By this assay the thresholds for aspartate and valine are $5 \times 10^{-9}M$ and $5 \times 10^{-3}M$, respectively. As in the capillary assay, a 50 percent effect is again observed when attractant and repellent are equally above threshold: roughly tenfold for Fig. 2, A and B.

Apparently bacteria have a "data-processing" system that receives opposing signals from the chemoreceptors for positive and negative chemotaxis, sums these signals, and sends the result to the flagella for action. The chemoreceptors for attractants might move the bacterial membrane potential in one direction while the chemoreceptors for repellents might move it in the opposite direction; or there may be a chemical whose level, influenced by the chemoreceptors, determines flagellar action (*12*).

The present and prior (*4*, *5*) results eliminate the following possibilities: (i) That the attractant might be dominant. Thus no matter how high the concentration of repellent, the bacterium ignores the repellent and is attracted. (ii) That the repellent might be dominant. Thus no matter how high the concentration of attractant, the bacterium will not be attracted so long as any repellent can be detected. (iii) Indecision. The bacterium might be neither repelled nor attracted, but vacillates ineffectively no matter what the concentration of attractant or repellent so long as they are detectable. Instead, the results show that bacteria presented simultaneously with both attractant and repellent respond to whichever is present in the more effective concentration.

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9. When the capillary contains saturating concentrations of attractant, the bacteria collect at some distance away from the mouth of the capillary where the concentration of attractant is low enough to be detected, but this does not lead to accumulation of bacteria inside the capillary in the time allowed. Mesibov *et al.* (*8*) showed that a gradient of attractant is most potent when it is nearest the dissociation constant of the chemoreceptor's recognition component; from Fig. 1B that would be 10^{-4} to $10^{-3}M$ aspartate.
10. In the case of D-galactose, the effect shown in Fig. 1A can be readily demonstrated, but the inhibition by $10^{-1}M$ valine can be overcome only slightly by increasing the concentration of galactose (as in Fig. 1B, left). This can be explained by the observation that the galactose chemoreceptor is relatively unresponsive; see (*8*).
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Mobility and the Restriction of Mobility of Plasma Membrane Lectin-Binding Components

Abstract. *Labeling by ferritin-conjugated agglutinins from* Ricinus communis *was used to demonstrate the relative mobilities of the agglutinin receptors located in specific regions on plasma membranes of rabbit spermatozoa. The relative mobility of lectin receptors was higher on postacrosomal regions of sperm than on acrosomal and tail regions. Lectin-induced clustering could not be demonstrated in the acrosomal and tail regions, an indication of the existence of localized restraints on the mobilities of lectin receptors. A system of transmembrane restraints may maintain the segregation of plasma membrane components into membrane domains on certain highly differentiated cells.*

Evidence has accumulated that the plasma membranes of animal cells are dynamic, fluid structures (*1*). For example, membrane lipids have been shown to undergo rapid lateral movements during electron paramagnetic and nuclear magnetic resonance experiments (*2*).

Other membrane components such as glycoprotein antigens and lectin-binding constituents are also capable of lateral motion. In experiments on the intermixing of surface antigens on heterokaryons after virus-induced cell fusion, Frye and Edidin were able by immunofluorescent methods to demonstrate the movement of membrane (glycoprotein) components (*3*). Also, fluorescent antibodies applied by micropipetting bind in small patches to cultured muscle fibers, but the patches quickly spread—an indication of rapid planar diffusion (*4*). Utilizing fluorescent antibodies (*5*, *6*) and fluorescent lectins (*7–9*), several investigators have described the movement of membrane components into "clusters" or "caps."

