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Dissimilatory Sulphate Reduction with Acetate as Electron Donor [and Discussion]

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Dissimilatory sulphate reduction with acetate as electron donor

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[Plate 1]

Acetate oxidation by sulphate was studied with *Desulfobacter postgatei*. Cell extracts of the organism were found to contain high activities of the following enzymes: citrate synthase, aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, fumarase, malate dehydrogenase and pyruvate synthase. It is concluded that acetate oxidation with sulphate in *D. postgatei* proceeds via the citric acid cycle with the synthesis of pyruvate from acetyl CoA and CO_2 as an anaplerotic reaction.

The apparent K_S for acetate oxidation by *D. postgatei* as determined *in vivo* was near 0.2 mM. The apparent K_S for acetate fermentation to methane and CO_2 by *Methanosarcina barkeri* was 3 mM. The significantly lower K_S for acetate of the sulphate reducer explains why methane formation from acetate in natural habitats is apparently inhibited by sulphate.

Introduction

Acetate (acetyl CoA) is probably the quantitatively most important physiological electron donor for dissimilatory sulphate reduction (for literature see Laanbroek & Pfennig 1981). In sulphate-sufficient habitats up to 70 % of the H_2S formed can come from sulphate reduction with acetate. Despite this fact organisms that can oxidize acetate with sulphate have been isolated only recently (Widdel & Pfennig 1977). Specific conditions are required for their enrichment and cultivation. Once these were recognized, however, many different fatty acid-oxidizing sulphate reducers were found. Seven new species in six genera have so far been described (Widdel 1980; Pfennig & Widdel 1981).

In this paper two problems are discussed: (i) How is acetate oxidized to CO_2 in these sulphate-reducing bacteria? (ii) Why is acetate oxidized to CO_2 in sulphate-sufficient habitats by sulphate-reducing bacteria rather than fermented to methane by methanogenic bacteria? The experiments described were performed with *Desulfobacter postgatei* (strain 2a c9), which is highly specialized in that it can only use acetate as electron donor for dissimilatory sulphate reduction (Widdel & Pfennig 1981). The paper begins with a short description of some relevant properties of this organism.

Acetate-oxidizing *Desulfobacter postgatei*

D. postgatei was first isolated by Widdel (1980) from anaerobic marine and brackish water habitats. It is a short oval to rod-shaped non-sporing bacterium (1.25 μm × 2 μm), which stains Gram-negative. Grazing sections of the organism revealed the presence of an outer membrane, of a cytoplasmic membrane, and of stacked intracytoplasmic membranes (figure 1). The membrane fraction contains an active ATPase (3 μmol min^{-1} mg^{-1} cell protein), which can

completely be inhibited by dicyclohexylcarbodiimide (DCCD). In the cytoplasmic fraction, high activities of adenylate kinase (3.4 μmol min^{-1} mg^{-1}) and of inorganic pyrophosphatase (10 μmol min^{-1} mg^{-1}) are found (unpublished observations). From the enzymes involved in dissimilatory sulphate reduction, APS reductase has been characterized and shown to be similar to the enzyme found in *Desulfovibrio* species (Stille 1982). A bisulphite reductase similar to desulfoviridin or desulforubidin appears to be absent (Widdel 1980). In the bacterium cytochromes *b* and *c*, ferredoxin (0.065 nmol mg^{-1} cells), and menaquinone (0.35 nmol mg^{-1} cells) rather than ubiquinone or demethylmenaquinone were found (Gebhardt 1981).

D. postgati grows on acetate and sulphate (or thiosulphate or sulphite) as sole energy source and acetate and CO_2 as sole carbon sources. Only biotin and 4-aminobenzoic acid are required as vitamins. NH_3 is the nitrogen source. Growth is dependent on rather high concentrations of NaCl and $MgCl_2$, reflecting the marine habitat of the organism. Maximal growth rates (t_d = 20–24 h) are observed at pH 7 and 28 °C in the dark. Daylight appears to inhibit cell proliferation. $Y_{SO_4^{2-}}$ was determined as being near 5 g mol^{-1} (Widdel & Pfennig 1981).

