translocated to the *trans* face of the membrane during insertion of toxin into the bilayer (see Fig. 1). This appears to be an essential step in the intoxication process in vivo and has been mimicked in systems in vitro by two groups, Donovan et al. (1982 *b*) and Kagan et al.[2]

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STUDIES OF PORINS

Spontaneously Transferred from Whole Cells and Reconstituted from Purified Proteins of *Neisseria gonorrhoeae* and *Neisseria meningitidis*

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Many human bacterial pathogens can invade and damage host epithelial surfaces in a process whereby the organism binds to and is transported across the boundary epithelial layer. The factors that define the virulence pattern and the invasive potential of a microbial pathogen are likely to be distinct bacterial products associated with the outer membrane surface or secreted into the extracellular environment. Postulating that invasive pathogens might elicit substances that could affect membrane permeability as a regulatory signal to cause their uptake into the cell, we exposed, under voltage-clamp conditions, artificial planar lipid bilayer membranes composed of phosphatidylethanolamine (PEA) or asolectin (ASO) to whole cells of several strains of invasive gram-negative cocci and noninvasive controls. The cells were maintained in culture under conditions described in reference 1. In bilayers contacted by these organisms, we observed the spontaneous transfer of ion-permeable channels that exhibited voltage-sensitive lifetimes. The ability to spontaneously transfer channel activity into the bilayer was compared in pathogenic strains of *Neisseria*, i.e., *N. meningitidis* and *N. gonorrhoeae* (isolated from patients with systemic and localized diseases) as well as a nonpathogenic strain of *N. sicca*.

Outer membrane proteins from the bacterial pathogens were purified (1) and reconstituted into proteoliposomes that were fused into the planar lipid bilayer (2). In the bilayer, channel-forming activity was determined to be associated with the strain specific protein I, the major outer membrane protein, analagous to the porin channel-forming protein described in *E. coli* (3). Channels transferred into the bilayer from whole cells and from vesicles containing purified protein I exhibited distinct properties that were characteristic of the strain of gonococcus or meningococcus used. For a given strain, the properties of channels reconstituted in the bilayer from whole cells or from the strain specific purified protein I in vesicles were similar.

RESULTS

Fig. 1 *A*, *C*, and *E* demonstrate the channel-forming activity as current jumps that spontaneously entered the model membrane at variable time (10 s–60 min) after the

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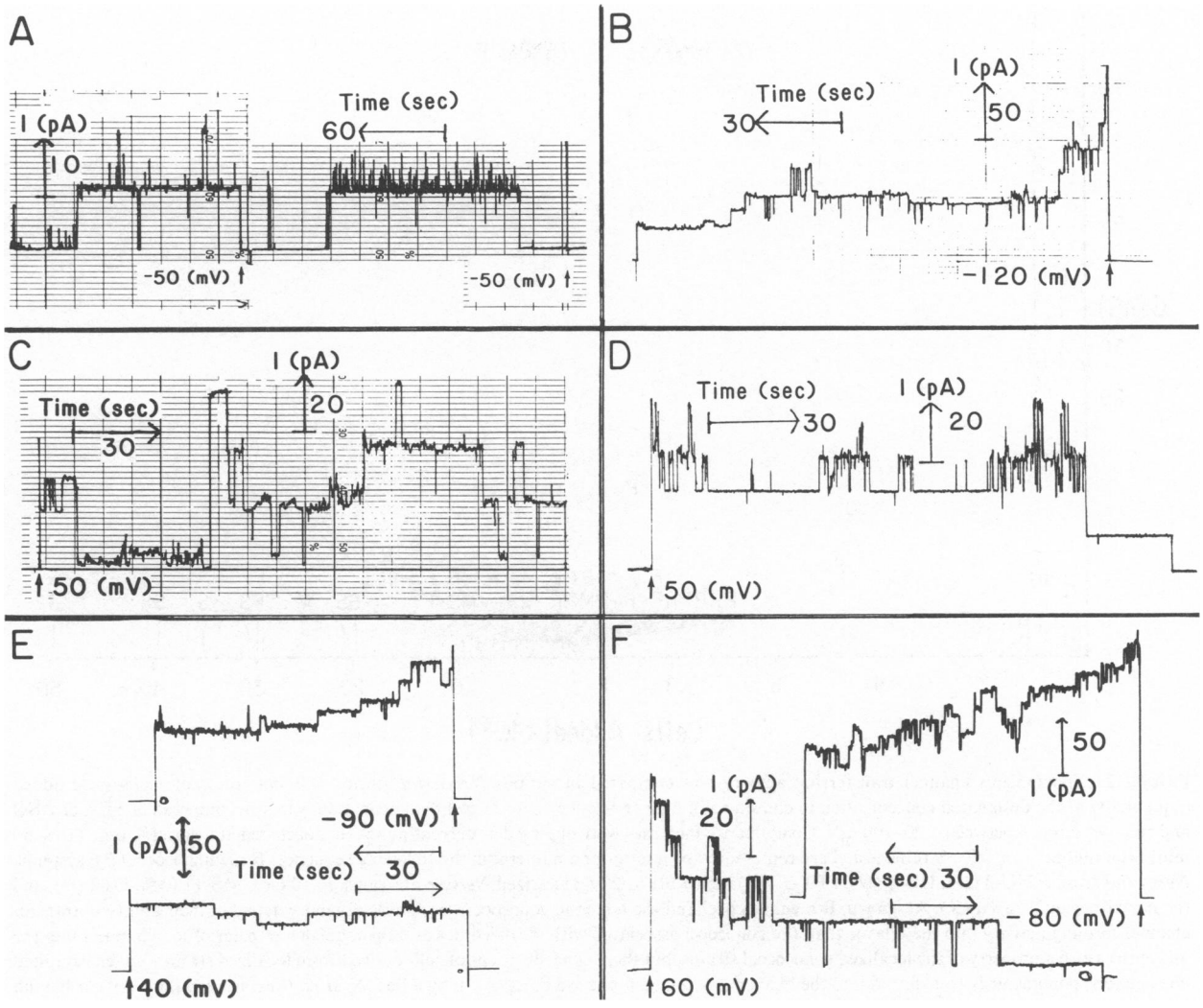