Electron microscopy has been used to determine small lateral movements (of the order of hundreds of nanometers) on cell plasma membrane surfaces. Ferritin-antibody (*5*, *10*), ferritin-lectin (*11*), and lectin-peroxidase (*12*) labeling of membrane components has demonstrated a change in the distribution of these receptors from uniform or random to aggregated or clustered distributions. Freeze-etch electron microscopy was used to study *p*H-dependent reversible changes in the distribution of human erythrocyte membrane-intercalated particles (*13*) that are associated with outer surface anionic groups such as sialic acid (*14*). Surface labeling with ferritin conjugates or hemocyanin markers have been used to show dynamic changes in the distribution of lymphocyte, fibroblast, and erythrocyte membrane antigens and saccharides (*10*, *11*, *15*). The movement of particles or lectins attached to cultured cells growing on substrate has suggested a flow process of mobile membrane components (*16*).

The lateral mobility of certain membrane components has been described (*1–16*), but there are indications that particular components may have specific rates of lateral motion. Frye and Edidin noticed that fluorescent antibodies against mouse and human antigens intermixed at different temperature-dependent rates on fused heterokaryons (*3*) and certain cell surface antigenic components form antibody-induced or lectin-induced temperature-dependent caps more readily than others (*5*, *9*). Edidin and Weiss have extended these findings to include the intermixing of mouse H-2 antigens and human membrane antigens on several normal and transformed fibroblasts and found that the antigenic sites on normal cell surfaces could not be easily clustered or capped by antibodies, while they were quickly capped on

transformed cells. When two transformed cell lines were fused by the Sendai virus technique to form heterokaryons, intermixing of surface components occurred rapidly. If normal cells or hybrids of normal cells fused with transformed cells (normal/transformed heterokaryons) were used, the times required for antigen intermixing were dramatically increased (*17*). Low temperature or chemical fixation prevents the lateral movement of conconavalin A (Con A)–binding sites on transformed cell surfaces visualized with replica techniques (*15*), correlating well with experiments in which fluorescent Con A was used (*7*).

Although the mobility of certain membrane components has been well established, other components may not be as readily mobile in the plane of the membrane. In studies on the distribution of anionic sites that bind colloidal iron hydroxide (CIH) on mammalian spermatozoa plasma membranes, we found that the inherent distribution of CIH-binding sites on glutaraldehyde-fixed sperm was discontinuous. On the head of several species of mammalian sperm the CIH sites were restricted to specific regions, although these cells are surrounded by a single continuous plasma membrane (*18*). For example, the CIH-binding site density on the plasma membranes of rabbit spermatozoa (isolated from the cauda epididymis and fixed with glutaraldehyde) was high on the tail and neck regions, but absent on the acrosomal and postacrosomal regions of the head (*18*). This finding was in marked contrast to the uniform CIH labeling on several fibroblast and lymphoid cell surfaces (*19*). Also, Edelman and Millette (*20*) have found that Con A–binding sites are disproportionately represented on mouse sperm heads as compared to tails, indicating a lack of free intermixing of Con A sites between these regions.

