same side of the same animal. Instead, the stripes are likely to be produced by some interaction that allows neighboring fibers in the retina to recognize one another in the tectum. If the fibers attempted to maintain maximum contact with their neighbors from the retina, periodic patterns, like the observed stripes, would probably result (*6*). Two good candidates for such neighbor recognition mechanisms are biochemical interactions (*7*) and neuronal activity (*8*). For example, nerve activity has been suggested to play a role in the patterning of nerve connections as a result of the observation that neighboring fibers have similar activity patterns, even in the absence of visual stimuli. If the fibers followed the rule, "fibers that fire together stay together," segregation on the basis of the eye of origin would be expected. Near neighbors in the same retina will have a much greater synchrony of firing than fibers originating from another eye but innervating the same area of the tectum. Biochemical oscillations could play a role similar to nerve activity, serving to keep near-neighboring fibers in the retina as near neighbors in the tectum. Recent experiments that block nerve activity with neurotoxin demonstrate an absence of fiber segregation into ocular dominance columns or stripes (*9*). Our experiments, taken with those that block nerve activity, implicate coincident nerve activity as a likely factor in the formation of ocular dominance stripes.

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10. Retinotectal mapping was accomplished by typical extracellular electrophysiologic techniques. The animals were anesthetized with Finquel (MS-222, Ayerst) and then paralyzed with a small injection of curare (tubocurarine). After limited craniotomy to expose the optic tecta, a platinum-tipped platinum-iridium microelectrode (5 Mohm before tipping) was lowered into the superficial neuropil of the optic tectum. The signals recorded by the electrode were amplified (×1000), filtered (100 Hz to 3 kHz bandpass; 60 Hz rejection), and monitored on an oscilloscope and loudspeaker. For each electrode position, the regions of visual space that would elicit activity at the electrode tip were determined through the use of an Aimark projection perimeter as a stimulus.
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Immunochemical Localization of NADP-Specific Isocitrate Dehydrogenase in *Escherichia coli*

Abstract. *The intracellular localization of isocitrate dehydrogenase was determined by immunochemical techniques with ultrathin sections of* Escherichia coli. *The thin sections, which were obtained by ultracryomicrotomy, were incubated first with antiserum specific for the enzyme and then with a protein A–gold complex. Transmission electron microscopy showed that the gold label was dispersed mainly in the cytoplasm.*

Intracellular antigenic sites have been located by electron microscopy in ultrathin sections of eukaryotic cells prepared by cryomicrotomy (*1–4*). Beesley and Adlam (*5*) reported, however, that immunolabeling when used with bacterial cryosection systems lacks specificity compared to its use with methacrylate preparations.

Tokuyasu (*6*) succeeded in using ultracryomicrotomy to prepare tissue for electron microscopy, and subsequently Painter *et al.* (*7*) used horse spleen ferritin as an electron-dense immunolabel to locate pancreatic ribonuclease by electron microscopy on frozen thin sections. Iron-dextran (Imposil) antibody conjugate was also reported to be a suitable immunolabel for transmission electron microscopy (*8*). Another electron-dense particle that has been used as an immunolabel is colloidal gold (*9, 10*) complexed with protein A, which specifically binds with the F_c portion of immunoglobin G (IgG) (*11*).

We used ultracryomicrotomy and labeling with the protein A–gold complex to investigate the localization of isocitrate dehydrogenase (E.C. 1.1.1.42) in *Escherichia coli*. This enzyme occupies a prominent position in the tricarboxylic acid cycle and has an absolute requirement for nicotinamide adenine dinucleotide phosphate (NADP). Although the enzyme has generally been assumed to be cytoplasmic, this has never been demonstrated directly.

Antibody specific for isocitrate dehydrogenase was raised in rabbits (*12*) injected with enzyme that had been purified by immunoaffinity in a column containing protein A coupled to IgG (*13*). The serum was collected, and the crude immunoglobulin fraction was precipitated with 33 percent $(NH_4)_2SO_4$. The precipitate was dissolved in 0.02*M* K_2HPO_4, *p*H 8.0, and desalted by passage through a Sephadex G-25 column equilibrated in the same buffer. The immunoglobulin was then applied to a DEAE–Affi-gel blue column equilibrated in the K_2HPO_4 solution, and the purified IgG was eluted by washing the column with the same solution. The purified IgG used in control experiments was obtained as above from serum from the same rabbits before they were immunized.

Antibodies specific for isocitrate dehydrogenase were then purified from the IgG fraction by affinity chromatography on Affi-gel 15 to which purified isocitrate dehydrogenase had been covalently bound (*14*). The IgG was applied to the column, which was then washed with 1.5*M* NaCl to remove all nonspecific IgG. The antibodies were eluted with 3*M* NaCNS, *p*H 6.6, and immediately desalted by passage through a Sephadex G-25 column equilibrated in phosphate-buffered saline at *p*H 7.2.