FIGURE 1 Channels transferred spontaneously from whole cells *A*, *C*, *E*, or from purified channel-forming protein reconstituted from proteoliposomes *B*, *D*, *F*, are compared in planar lipid bilayers bathed by 0.1M NaCl, 10 mM $CaCl_2$ that were made of PEA or ASO. Channels reconstituted from whole cells or from purified proteoliposomes from a localized gonococcal strain R-10 (compare *A* to *B*), from a systemic gonococcal strain 120176-2 (compare *C* to *D*), and from a Group-B meningococcus (compare *E* to *F*) exhibited similar unit channel conductances, voltage-dependent lifetimes, and anion preferences (data not shown).

addition of a quantity (10^5–10^7) cells of the designated strain to a 4-ml bath volume on one side of a high-resistance planar lipid bilayer. In other experiments, liposomes containing purified proteins-I from each of these strains were fused into the bilayer membranes; the resultant channels displayed unit channel conductances, voltage dependent lifetimes, and open-to-closed-conductance transitions that were similar to channels derived from the whole cells extracts of that strain (Fig. 1 *B*, *D*, *F*).

The spontaneous transfer of channels as a function of the concentration of the bacterial cells added into the bilayer was examined comparatively for a number of strains of pathogenic cocci. As shown in Fig. 2, invasive B-meningococcus, and gonococcal strains UU-1, UU-3, PFT-35, and 120176-2 that exhibit the characteristic (32K) phenotype of protein I associated with systemic gonococcal infections (1) have a high spontaneous rate of transfer of ion channels into the lipid bilayer. However, gonococcal strains such as R-10, MS-11, and F-62, isolated from patients with a localized infection, have a larger molecular species (36K) of protein I (1). These strains have a significantly decreased activity of spontaneously transferred channels even when a concentration of cells greater than 10- to 100-fold of that required for transfer of channels from more invasive strains of *N. gonorrhoeae* and *N. meningitidis* is used (Fig. 2).

High concentrations (up to 10^8) of cells from the nonpathogenic, noninvasive commensal strain *N. sicca* did not display transfer of channel-forming activity into the planar lipid bilayer.

By introduction of purified proteins I into liposomes that were fused into the planar lipid bilayer, membranes were obtained with multiple channel conductances from the meningococcus, disseminated and localized gonococcal

