expression (4), is included in Figs 1 and 2. Comparison with the earlier fit based on equation (10), which may be regarded as the limiting case for large b, shows that $b = 21$ represents a lower limit for b. Such a high number of binding sites does not seem unlikely because such binding is only a loose association between LPS and protein, comparable with lipid–protein association, where even higher numbers of boundary lipids associated with protein are known.

In contrast to the evaluation presented here, Schindler *et al.*[1] restricted themselves to $b = 3$ and $K_d = 0$. With these values equation (6) yields $\tilde{D}_{LPS} = n_f = 0$ at high protein concentration, and at low protein concentration equation (5) yields a straight line for $\tilde{D}_{LPS}$ (P/LPS). The latter is included in Fig. 1, showing its serious disagreement with the experimental data, which led Schindler *et al.*[1] to propose a new membrane model. They adopted the number of three binding sites from studies of the *E. coli* outer membrane, where it was found that three LPS molecules are associated with one matrix protein[2]. However, the assumption $b = 3$ would imply that the matrix proteins are saturated with LPS at the relatively high protein concentration of the outer membrane, which seems rather unlikely. Within our evaluation we would predict from equation (10), with $\kappa b = 325$ and the outer membrane molar ratios P/LPS = 1/3 and PL/LPS = 6, that $n_f = 0.06$, or 2.82 LPS molecules are associated with 1 matrix protein. This number for the site occupation agrees well with the experimental result, while the number of binding sites is expected to be much higher, as discussed. Note, however, that the evaluation of Schindler *et al.* and the one presented here differ not only in the number of binding sites, but also in the binding constant, producing a different dependence of $\tilde{D}_{LPS}$ on protein concentration.

Because the evaluation of Schindler *et al.* is too restricted, their conclusions concerning old and new membrane models are invalid. However, I hope I have made it clear that lateral diffusion measurements such as those of Schindler *et al.* are useful in studying the association behaviour of membrane constituents.

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Reply from D. E. Koppel*, M. J. Osborn† & M. Schindler‡

Departments of Biochemistry* and Microbiology†, University of Connecticut Health Center, Farmington, Connecticut 06032
‡ Department of Biochemistry, Michigan State University, East Lansing, Michigan 48823

Some comments are in order to put Dr Jähnig's re-evaluation of our data in a proper perspective. We determined the lateral diffusion coefficients of lipopolysaccharide (LPS), phospholipid (PL) and *Escherichia coli* matrix porin protein in reconstituted multibilayer membranes[1], using the technique of fluorescence redistribution after photobleaching. We compared the results from a series of samples of constant relative LPS and PL content (1:1 by weight), but different protein content (0–60% by weight). It was found that as the protein concentration increased, the diffusion coefficient of LPS decreased by an order of magnitude, whereas that of PL decreased to a much smaller extent. The protein molecules at the higher concentrations were essentially 'immobile', with little or no redistribution observable over the distance scale (~3 μm) and maximum time scale ($\sim 3 \times 10^3$ s) of these experiments. This immobility would be a surprising result in the context of the fluid mosaic model, but is not unexpected in view of considerable evidence demonstrating the self-association of porin trimers into ordered aggregates *in vivo* as well as *in vitro*[2].

Jähnig has chosen to interpret these results solely in terms of specific LPS binding to the immobile protein, leaving both the dissociation constant and the stoichiometry of binding as floating parameters. This analysis can yield results consistent with the data, but only if one allows a total of at least 21 LPS binding sites per protein monomer (that is, ~63 sites per trimer).

In contrast to the above, we[1] did not assume that in the absence of specific binding the diffusion rates would be necessarily unaffected by the presence of an immobile protein matrix in the membrane, but set out to try to determine what other factor, if any, could be contributing to the observed diffusion characteristics. We evaluated the possible effect of LPS binding consistent with that which we considered to be the best available value of binding stoichiometry. Based on evidence of co-purification, we estimate this as 9 LPS molecules per protein trimer[3,4]. Under this constraint, the binding model of diffusion could not account for the magnitude of the observed decrease in LPS diffusion. As a possible explanation of this difference we speculated that the translational diffusion of membrane components in these conditions might bear a closer resemblance to diffusion through a polymeric network than to diffusion in a simple viscous fluid. The differential effects observed for LPS and PL would be explained by the different sizes of the diffusing species. The part of the polymeric network in this scenario is played by the immobile protein matrix. Subsequent experiments[5] have suggested that the peripheral protein matrix of erythrocyte membranes may exert a similar control over the diffusion of integral membrane proteins projecting beyond the membrane bilayer.

Evidence is not yet available to allow a final definitive analysis. The value of the stoichiometry we have selected is, admittedly, a relatively soft number—it could be higher. At the same time, the value deduced by Jähnig seems unreasonably high for a specific binding interaction. The truth probably lies somewhere in between.

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Molecular cloning of the K1 capsular polysaccharide genes of *E. coli*

Richard P. Silver*, Charles W. Finn*, Willie F. Vann*, Wendy Aaronson*, Rachel Schneerson*, Peter J. Kretschmer†§ & Claude F. Garon‡

* Division of Bacterial Products, Bureau of Biologics, FDA, Bethesda, Maryland 20205
† Molecular Hematology Laboratory, National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland 20205
‡ Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland 20205

Epidemiological and immunological evidence indicates that the K1 capsular polysaccharide confers the property of virulence on *Escherichia coli*. *E. coli* K1 is associated with invasive diseases in humans and in laboratory and domesticated animals[1]. K1 isolates account for 80% of *E. coli* neonatal meningitis and comprise the majority of capsular types in neonatal septicaemia without meningitis and in childhood pyelonephritis[2,3]. Passive administration of K1 antibodies prevented bacteraemia and meningitis in infant rats fed *E. coli* K1[4]. Nonencapsulated

§ Present address: Bethesda Research Laboratories, Rockville, Maryland 20850.