Pathway of acetate oxidation to CO_2

In aerobic and in phototrophic organisms acetate is oxidized to CO_2 via the citric acid cycle. α-Ketoglutarate dehydrogenase and succinate dehydrogenase are considered key enzymes since all the other enzyme of the cycle are generally also found in anaerobic organisms not capable of oxidizing acetate. Here the enzymes have an anabolic function rather than a catabolic function. The presence of α-ketoglutarate dehydrogenase and of succinate dehydrogenase is therefore diagnostic for the operation of the citric acid cycle in an organism. A recent review on bacterial citric acid cycle enzymes is by Weitzman (1981).

Cell extracts of *D. postgatei* were found to contain high activities of both α-ketoglutarate dehydrogenase and of succinate dehydrogenase (table 1). The α-ketoglutarate dehydrogenase activity was soluble and specific for ferredoxin as electron acceptor. Normally the enzyme is NAD-specific but exceptions from this rule have been reported (Kerscher & Oesterhelt 1981). Succinate dehydrogenase was characteristically membrane-bound and catalysed the oxidation of succinate to fumarate with 1,4-naphthoquinone. Since *D. postgatei* contains menaquinone rather than ubiquinone or demethylmenaquinone, menaquinone may be the physiological electron acceptor. This would, however, be the first succinate dehydrogenase that physiologically couples with menaquinone (Kröger 1978). The reverse reaction, the reduction of fumarate to succinate, was only very slowly mediated by the membrane fraction, indicating that the enzyme is functionally a succinate dehydrogenase and not a fumarate reductase.

All other enzymes of the citric acid cycle (with exception of succinate thiokinase) have also been detected. It was difficult to find some of these enzymes. Citrate synthase of *D. postgatei* is only active in the presence of AMP; aconitase is inhibited by ammonia; fumarase is inactivated upon freezing; malate dehydrogenase is membrane bound and couples with 1,4-naphthoquinone as in *Bacillus* species (Kröger & Dadak 1969) rather than with pyridine nucleotides. All the enzymes were present in sufficiently high activities to account for the rates of acetate oxidation *in vivo* (table 1). We conclude that acetate is oxidized to CO_2 via the citric acid cycle in *D. postgatei*.

For the operation of the citric acid cycle an anaplerotic mechanism is required to replenish those intermediates that are continuously removed for the biosynthesis of asparate, glutamate,

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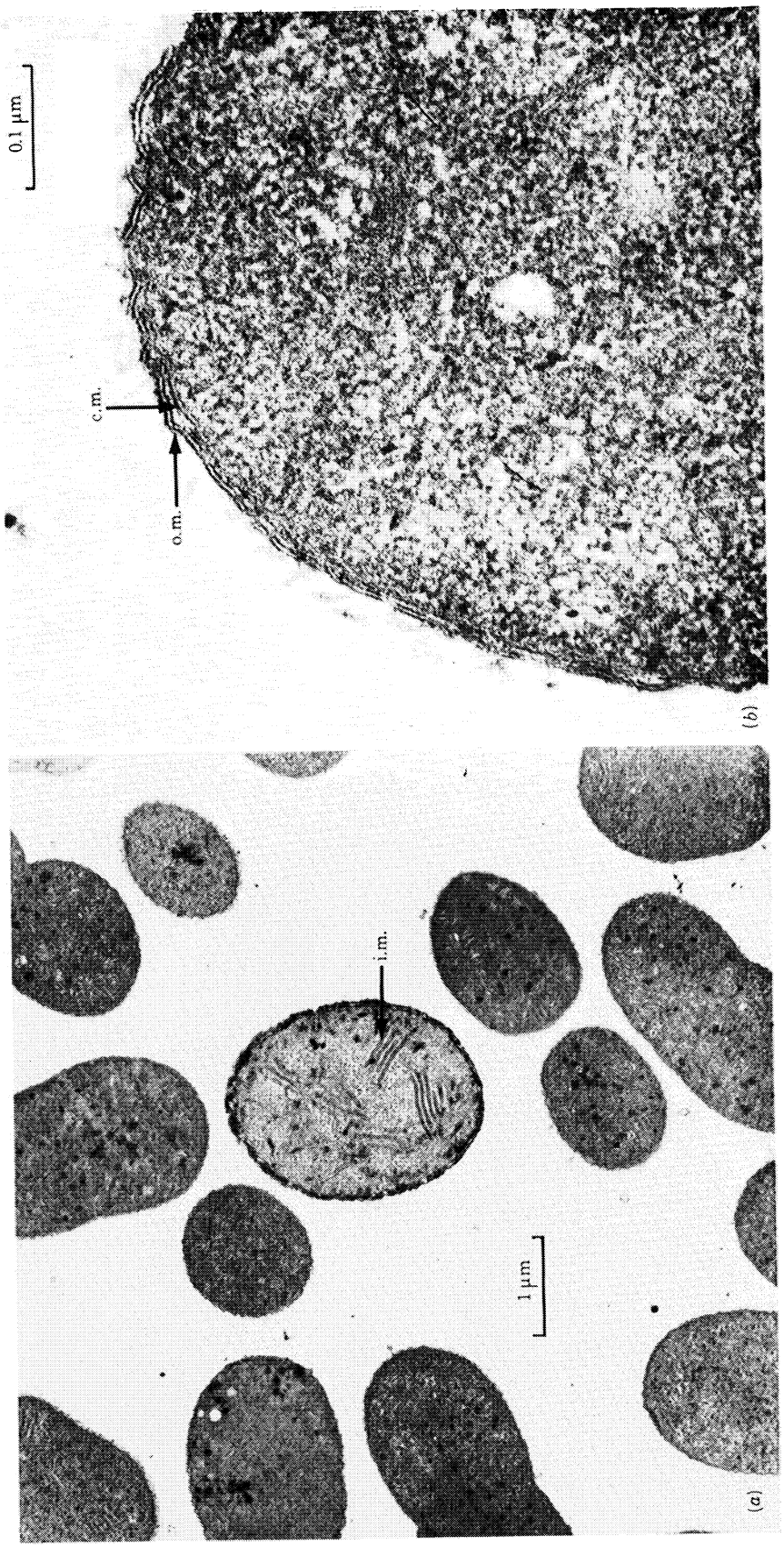