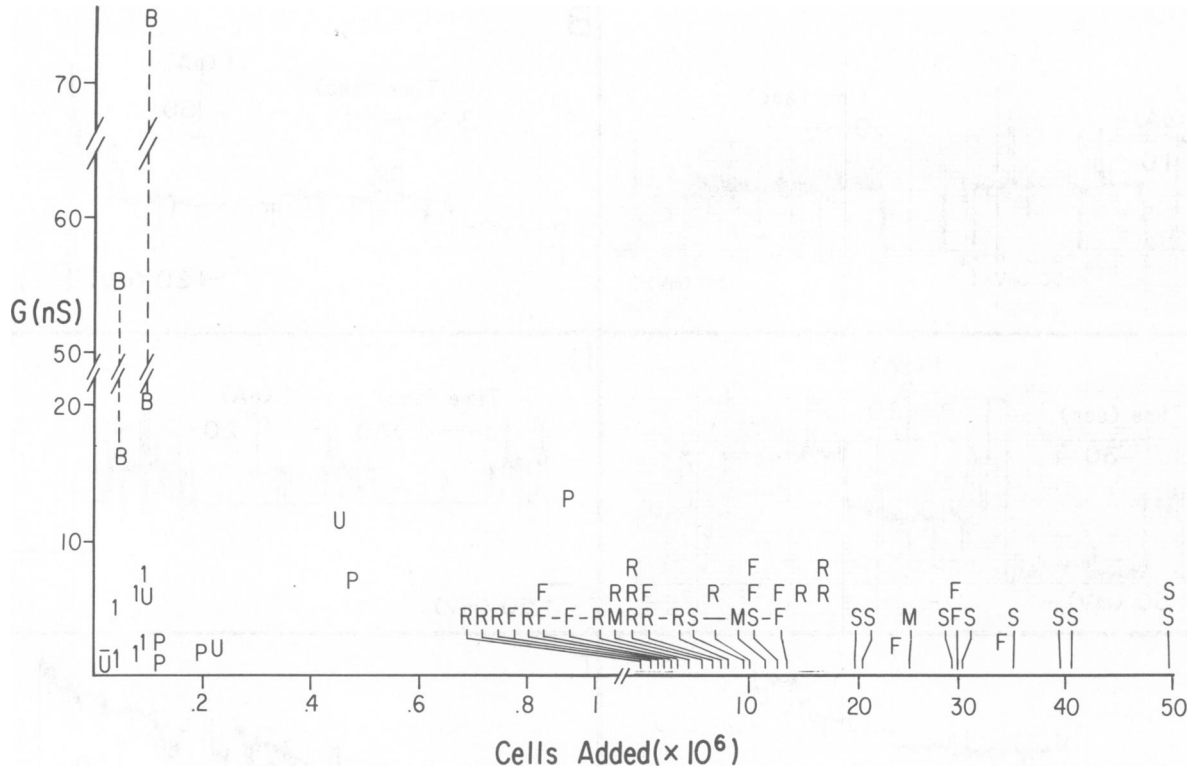

FIGURE 2 Spontaneous channel transferring activity was compared in various *Neisserial* strains. Aliquots of whole cells were added sequentially at the designated concentration to one side of a high resistance ($G < 25$ pS) planar lipid bilayer film composed of PEA or ASO and voltage clamped between ±20–100 mV. Conductance increases were observed as current jumps that occurred at a variable time (10 s–60 min) after additions of cells. Strains tested are represented by a letter or a numeral in the following sequence: B-meningococci (*B*); systemic *Neisserial* strains, UU-3 (*U*), UU-1 (*U*), PFT-35 (*P*), and 120176-2 (*1*); localized *Neisserial* strains, R-10 (*R*), MS-11 (*M*), F-62 (*F*), and the nonpathogen *N. sicca* (*S*). As shown, B-meningococci and the systemic gonococci were markedly more active in their ability to transfer channel-forming activity into the bilayer than the gonococci associated with localized disease. Spontaneous transfer of ion channels into the bilayer was also a property of the localized gonococcal strains, but those concentrations of cells derived from localized strains (which exhibited no channels spontaneously transferred into the bilayer) are joined to the *x*-axis at $G = 0$ by a line. *N. sicca* (at concentrations $>10^8$ cells) did not introduce channels into the bilayer.

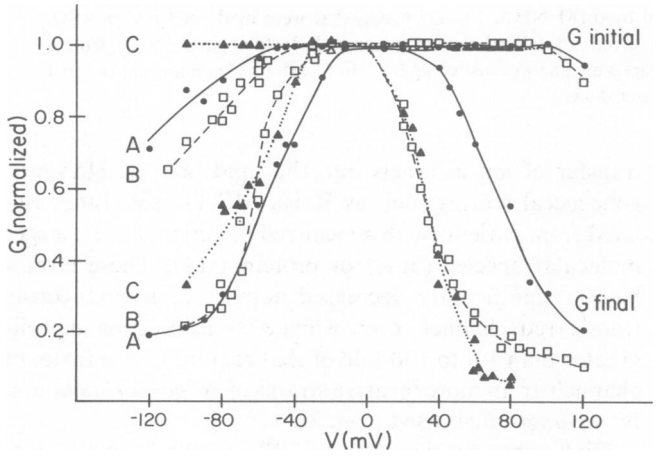

FIGURE 3 Proteoliposomes containing purified protein I from *A*, gonococcal localized strain R-10 (—, •); *B*, gonococcal systemic strain 120176-2 (---, □); and *C*, B-meningococcus (···, ▲) were fused to the bilayer and multiple channel containing membranes were obtained. At 5–10 mV intervals, membranes were subjected to 90-s voltage pulses between 0 ± 120 mV with 2–3 min periods at 0 mV intervening between voltage pulses. Conductance was monitored at the beginning (*G* initial) and end (*G* final) of each voltage pulse.

