showed the growth of cocci (attributable to various strains of *Staph. epidermidis)* and/or microbial forms looking like corynebacteria.

Concerning corynebacteria-like forms, optical microscopy in every case demonstrated a high grade of pleomorphism, Gram positivity with a notable variation in the ease of decolorization among the cells within the same culture, very rare acid-fast cells. Many cells carried metachromatic granules, often very large and in terminal situations, easily recognizable on the basis of their staining properties and UV absorption.

Notwithstanding the variability of such features, referable to the growth cycle of the coryneform bacteria, distinctive morphological and physiological characters have been recognized and considered to be indicative of the presence of at least 4–5 different well defined bacterial strains: it has to be noted that each patient carried a unique form. The possible meaning of such observations will be the scope of further research.

Electron microscopy applied to the primary cultures confirmed and completed the data obtained by means of optical microscopy, concerning the behaviour of the bacterial forms free in the liquid medium. Moreover, the evolution of structures not detectable by means of optical microscopy, appeared to be indicative of a developmental cycle taking origin from very small compact osmiophilic granules carried within the erythrocytes.

Because of the strong opacity of the red cells towards the electronic beam, the presence, the increase in size and the evolution of the granules towards conventional bacterial forms were easily recognizable only within the erythrocytes undergoing a process of lysis, or when they had been set free from the erythrocytic stroma. In figures 1 and 2, some

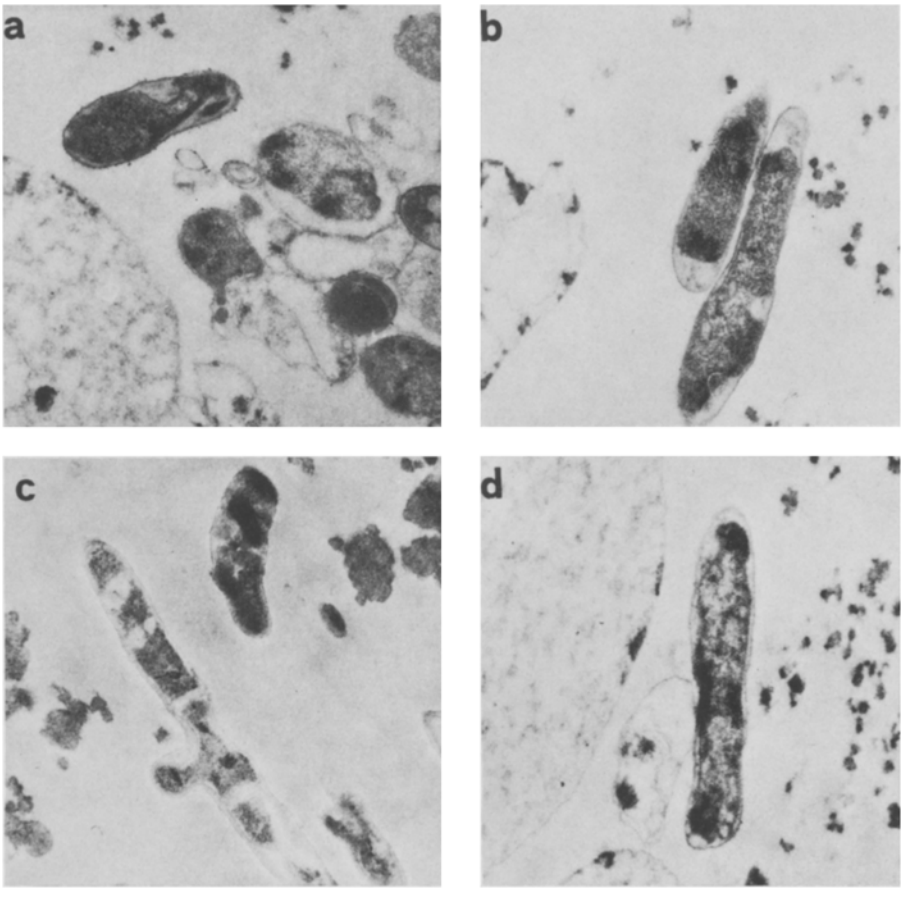

Fig. 2. Corynebacteria-like microorganisms free in the medium between erythrocytes ghosts. a, × 13,000; b–d, × 20,000.

aspects observed following 45 days' incubation of 4 blood specimens are reported.

