ent results are consistent with theories of short- and long-term stores, in particular the models in (*6*) and (*15*). On the other hand, it is difficult for models like those in (*2*), (*8*), and (*9*) to accommodate them.

An interesting additional result is seen if we examine the average vividness rating of recalled slides for each list type (*16*). For the 10-1, 10-2, 20-1, 20-2, and 40-1 lists (referring to number of slides and seconds per slide) these ratings are, respectively, 4.2, 4.4, 4.2, 4.4, and 4.2. Thus average vividness was higher for recalled words which had been presented for longer durations, but average vividness did not depend on list length. To the degree that vividness ratings represent long-term trace strength we may conclude that strength varied with presentation time, but not list length. This conclusion is compatible with the theory proposed in (*6*) and (*15*).

One final note is worth emphasizing. Aside from the particular theoretical conclusions reached in the present study, the technique used to test recall for complex visual materials would appear to have quite general value. A similar technique could be used to examine recall in other free-recall tasks, in paired-associate tasks, in serial-learning tasks, or in any other recall tasks which have previously been investigated by using solely verbal materials. Such visual investigations may be contrasted with related verbal ones, and the comparisons can be expected to have considerable import for theories of memory.

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12. The data were also analyzed in terms of the ratings of vividness in many ways. For example, we graphed the probability of a vividness rating of 5 as a function of serial position. All these analyses looked qualitatively equivalent to the recall scores.
13. While the results in (*10*) lead one to expect minimal serial-position effects, it could also be argued that it is the act of writing descriptions that interferes with short-term memory and causes small recency effects.
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16. Average vividness is the sum of the vividness ratings divided by the number of non-zero vividness ratings.
17. Supported by PHS grant MH 12717-05. I thank B. Shaffer and S. White for collecting and analyzing the data.

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Are Cell Membranes Fluid?

Singer and Nicolson (*1*) have suggested that the majority of cell membranes can be considered as a fluid mosaic structure, and that "the lipids of functional cell membranes are in a fluid rather than a crystalline state."

At present, however, there appears to be evidence accumulating in support of lipid heterogeneity, specifically, that some organisms actually contain crystalline or gel-state lipids in addition to the familiar "fluid" liquid crystalline domains that have been widely accepted as a central feature of many membrane models.

The techniques of differential scanning calorimetry, x-ray diffraction, nuclear magnetic resonance (NMR), electron spin resonance (ESR), and monolayer studies have yielded evidence that appears to be consistent with the notion that perhaps only a small fraction of the lipids of a cell membrane need be in this fluid condition for organism growth to occur, that is, large quantities of lipid may be quite rigid or crystalline. I consider, in particular, evidence pertaining to the plasma membrane of *Acholeplasma laidlawii B* and *Escherichia coli*.

The plasma membranes of both these organisms undergo reversible thermotropic gel-to-liquid crystal phase transitions of low cooperativity (*2–4*). With unsupplemented or stearate-supplemented cells of *A. laidlawii B*, the thermal transition encompasses the growth temperature, 37°C (*2–4*). Indeed, for stearate-supplemented membranes, the transition extends from about 35° to 73°C. This must indicate that the lipids are predominantly in a crystalline gel state at the growth temperature (37°C).

Similarly, with *E. coli* grown on "normal" media at 37°C, the membranes and their isolated lipids exist on a broad thermal transition (*4*), which extends from about 15° to 43°C. These broad thermal transitions are analogous to the melting of a highly impure crystal. Both crystalline gel ("solid") and liquid crystalline ("liquid") phases, are present "on" the transition. This type of phase behavior has been well investigated in simple model systems (*5*) and is a result of heterogeneity of both lipid polar head groups and hydrocarbon chains.

This interpretation is supported by x-ray data for both membrane systems (*6, 7*). Engelman (*6*) has demonstrated that, in palmitate-supplemented membranes from *A. laidlawii B*, the thermal phase transition—as detected by x-rays (a sharp 4.15-Å band changing to a diffuse 4.6-Å band)—encompasses the growth temperature of 37°C, and has also shown that the crystalline 4.15-Å spacing disappears only at 44°C, that is, well above the growth temperature.

Similarly, Esfahani *et al.* (*7*) have demonstrated that both 4.2-Å and 4.6-Å spacings are obtained from *E. coli* K12, supplemented with elaidate and linolenate, at the growth temperature. Their results also indicate that some sort of "fluidity feedback" system may be in operation.