FIGURE 1. Grazing section of *Desulfobacter postgatei* (strain 2 a c 9) revealing outer membrane (o.m.), cytoplasmic membrane (c.m.) and intracytoplasmic membranes (i.m.). Magns: (*a*) ×16000; (*b*) ×200000.

(*Facing p.* 468)

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TABLE 1. ENZYMES OF THE CITRIC ACID CYCLE IN *DESULFOBACTER POSTGATEI*

enzyme	coenzyme	U/mg†	localization
citrate synthase	—	> 0.1	cytosol
aconitase	—	0.2	cytosol
isocitrate dehydrogenase	NADP	8.5	cytosol
α-ketoglutarate dehydrogenase	ferredoxin	1.0	cytosol
succinate dehydrogenase	menaquinone?	0.8	membrane
fumarase	—	3.5	cytosol
malate dehydrogenase	menaquinone	0.1	membrane
pyruvate synthase	ferredoxin	0.3	cytosol

† Specific activity (μmol min^{-1} mg^{-1} cell protein).

TABLE 2. REDOX POTENTIAL OF ELECTRON DONORS AND ELECTRON ACCEPTORS INVOLVED IN ACETATE OXIDATION BY SULPHATE VIA THE CITRIC ACID CYCLE (FROM THAUER *ET AL.* 1977).

redox couple	$E^{\circ\prime}$/mV
fumarate/succinate	+ 33
oxaloacetate/malate	−172
α-ketoglutarate/isocitrate	−432
succinyl CoA/α-ketoglutarate	−498
APS/HSO_3^- + AMP	− 60
HSO_3^-/HS^-	−116
$S_3O_6^{2-}/S_2O_3^{2-} + HSO_3^-$	+225
$HSO_3^-/S_3O_6^{2-}$	−173
$S_2O_3^{2-}/HS^- + HSO_3^-$	−402

and of other cell compounds. In aerobic organisms growing on fatty acids the glyoxylate bypass performs this function. The enzymes of this bypass appear not to be present in *D. postgatei*. Instead, an active pyruvate synthase was found (table 1), which catalyses the reductive carboxylation of acetyl CoA with reduced ferredoxin as electron donor. It is therefore likely that synthesis of oxaloacetate from acetate and 2 CO_2 via pyruvate is the anaplerotic mechanism of *D. postgatei*.