To determine the effect of glutaraldehyde fixative on the antigen, we incubated purified isocitrate dehydrogenase at room temperature with increasing concentrations of the aldehyde for different time periods. A sample from each tube was then tested for its capacity to agglu-

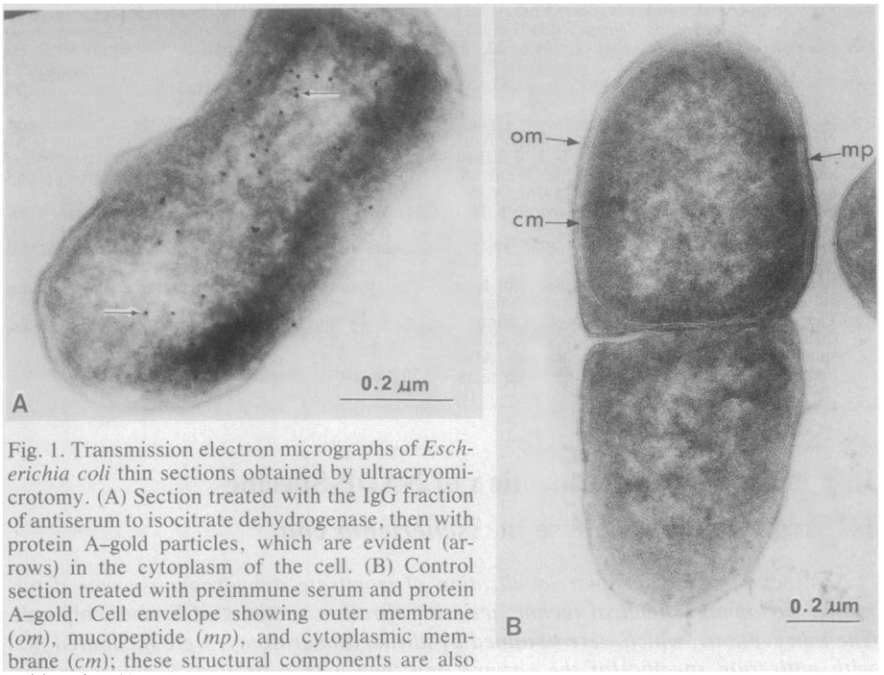

Fig. 1. Transmission electron micrographs of *Escherichia coli* thin sections obtained by ultracryomicrotomy. (A) Section treated with the IgG fraction of antiserum to isocitrate dehydrogenase, then with protein A–gold particles, which are evident (arrows) in the cytoplasm of the cell. (B) Control section treated with preimmune serum and protein A–gold. Cell envelope showing outer membrane (*om*), mucopeptide (*mp*), and cytoplasmic membrane (*cm*); these structural components are also evident in (A).

tinate *Staphylococcus aureus* cells that had been incubated with antibody specific for isocitrate dehydrogenase.

On the basis of the results from these studies, *E. coli* strain K-12 cells suspended in 0.1*M* sodium phosphate buffer, *p*H 7.5, were treated with 0.1 percent glutaraldehyde for 30 minutes at 26°C. The cells had been grown at 37°C with shaking in a mineral salts medium containing 0.1 percent glycerol as the carbon source; they were harvested by centrifugation at 4°C for 10 minutes at 12,000*g* when the culture had reached a turbidity at 660 nm (red filter) of 120 Klett units. After fixation, cells were washed in the same buffer and enrobed in 10 percent gelatin. Small pieces of this mass were infused with 1.0*M* sucrose in 0.1*M* sodium phosphate buffer, *p*H 7.5, for 1 hour at room temperature, then mounted on copper specimen pegs and rapidly frozen in Freon 12 slush. Ultrathin frozen sections were obtained at −90°C on glass knives (*15*) with an ultramicrotome (Porter-Blum MT-2B) equipped with a Cryokit (DuPont).

Carbon-stabilized, support-filmed grids with the sections adhering to them were treated with 2 percent gelatin prepared in 0.1*M* glycine phosphate buffer for 10 minutes to inhibit nonspecific antibody binding. The grids were floated, section side down, on 10 μl of the antibody (0.194 mg/ml) for 10 minutes at room temperature. Control sections were incubated in an identical manner with the purified IgG obtained from the preimmune serum. After being thoroughly rinsed, each grid was floated for 10 minutes at room temperature on 10 μl of protein A–gold particles (10 mg/ml; diameter, 20 nm) (E. Y. Laboratories) diluted 1:10 with 5 percent bovine serum albumin in 0.1*M* sodium phosphate buffer.

The immunocomplexes were washed and then stabilized with 2 percent glutaraldehyde, and sections were stained and embedded as described (*16*). Samples were examined with a transmission electron microscope at an acceleration of 200 keV. In bacterial sections treated with affinity-purified antibody and protein A–gold, most of the label was located in the cellular cytoplasmic regions (Fig. 1A). No label was found on control sections (Fig. 1B).

In our preliminary experiments, excessive nonspecific labeling was observed on grid surfaces. The nonspecificity was eliminated by enrobing the cells in 10 percent gelatin before sucrose infusion and by diluting the protein A–gold suspension 1:10 with 5 percent bovine serum albumin in 0.1*M* sodium phosphate buffer. Bernhard and Viron (*17*) had reported earlier that 20 percent gelatin was indispensable when used as a molten embedment for bacteria and other tissues.

Because of its high atomic number, colloidal gold has become a powerful alternative to labels such as ferritin for providing high contrast in the electron microscope. Gold particles were easily visible on sections of less than optimum thickness as compared with thin sections routinely obtained from plastic embedment. Furthermore, in contrast to ferritin (data not shown), gold particles were clearly recognizable on sections observed with high-voltage (200 keV) electron microscopy.

Although the bacteria were lightly fixed with aldehyde, rather than the more rigorous fixatives routinely used for morphological preservation, membranes and cell walls retained their integrity (Fig. 1, A and B). The use of hydrophilic embedment and adsorption staining (*18*) reliably delineated plasma membrane and cell wall profiles. Neither we, nor others (*19, 20*), have demonstrated the presence of ribosomes.

Our experiments thus provide a basis for the ultracryomicrotomy of bacterial cells. This technique produces sections with excellent retention of structural integrity and can be used with immunolabeling to determine the localization of intracellular proteins.

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