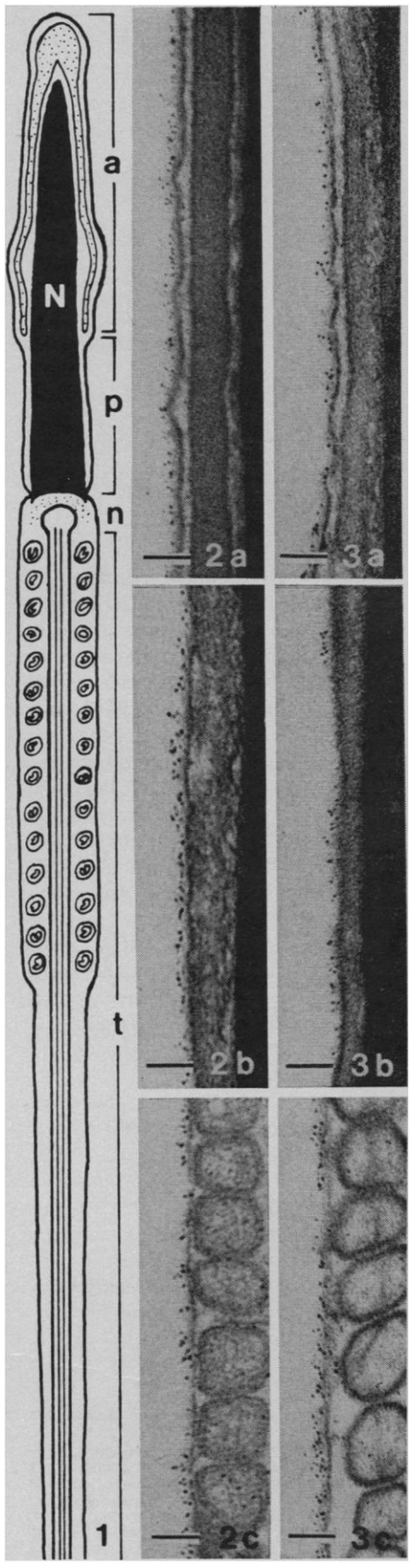

Fig. 1. Diagram of a rabbit spermatozoon (side view): *N*, nucleus; *a*, acrosomal region; *p*, postacrosomal region; *n*, neck region; *t*, tail region. Fig. 2. Ferritin-conjugated *Ricinus communis I* labeling of rabbit spermatozoon at 0°C. Sperm were collected from caput epididymis and washed twice by centrifugation in 1 m*M* tris·HCl–0.9 percent sodium chloride, *p*H 7.4, containing 0.1 percent bovine serum albumin. Sperm were labeled with the ferritin conjugate (< 1 mg of protein per milliliter) at 0°C for 14 minutes, washed, and then incubated further at 0°C for 15 to 20 minutes. At that time the sperm were fixed in 1 percent buffered glutaraldehyde for 1 hour, and then postfixed in 1 percent buffered osmium tetroxide for 1 hour. Finally, the sperm were dehydrated in a mixture of ethanol and propylene oxide, embedded in Epon 812, and sectioned with a diamond knife. (a) Acrosomal region; (b) postacrosomal region; (c) tail region. Bars represent 0.1 μm. Fig. 3. Legend is the same as in Fig. 2, except that sperm were labeled for 14 minutes at 0°C, washed, and then incubated at 37°C for 15 to 20 minutes prior to fixation. Bars represent 0.1 μm.

We report here on the relative mobility of the plasma membrane lectin-binding sites on rabbit spermatozoa (*21*) for *Ricinus communis* agglutinin (RCA_I), an affinity purified plant lectin that binds to oligosaccharide residues with terminal structures similar to β-D-galactose (*22*). Rabbit spermatozoa were collected from caput epididymis and washed twice by centrifugation in tris · HCl buffered saline containing 0.1 percent bovine serum albumin. RCA_I was conjugated to ferritin (Fer-RCA_I) in a buffer containing 0.05*M* β-lactose and purified by affinity chromatography (*23*). Some of the sperm samples were first fixed with 3 percent buffered formaldehyde for 15 minutes and washed in a buffer containing glycine before they were labeled. Unfixed or formaldehyde-fixed cells were labeled with Fer-RCA_I for 7 to 14 minutes at 0°C, washed once, and then incubated at 0°, 20°, or 37°C for 10 to 15 minutes. At that time the cells were washed and fixed in 1 percent glutaraldehyde and postfixed in 1 percent osmium tetroxide and processing for Epon embedding.

If unfixed rabbit spermatozoa were labeled with Fer-RCA_I at 0°C and then incubated further at 0°C, the distribution of Fer-RCA_I on the sperm heads and tails (see Fig. 1 for diagram) was continuous (Fig. 2, a to c). If sperm were labeled at 0°C and subsequently incubated at 20° or 37°C, the Fer-RCA_I molecules were clustered in the postacrosomal region of the plasma membrane (Fig. 3b). However, on the acrosomal (Fig. 3a) and tail regions (Fig. 3c) the distribution of Fer-RCA_I remained rather uniform and clustering of RCA-binding sites was not evident. Occasionally the "clustering" of RCA-binding sites at 20° or 37°C in the postacrosomal region left this region almost bare on some sperm (not shown). Prior fixation of sperm with formaldehyde resulted in uniform Fer-RCA_I distributions in all sperm regions, regardless of subsequent incubation temperatures [in agreement with other studies (*7, 15, 24*)]. This result indicates that discontinuous distributions of RCA_I-binding sites on sperm plasma membranes in postacrosomal regions after labeling at 0°C and incubating at higher temperatures was due to lectin-induced redistribution and that the mobil-

