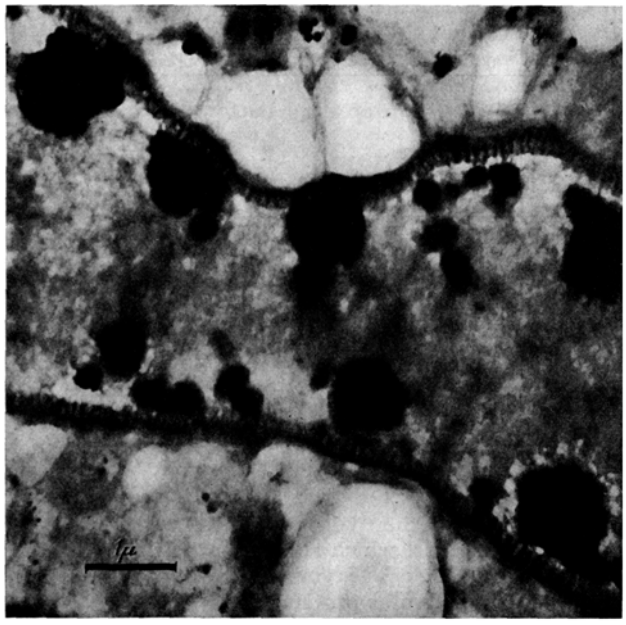

Fig. 1.–Cross section of part of *Amoeba nucleus*. Fixed in osmic, dichromate, lanthanum mixture.

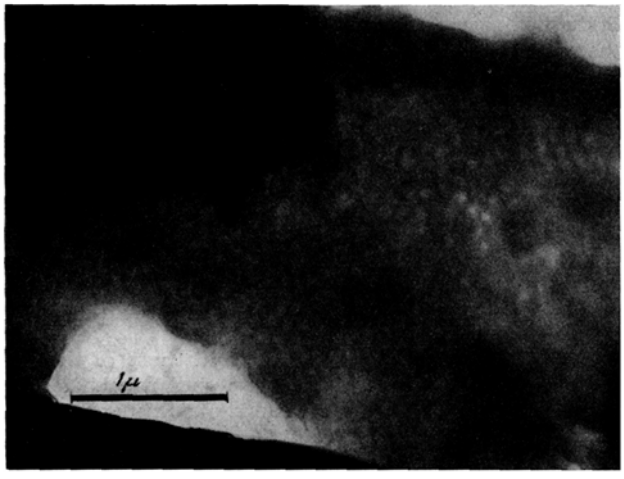

Fig. 2.–Section of nuclear membrane cut parallel to the surface, showing arrangement of pores. Fixed in 2% osmic acid.

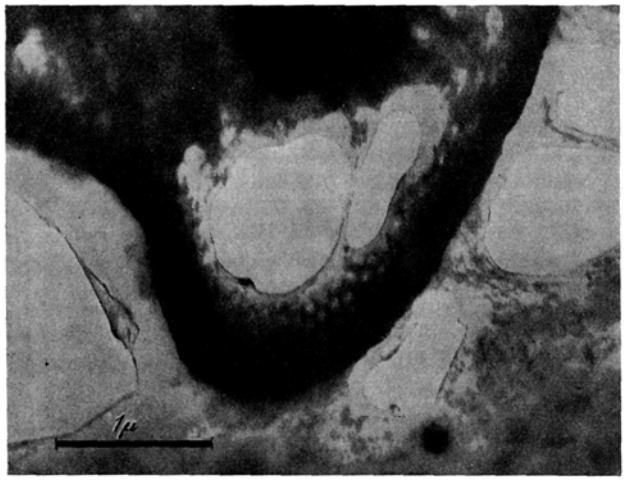

Fig. 3.–Oblique section through nuclear membrane. Fixed in 2% osmic acid.

Electron Microscope Study of the Nuclear Membrane of *Amoeba Proteus* in Thin Section

The contribution of nuclear material to the cytoplasm during certain stages of cellular activity has given rise to an interest in the structure of the nuclear membrane across which this material must pass, and which might give a clue as to the nature of this exchange. Electron microscope investigations have already been made, employing a variety of techniques. CALLAN and TOMLIN[1], using nuclei of *Triturus* and *Xenopus* oocytes, spread out individual membranes on microscope specimen supports; BAUD[2] made replicas of nuclear surfaces in rat liver; and BAIRATI and LEHMANN[3] studied fragmented nuclei of *Amoeba proteus*. In our investigation of the nuclear membrane of *Amoeba proteus* we used the method of thin sectioning for the electron microscope.

Amoebae were fixed in two different fluids: 2% osmic acid, and a mixture of equal parts of 2% osmic acid, 3% potassium dichromate, and 2% lanthanum nitrate. After dehydrating in alcohols, they were embedded in n-butyl methacrylate[4] and sectioned at 0·1 to 0·2 microns with a modified Spencer microtome[5]. The sections were floated off onto a dioxane-water surface as they were cut and picked up on a glass microscope slide. The embedding material was removed with amyl acetate and the slide was then immersed in a dilute solution of collodion in amyl acetate. After drying, the resulting film was stripped off onto a water surface and mounted on an electron microscope specimen screen. The sections were observed with an R.C.A. type E.M.U. electron microscope.