The observations here described indicate that the corynebacteria-like forms taken under consideration in the course of the present research undergo, through a great variety of morphological appearances, a developmental cycle fairly referable to the one already described in the literature[14].

The present observations appear to be in accordance with the point of view of Pease[9,11,12] concerning the association of the infection with the erythrocytes, and also with previous data[15] related to the intraerythrocytic growth patterns of the cell wall deficient variants of other microbial forms.

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The ultrastructure of the spiral notosetae of *Nicomache maculata* Arwidsson (Polychaeta, Maldanidae)

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Summary. Scanning- and transmission electron microscopy show that the long, thin notosetae of *Nicomache maculata* are helical over much of their length, and that their finely feathered appearance is produced by a series of minutely spiny scales. The internal structure varies considerably from one end of the seta to the other. While a helical shape could result from a rotating or asymmetrical secretion-rate gradient across the chaetoblast, we raise the possibility that the spiral represents a warp in the seta following deposition of the setal material.

A tremendous variety of setae are produced by polychaetes. Among the more spectacular of these are the extremely long and thin spiral notosetae on the posterior segments of some Maldanidae. The setae are visible in a good dissection microscope, and they appear in some of the older figures of *Nicomache* species[1]. They are not always figured as having a helical shape, however, and the details of their surface features, beyond the resolving capacity of even the best light microscopes, have been variously interpreted. Here we present some results of an examination using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) of these remarkable setae in *Nicomache maculata*, with some measurements and comments on their structural properties.

We collected the specimens from the shore near the Dove Marine Laboratory[2], Cullercoats (England) and processed them for SEM and TEM as described elsewhere[3,4].

The long, thin setae of species of *Nicomache* have been described as wavy or sinuous[5,6]. Viewed from different angles in the dissection microscope, these setae in *Nicomache maculata* appear helical rather than wavy in either living or fixed material, and our SEMs (figure, A and B) show they are spiral over much of their length. In our larger specimens, whose perianal funnel is about 2 mm in diameter, the longest of the spiral setae may be combed straight with a forceps to a length of more than 3 mm.

The feathered[5,7] or pinnate[6] appearance of these setae in the light microscope is produced by an arrangement of minute spines (figure, C–G). At their fullest development, these spines are grouped to form a series of partially overlapping, distally pointed, featherlike scales, each wrapped around the setal axis. Each scale is grossly asymmetrical, such that the shorter edge of each scale (figure, E, s) meets the longer edge of a more distal scale (figure, E, l) at a point only part way around the seta (figures E, p; J, f).

Near its proximal end (not figured), a fully-grown spiral seta has a structure characteristic of setal shafts from other polychaetes[8–11]. The shaft is a cylindrical bundle of tubules of various diameters, from up to 0.8 µm near the axis to 0.3 µm or smaller at the periphery. The tubules are cylindrical, so that while they are closely packed, there are electron-translucent spaces among them. The electron opaque wall of each tubule encloses an electron-translucent lumen irregularly traversed by fine strands.

More distally, where the shaft emerges from the body wall (figure, H), the boundaries of most of the tubules are obscured by electron-opaque material, and the lumens are narrower than near the proximal end. The spatial arrangement of the centres of the tubules is like that more basally, however, and a few electron-translucent packing faults are visible. Still more distally (figure, I), nearly all the lumens are reduced to small points in section. Here, however, the boundaries of the tightly-packed tubules appear as electron-translucent polygonal outlines. Some of the outer polygons appear to be joined, and large numbers of very small ones gather just within the margin.

Over most of its length, the seta is decorated by spines, and throughout this region (figures, J and K) the tubules described from the shaft are either absent or obscured by other features. Small irregular or circular electron-translucent spaces appear, but they are not arranged as described above, nor can they be traced very far through serial sections. Each spine contains a tiny lumen (figures, K and L) which can be traced to the point where the spine merges with the setal axis.

The specific shape of a polychaete seta is produced by changes in the form of the apical surface of the chaetoblast upon which it is molded during basal appositional growth[12].