A thermodynamically problematic step in the citric acid cycle is the oxidation of succinate to fumarate. The standard redox potentials ($E^{\circ\prime}$) of the fumarate/succinate couple is +33 mV. Acetate oxidation via the citric acid cycle can thus only proceed if electron acceptors are available with a redox potential more positive than +33 mV. Adenylyl sulphate (APS) and bisulphite (HSO_3^-) and possibly also trithionate ($S_3O_6^{2-}$) and thiosulphate ($S_2O_3^{2-}$) have to be discussed as the terminal electron acceptors available in *D. postgatei*. From these only the $S_3O_6^{2-}/S_2O_3^{2-} + HSO_3^-$ couple has a potential suitable for the oxidation of succinate (table 2). The formation of free trithionate as intermediate in dissimilatory sulphate reduction is, however, disputed. For reviews on the subject see Thauer *et al.* (1977) and Akagi (1981).

Desulfuromonas acetoxidans oxidizes acetate to CO_2 with elemental sulphur as electron acceptor, which is reduced to H_2S (Pfennig & Biebl 1976). Enzymatic studies indicate that in this anaerobic organism acetate oxidation also proceeds via the citric acid cycle (Kaulfers 1976). The $E^{\circ\prime}$ of the S^0/HS^- couple is −270 mV (table 2). Even assuming large deviations from the standard conditions *in vivo* an oxidation of succinate with S^0 will require the input of energy. An energy-driven reversed electron transport (Klingenberg & Schollmeyer 1960) from succinate

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to sulphur has therefore to be postulated for *D. acetoxidans* and has to be considered in the case of *D. postgatei* also.

Evidence is available that dissimilatory sulphate reduction with H_2 is coupled with the net synthesis of only 1 mol ATP (Badziong & Thauer 1978, 1980; Thauer & Badziong 1980, 1981). If succinate oxidation is an energy-dependent process in acetate-oxidizing sulphate reducers then more than 1 mol ATP (or energy equivalent) must be formed during sulphate reduction to H_2S in these organisms.

Apparent K_S for acetate oxidation *in vivo*

In sulphate-sufficient habitats acetate is oxidized to CO_2 by sulphate-reducing bacteria rather than fermented to methane and CO_2 by methanogenic bacteria (for literature see Kristjansson *et al.* 1982). Neither sulphate nor H_2S inhibit methane formation or the growth of methanogenic bacteria in pure cultures. A lower K_S for acetate of the sulphate reducers has been proposed to explain the phenomenon (Winfrey & Zeikus 1977). To test this proposal the K_S for acetate of *D. postgatei* and of a methanogen (*Methanosarcina barkeri*, strain Fusaro) were determined. The apparent K_S for acetate of *D. postgatei* was lower than 0.2 mM, that of *M. barkeri* near 3 mM (Schönheit *et al.* 1982). In mixed cell suspensions of the two organisms (adjusted to equal V_{max}), methane formation from acetate was not inhibited by sulphate at high acetate concentrations (more than 10 mM) but was almost completely suppressed at low acetate concentrations (less than 0.5 mM). Different substrate affinities can thus account for the inhibition of methanogenesis from acetate in sulphate-rich environments, where the acetate concentration is well below 1 mM (Zehnder *et al.* 1982).

The reason why sulphate reducers have a lower K_S for acetate than the methanogens can only be speculative. The free energy change of acetate oxidation by sulphate $\Delta G^{\circ\prime} = -57$ kJ mol^{-1}) is considerably more negative than that of acetate fermentation to methane and CO_2 ($\Delta G^{\circ\prime} = -36$ kJ mol^{-1}). The Haldane equation (Fersht 1977) predicts that K_S of an enzyme is in part determined by the free energy change associated with the particular reaction.

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Discussion

J. R. Postgate, F.R.S. (*U.N.F., University of Sussex, Brighton, U.K.*). Is there any clear evidence that sulphate enters the cells of sulphate-reducing bacteria? Or is it converted to APS at the protoplast surface?