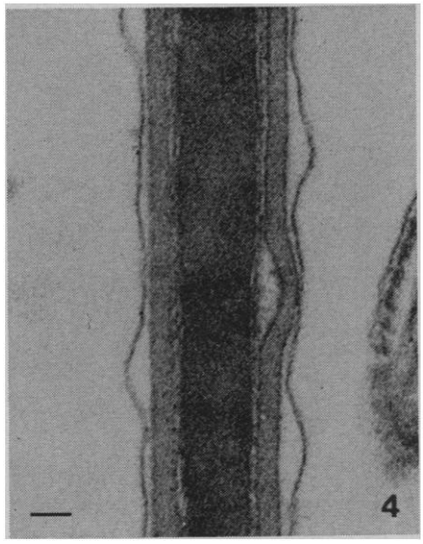

Fig. 4. Saccharide control for ferritin–*Ricinus communis I* labeling. Legend is the same as in Fig. 2, except that 0.1*M* β-lactose was included in the incubation and wash solutions. Area shown is in the acrosomal region. Bar equals 0.1 μm.

ities of plasma membrane RCA receptors in the sperm acrosomal and tail regions were relatively lower than in the postacrosomal region. Labeling to sperm was specific as β-lactose blocked all Fer-RCA_I labeling (Fig. 4).

The most reasonable explanation for the difference in relative lectin-induced aggregation or lateral shifting of RCA receptors on postacrosomal regions of sperm heads is that in these membrane regions the RCA receptors are freely mobile in a fluid membrane environment and are easily aggregated by polyvalent RCA_I molecules. However, on the sperm plasma membrane tail and acrosomal regions, RCA receptors are less mobile and not as susceptible to lectin-induced aggregation. Alternatively, lectin-binding sites in these regions may be more easily cross-linked by lectin molecules, leading to inhibition of receptor mobility (*6*). The relative immobilization of certain plasma membrane components is not in opposition to the principles of the fluid mosaic membrane model of Singer and Nicolson (*1*). The sperm plasma membrane lipids in the tail and acrosomal regions may exist in a fluid state while certain other integral membrane components remain relatively immobilized. For example, certain integral glycoprotein components may have their positions relatively "frozen" by peripheral membrane restraints at the inner surface of the plasma membrane. A system of specific peripheral membrane restraints may explain the existence of highly ordered membrane structures such as junctional and budding viral macromolecular complexes (*25*). In the case of the specialized spermatozoa structure, a system has evolved that efficiently maintains membrane domains so that specific plasma membrane components are restricted to certain defined (and morphologically identifiable) regions of the head and tail.

There is some precedence for peripheral membrane restraint of plasma membrane components. Berlin and coworkers have found that microtubule-disrupting drugs affect the agglutinability of fibroblasts and polymorphonuclear leukocytes by Con A (*26*). In a direct study of peripheral membrane component interaction with an integral membrane glycoprotein, Nicolson and Painter (*27*) have shown that immunoglobulin G antibodies against spectrin (*28*) [a peripheral membrane protein located on the inner surface of the human erythrocyte ghost (*29*)], upon binding to the inner surface of the membrane and aggregating spectrin, cause a concomitant aggregation of linked integral sialoglycoproteins expressed on the outer surface through, presumably, a transmembrane phenomenon (*1, 28*). In an analogous fashion peripheral plasma membrane proteins may restrain the mobility of RCA receptors on specific regions of the sperm surface by transmembrane control, thereby maintaining the segregation of plasma membrane components on this highly differentiated cell.

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