TABLE I
COMPARISON OF CHANNEL PROPERTIES IN NEISSERIAL STRAINS

	Localized strain R-10	Systemic strain 120176-2	B Meningococcus
Unit channel size*	210–230 pS	150–400	500–700
Ratio $\rho \frac{Cl^-}{Na^+}$§	6	3	1.2
N; + side, − side ‖	2–3	2–3	2–3
V_o; + side, − side¶ (mv)	49, −48	38, −59	35, −57
$\frac{G_{max}}{G_{min}}$**	3–5	5–10	14–18

*Channel conductance determined in 0.1M NaCl, 10 mM $CaCl_2$ solutions.
§Determined from Goldman equation in 6–10 fold concentration gradient.
‖N is the number of phenomenological gating particles determined from the Boltzmann relationship.
¶V_o is the voltage at which the channels have an equal probability to be open or closed.
**Ratio of the maximal to the minimal conductance.

strains. Conductance-voltage plots, Boltzmann analysis, and ion selectivity data were determined for channels derived from each strain; the results are summarized in Fig. 3 and Table I.

These studies demonstrate that microbial outer membrane proteins can be transferred spontaneously as functioning channels into model membranes. These channels can be identified in foreign membranes through the changes in specific electrical characteristics that they impart. In their transferability, conductance properties, ion selectivity, and voltage-dependent behavior, these porins produced by pathogenic *Neisserial* cocci differ markedly from those porins that have been described from *E. coli* studies (3).

The introduction of ion channels into host membranes by cytopathic organisms may be a central process to many diseases such as the cytolytic destruction of cells by the *Entamoeba histolytica* described by Lynch et al. (4). The insertion of ion channels into host cell membranes may also supply an electrical signaling mechanism that results in the resident uptake of a microorganism within a host cell phagosome. While the ability to transfer ion channel proteins into foreign membranes may be a general property of many pathogens, our studies suggest that only those highly virulent gram-negative cocci that can effect an intracellular invasion process (such as the *Neisseria meningitidis* and disseminated strains of *Neisseria gonorrhoeae*) exhibit a significant ability to effect an intermembraneous transfer of their outer membrane porin channels.

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Note added in proof: *N. gonorrhoeae*, strain 120176-2, used in our experiments, was originally isolated from the cervix but has the 32 KD Protein I that is characteristic of disseminated gonococcal strains.

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DIPHTHERIA TOXIN FRAGMENT CHANNELS IN LIPID BILAYER MEMBRANES

Selective Sieves or Discarded Wrappers?

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The relationships of protein structure to the gating and selectivity of transmembrane ion conducting channels can be investigated using sequenced and mutatable bacterial-toxin proteins that form channels in lipid bilayer membranes. This analysis might also offer some hints as to the role of channel-forming regions of these proteins in the mechanisms of action of the toxins.

Diphtheria toxin (DT) consists of two subunits joined by a peptide and a sulfhydryl bond: an enzymatic *A* subunit, which blocks protein synthesis by inhibiting nascent chain elongation, and a carrier *B* subunit, which introduces *A* into the cell. The COOH-terminal region of *B* binds to the host's plasma membrane surface; the NH_2-terminal region, called B45, contains highly hydrophobic sequences. The cytotoxicity of DT, after membrane binding, requires the presence of pH gradients or acidic vesicles within the cell (1). The B45 region itself appears to form ion channels of sufficient diameter to accommodate an unbranched polypeptide chain such as *A* (2). To make its devastating cytoplasmic appearance, subunit *A* might either traverse an open *B* channel, just as the signal sequence of a nascent polypeptide chain crosses the rough endoplasmic reticulum (3), or, at a most propitious moment, the *A* subunit might merely "pop out" of a cavity in *B*, into which it was packaged during toxin synthesis or membrane insertion.

Using standard bilayer techniques and asolectin bilayers (2, 4), I have examined aspects of the voltage and pH gating by single channels made by both the B45 region and CRM45, a mutant DT which consists of B45 plus the entire *A* subunit. CRM45 had either an intact or "nicked" sulfhydryl bridge between the *A* and *B* subunits. These DT fragments were kindly provided to the lab by Dr. A. M. Pappenheimer, Jr. Their structural stability was periodically checked by molecular weight determination using SDS polyacrylamide gel electrophoresis. These experiments have partially characterized the voltage and pH gating of B45 and CRM45 single channels and suggest that the addition of a large hydrophilic domain to the