R. K. Thauer. There is no clear evidence that sulphate enters the cell of sulphate-reducing bacteria and is there activated to APS. It could be that sulphate is transported into the cell by being chemically modified to APS (transport via group translocation). It could also be that sulphate enters the cell by facilitated diffusion through a gate, which is opened only when the membrane is energized. The problem is that sulphate uptake is observed only when the ATP concentration in the sulphate-reducing bacteria (*Desulfovibrio vulgaris*) is high.

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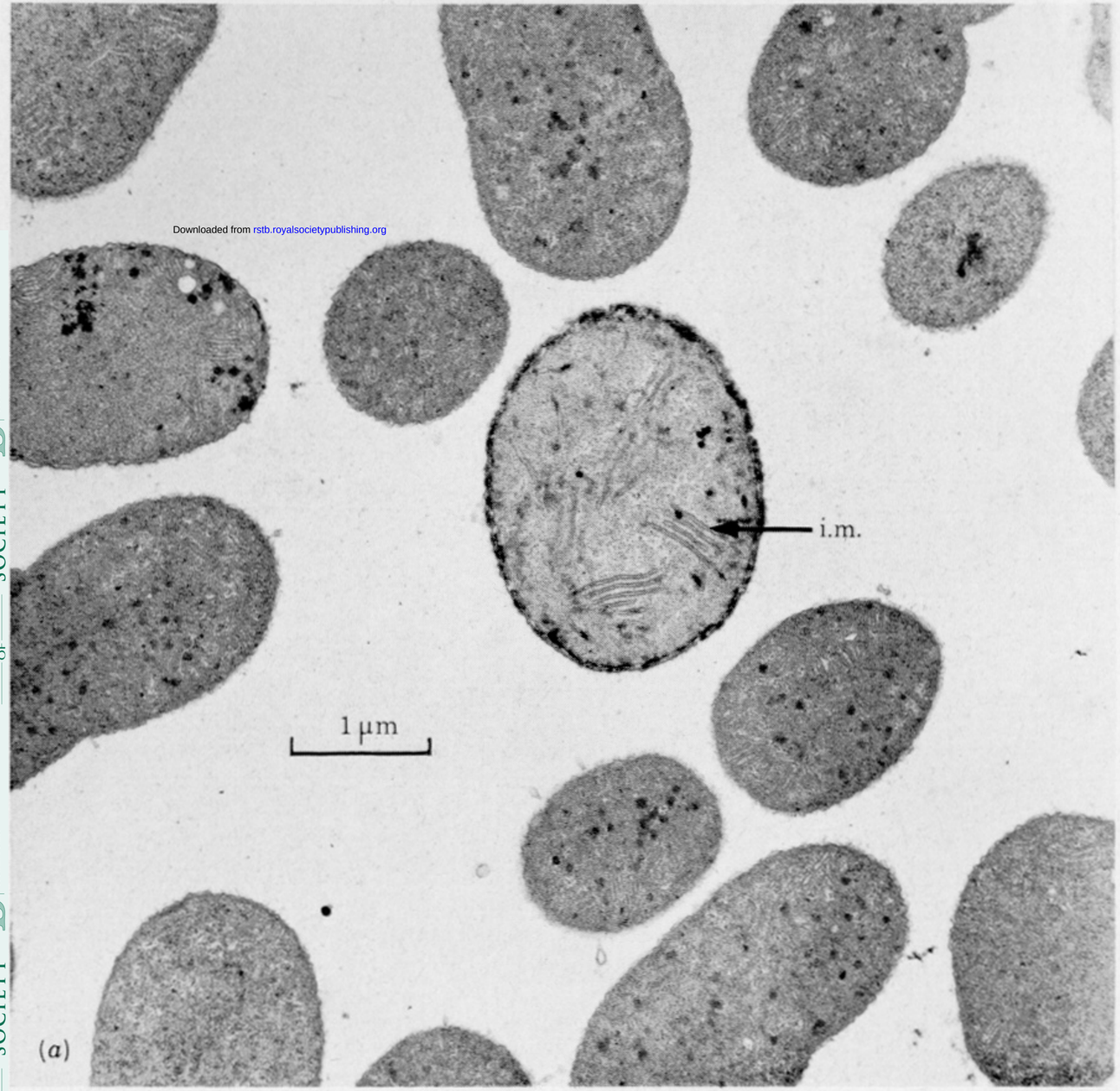