The double nature of the nuclear membrane, as first described by CALLAN and TOMLIN in amphibian oocytes and later by BAIRATI and LEHMANN in *Amoeba proteus*, can easily be seen in cross section as shown in Figure 1. There is an outer continous layer approximately 1000 Å thick and an inner porous layer of about 2000 Å. Figure 2 shows a section of the membrane cut parallel to its surface where the characteristic pattern of pores may be seen. In Figure 3 an oblique section at the edge of the nucleus again shows the inner position of the porous layer. The average spacing between pore centers is approximately 1200 Å as measured in both cross sections and sections cut parallel to the surface of the membrane. The average pore diameter is around 800 Å. There was no noticeable difference between specimens fixed in osmic acid and those fixed in the osmic, dichromate, lanthanum mixture.

In contrast to the arrangement found in the amphibian oocyte nuclear membrane, the continuous layer of the amoeba nucleus lies on the outside, while the porous layer lies inside. The average pore size and spacing found here are somewhat less than that found by BAIRATI and LEHMANN who reported an average diameter of 1200 Å and 1500 Å between centers. However, if one considers the difference in techniques employed and the many places where preparation artifacts may occur, the size measurements are in surprisingly good agreement.

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We wish to thank Dr. DANIEL MAZIA for his encouragement and direction.

Department of Zoology, University of California, Berkeley, May 19, 1952.

Zusammenfassung

Man findet in den elektronenmikroskopischen Bildern dünner Schnitte der Kernmembran von *Amoeba proteus* eine charakteristische Porenstruktur, wie sie BAIRATI und LEHMANN (1952) mit einer andern Technik gezeigt haben. Querschnitte und semitangentiale Schnitte durch die Kernmembran zeigen eine äussere kontinuierliche und darunter eine innere Porenschicht. Bestandteile des Kerninhaltes können unterschieden werden.

DISPUTANDA

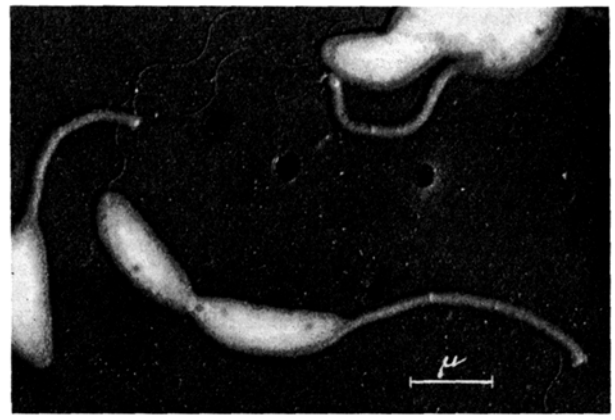
Contamination of Electron Microscope Preparations

Some Remarks to the Brief Report on *Metabolic Chromosomes Isolated from Blood Cell Nuclei of Various Animals* by G. YASUZUMI *et al.*[1]

In a recent paper by YASUZUMI *et al.*[1] some electron micrographs are included which supposedly represent chromosomes of various vertebrate animals. The fourth one of these micrographs looks very familiar to me since the "chromosome" closely resembles a bacterium I have been cultivating in pure culture for some years: a stalked bacterium, *Caulobacter* spec. Up to the present this genus has received little attention, though one species had been isolated as early as 1905 by JONES[2]. The genus was described by HENRICI and JOHNSON[3]. Electron micrographs and a short description of this bacterium have been given by HOUWINK and VAN ITERSON[4] and, with more particulars, by HOUWINK[5]. Figure 1 shows that the stalk may bear a number of cross-bars the nature of which I have not been able to elucidate. The fact that the latter are similar to the two cross-bars shown on YASUZUMI's micrograph adds to the degree of certainty with which the organism may be identified.

The genus is probably common in fresh-water. My first strain, however, was isolated from distilled water. As every electron microscopist uses distilled water in the preparation of his specimens, I am not surprised at *Caulobacter* turning up in an E. M. study on a subject not in the least related to bacteriology.

Only rarely, however, does *Caulobacter* occur in E. M. preparations. Another contamination of organic origin is found much more frequently. On electron micrographs of shadowed specimens it looks a double-stranded spiral (Fig. 3). Usually one of the ends is rounded and here one or a few "flagella" seem to be inserted. With many specimens, the square cut appearance of the other end suggests that they have been broken in two parts. The diameter is 5000–7000 Å. A description and some micrographs of these as yet unidentified "organisms" have



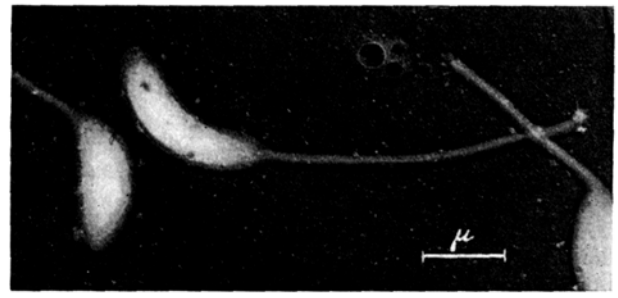


Fig. 1 and 2.–*Caulobacter* spec.

Fig. 3.–Unidentified "micro-organism".

been published by WIGAND and PETERS[1]. For further particulars I refer to their paper. Electron microscopists would be well advised to make themselves acquainted with the appearance of this common contamination.

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Zusammenfassung

In dieser Zeitschrift wurden vor kurzem von YASUZUMI *et al.* elektronenmikroskopische Aufnahmen von Chromosomen veröffentlicht. Auf den Bildern sind gewisse merkwürdige Teilchen zu sehen. In der vorliegenden Notiz macht der Verfasser darauf aufmerksam, dass Mikroorganismen gelegentlich Präparate für elektronenmikroskopische Untersuchungen verunreinigen können.

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