surface is spined (Figure 4). The ventral lobe is free at its apex and lateral margins, but is fixed to and arises from the mid-ventral cup wall at its base. The host villus comes to lie between the apposing surfaces of the 2 adhesive organ lobes.

The outer and inner surfaces of the fore-body cup and the surface of the adhesive organ lobes adjacent to the cup wall are covered with 'normal'[8] cytoplasmic tegument. In contrast, the apposing surface of the lobes is covered with a specialized, chambered cytoplasmic surface (Figure 2). The chambers are covered with a unit plasma membrane and from the walls arise long slender microvilli (Figure 3). The walls of the cup and the adhesive organ lobes contain extensions of the excretory lacuna system and the walls of this are cytoplasmic and bear lamellae[9]. The host tissue plug, lying between the lobes, shows considerable lysis and in many cases the host capillaries come into close contact with the specialized adhesive organ surface (Figure 4).

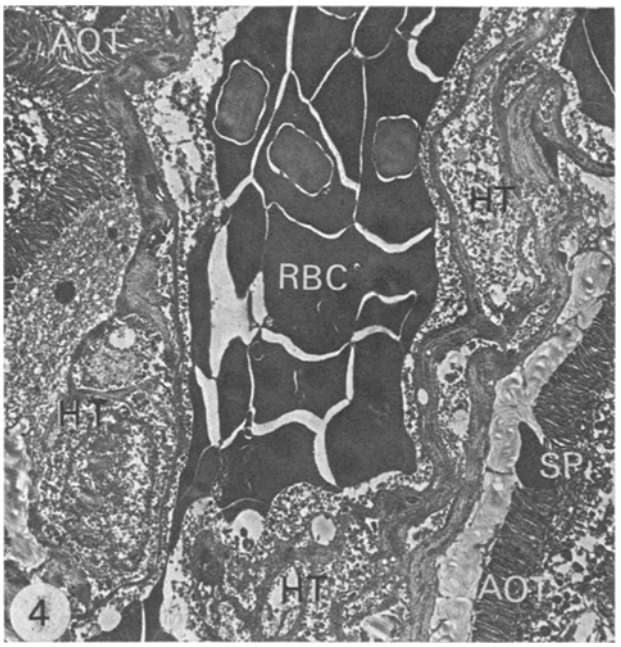

Fig. 4. Electronmicrograph of host tissue plug (HT) lying between the specialized surfaces of the adhesive organ lobes (AOT). Note that the host tissue contains capillaries and erythrocytes (RBC), which lie very close to the adhesive organ surface. Spines (SP) are present on a portion of this surface. Stained as in Figure 2. × 2000.

Surface specialization therefore, also exists in *Cardiocephaloides* and the nature of this surface suggests that it might play a vital, possibly placental role, in the absorption of nutrients from the host. The excretory lacunae, present in these lobes, might also aid in the translocation of absorbed nutrients. It is also worth mentioning that it is possible to use, for ultrastructural studies, material fixed in the field for other purposes, although it is suitable for the study of the more obvious ultrastructural features only[10].

Zusammenfassung. Die ultrastruktuelle Untersuchung des Haftorgans von *Cardiocephaloides physalis* (Trematoda) lässt eine mit Aushöhlungen versehene zellplasmatische Oberfläche erkennen, die mit der lamina propria der Wirtsdarmzotte (*Phalacrocorax bougainvillei*) in engstem Kontakt steht und möglicherweise Plazentafunktion besitzt.

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[7] J. G. Baer, Parasitologieskii Sbornik, XXIV. (Academia Nauk SSSR, Moskwa 1969), p. 7.
[8] D. A. Erasmus, J. Parasit. *53*, 703 (1967b).
[9] D. A. Erasmus, J. Parasit. *53*, 525 (1967a).
[10] I am extremely grateful to Prof. Baer for his interest and generosity in sending me the specimens, and to Mr. T. Davies for his technical assistance.

Properties of a Blue-Green Algal Sheath

Structures external to the cell wall (i.e. sheaths and capsules) are common among the Procaryota, although they are probably more typical of the blue-green algae (Myxophyceae) than the bacteria. In the latter group the sheath is a fairly well defined morphological feature – a hollow structure enclosing a chain of cells or a trichome[1]. It is chemically distinct from the capsule in *Sphaerotilus natans*[2]. In the blue-green algae all structures external to the cell wall, whether they be hollow tube-like structures with defined margins visible under phase-contrast microscopy (e.g. *Tolypothrix*) or less obvious, more mucilaginous, structures with indistinct outer edges (as in *Nostoc, Anabaena*), are referred to as sheaths. In studies of the sheath of an isolate prepared in this laboratory, referred to as strain FP23 (it is probably a *Nostoc* sp.), the sheath showed reactions typical of a bacterial capsule, and nigrosin showed a surprising affinity for protein absorbed by the sheath.

Filaments of a culture of FP23 in Knop's medium[3] possessed thick gelatinous adherent sheaths up to 25 μ in diameter. These sheaths become clearly visible under phase-contrast microscopy when 'stained' non-specifically with 0.1% bovine serum albumin (BSA) at pH 3.4 (Tomcsik and Guex-Holzer[4]). Presumeably, as suggested by Tomcsik and Guex-Holzer[4], the protein was forming a salt-like compound or precipitate in the sheath. Flushing such preparations with McIlvaines buffer at pH 7.2 removed the protein and the sheaths reverted to their normal poorly visible state under phase-contrast microscopy.