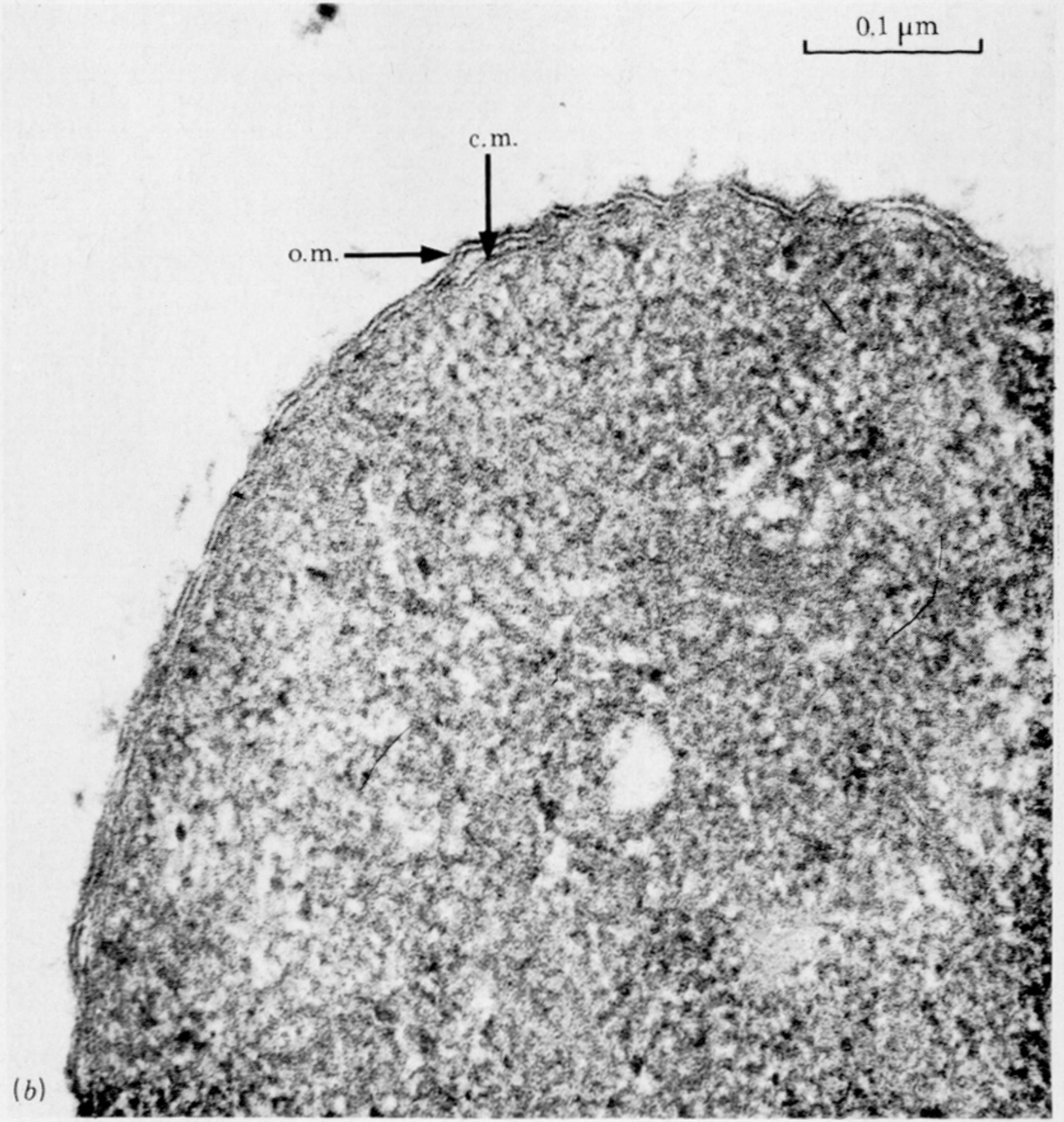

FIGURE 1. Grazing section of *Desulfobacter postgatei* (strain 2 a c 9) revealing outer membrane (o.m.), cytoplasmic membrane (c.m.) and intracytoplasmic membranes (i.m.). Magns: (*a*) × 16 000; (*b*) × 200 000.