Organisms supplemented with linolenate or oleate, which would be expected to produce more fluid lipids (mainly phosphatidylethanolamines), were shown to incorporate large amounts of palmitate, a saturated acid. For example, linolenate-supplemented membranes contained 67 percent palmitate and only 23 percent linolenate. However, membranes supplemented with elaidate incorporated 75 percent elaidate and only 14 percent palmitate. This might be predicted on the basis of a model in which many lipids need to be quite rigid, since the physical properties of dielaidoylphosphatidylethanolamine are quite similar to those of dipalmitoylphosphatidylethanolamine (*8*). Esfahani *et al.* (*7*) demonstrated, with elaidate-supplemented membranes, that the x-ray detected thermal phase transition extended from 30° to 40°C for intact membranes (organisms grown at 37°C) and for linolenate- and myristoleate-

supplemented membranes, from 36° to 46°C. It thus appears that, especially for elaidate-supplemented membranes, at the temperature of growth many of the membrane lipids are in a crystalline gel state.

Recent results from experiments, in which deuterium NMR was used, on membranes supplemented with highly deuterated fatty acids (*9*) have indicated that, with palmitate-supplemented *A. laidlawii B*, most of the lipids at the growth temperature are in a state of mobility akin to that found in the crystalline gel state of lecithin; this view has been reinforced by recent carbon-13 NMR data (*10*).

Electron spin resonance results (*11*) might at first appear to contradict this suggestion of rigid lipid domains. However, they do not, because spin-labels tend to probe only the most fluid regions in these heterogeneous systems; that is, they will only detect the liquid crystalline phase (*12*).

In monolayer experiments, Overath *et al.* (*13*) have presented evidence for the liquid crystalline nature of the membrane lipids of *E. coli*. They did find, however, that, when *E. coli* was supplemented with elaidate at 40°C, the condensed-to-expanded monolayer transition of the extracted phosphatidylethanolamines—the criterion of fluidity used—occurred at 41° to 44°C, some 4°C above the growth temperature. This would appear to actually demonstrate the presence of rigid lipids at the growth temperature, in good agreement with my interpretation of the results of Seim (*4*) and Esfahani *et al.* (*7*).

It is, of course, reasonable to suppose that crystalline regions of a membrane may limit movement of molecules through the membrane, and Krasne *et al.* (*14*) have demonstrated that the enhancement of potassium-ion transport by valinomycin is interrupted when a model bilayer is frozen by lowering the temperature. However, they also demonstrated that gramicidin A continued to function under these conditions and postulated that this occurs by the molecule forming a channel across the bilayer, which might suggest that a completely fluid membrane may not be a prerequisite for in vivo transport processes to occur.

Thus some microorganisms, significantly perhaps those that lack cholesterol, appear to be capable of growth with large quantities of their lipids in a rigid crystalline gel state.

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Yes, cell membranes are fluid.

Oldfield (*1*) makes the useful point that it is possible for the plasma membranes of prokaryotes to contain significant portions of their lipid in a crystalline state under physiological conditions. However, the title of his comment, "Are cell membranes fluid?" is a non sequitur, because none of the evidence he quotes, or any that I am otherwise aware of, shows that *all* of the lipid of the membrane can be found in the crystalline state at the growth temperature of the organism. In our original article (*2*, p. 722) we specifically stated that it is "not excluded that some significant fraction of the phospholipid . . . is physically in a different state from the rest of the lipid." If part of the lipid is crystalline and the remainder is fluid, there may be functional consequences worth investigating; but there is no conflict with our fluid mosaic model of membrane structure.

With respect to the conclusion that the membrane lipids may be very largely in the crystalline state under physiological conditions, a great deal rests on x-ray diffraction evidence defining the low temperature end of the solid-liquid transition. An example quoted by Oldfield from Esfahani *et al.* (*3*) is that of membranes from suitably supplemented *Escherichia coli* cells grown at 37°C, whose phase transition appeared to extend from 36° to 46°C. If correct, this would suggest that very little of the membrane lipid was fluid at 37°C. However, in the very careful analysis of Engleman (*4*), it is explicitly stated that one cannot accurately define the low temperature end of the phase transition by the x-ray method, because it is difficult to determine when the diffuse band characteristic of the liquid state of the lipid is entirely absent. This always produces an overestimate of the low temperature end of the transition, easily by as much as 10°C. In other words, in the case just mentioned, the transition range might well have been 26° to 46°C, rather than 36° to 46°C as quoted.

Finally, since our article was published, there have appeared numerous reports (*5*) which not only establish that the molecular components of many cell membranes are rotationally and translationally mobile, but that such mobility almost certainly has profound biological significance.

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