When BSA 'stained' sheaths at pH 3.4 were mounted in 1/3000 nigrosin they were found to be lightly positively stained. The depth of staining with nigrosin was variable,

[1] V. B. D. Skerman, *A Guide to the Identification of the Genera of Bacteria* (Williams and Wilkins Co., Baltimore, 1967), p. 19.
[2] A. H. Romano and J. P. Peloquin, J. Bact. *86*, 252 (1963).
[3] E. G. Pringsheim, *Pure Cultures of Algae* (Cambridge University Press, 1949), p. 35.
[4] J. Tomcsik and S. Guex-Holzer, J. gen. Microbiol. *10*, 97 (1954).

both between filaments and within the same filament. Areas with a striated appearance, which were most opaque in phase-contrast microscopy also stained most intensely. This staining was associated with the presence of the protein (or protein-sheath complex) as shown by the following observations.

A suspension of the alga was washed and resuspended in buffer at pH 3.4 containing 0.1% BSA. To this was added a volume of 1/3000 nigrosin (G. T. Gurr 'Nigrosin W.S.') in buffer at pH 3.4. The amount of nigrosin taken up by the protein-sheath complex was estimated by comparing the optical density of the nigrosin solution at 750 nm with the optical density of the supernate of the centrifuged nigrosin/alga mixture. Control experiments were conducted under identical conditions, except that BSA was omitted. These experiments showed that consistently more nigrosin was absorbed by BSA-treated filaments at pH 3.4 than by untreated filaments at pH 3.4, and very much more than BSA-treated filaments at pH 7.2. From 50–70% of this absorbed nigrosin could be removed by 1 or 2 washes with buffer at pH 7.2, leaving the filaments quite unstained under the microscope. (The greater the absolute amount of nigrosin taken up, the greater was the percentage recoverable in buffer at pH 7.2.) Washes at low pH released less than 10% of the absorbed nigrosin from BSA-treated filaments. A similar experiment with a capsulate *Bacillus* sp. gave similar results. Typical experimental findings are shown in the Table.

Stainability of *Nostoc* and *Bacillus* capsules with nigrosin

Organism	BSA	pH	% available nigrosin absorbed	% of absorbed nigrosin released at pH 7.2
Nostoc FP23	+	3.4	58%	69%
	—	3.4	29%	36%
	+	7.2	7%	–
Bacillus sp.	+	3.4	80%	77%
	—	3.4	27%	52%
	+	7.2	<10%	–

The sheath of FP23 is likely to be chiefly polysaccharide[5]. Its reaction with proteins at pH values near their isoelectric points is very similar to that displayed by bacterial capsules[4]. From a morphological point of view also, it would seem that the 'sheath' of FP23 is more comparable with bacterial capsules than the sheaths found in Chlamydobacteria. Whether this can be said for all blue-green algal sheaths is not clear, but it is unlikely to apply, for example, to genera like *Tolypothrix*.

The affinity of nigrosin for protein absorbed by polysaccharide gels was quite unexpected. Possibly the experiments reported here may afford a means of estimating such protein. Nigrosin is widely used in bacteriology as a negative stain, and it is occasionally used in histology as a positive stain for nervous tissue, but it does not seem to have any recorded affinity for particular cell components[6,7].

Zusammenfassung. Die Polysaccharid-Schale, resp. Kapsel der Schleimalge *Nostoc* sp. (Myxophyceae) kann nach Serumalbuminbehandlung mit Nigrosin bei niedrigen pH-Werten gefärbt werden.

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[6] H. J. CONN, *Biological Stains* (Williams and Wilkins Co., Baltimore 1961), p. 125.
[7] E. GURR, *Encyclopedia of Microscopic Stains* (Leonard Hill Books Ltd., London 1960), p. 297.

PRO EXPERIMENTIS

A Simple Method for the Estimation of Yeast-Growth in Hydrocarbon-Substrates by Determination of Turbidity

When microorganisms are cultivated in substrates containing hydrocarbons, the estimation of growth is in general more difficult than it is in the case of a sugar substrate. The reason for this disadvantage lies in the fact that those culture media always consist of an aqueous and of a hydrocarbon phase. As long as the culture is agitated mechanically the hydrocarbon phase is emulsified and a more or less homogeneous suspension can be maintained. This emulsion is supported and stabilized to a certain degree by the presence of fatty acids and proteins, which are produced during the growth of the yeast population. Whenever agitation ceases, the emulsion is decomposed and the largest part of the hydrocarbon phase, containing the bulk of the microorganisms, accumulates as a surface layer. Under these circumstances, sufficient mixing of the culture medium is necessary during the sampling procedure. Furthermore, representative sampling is rendered more difficult by the strong adhesion of the hydrocarbon phase to the glassware, and inhomogeneity is again the result.

GATELLIER et al.[1] reported several methods suitable for the estimation of growth. In their experiments the only source of nitrogen supplied to growing yeasts in the medium were ammonium salts. The amount of NH_4OH supplied, which was necessary for maintenance of a constant pH, was recorded continuously. By plotting the logarithm of volumes of ammonia solution consumed as a function of time, a straight line was obtained representing exponential growth. For the determination of cell concentration a method using filtration on a millipore membrane was described. The cells were washed by isopropyl alcohol and weighed after drying at 100 °C. Consumption of oxygen and production of carbon dioxide were also measured. It has been shown by the authors that all these methods gave the exact same value for the generation

[1] C. GATELLIER, G. GLIKMANS and D. BALLERINI, Kinetics of Alkane Oxidation and Assimilation by Yeasts. Presented at the 154th Congress of the Am. chem. Soc., Chicago Meeting, Sept. 1967.