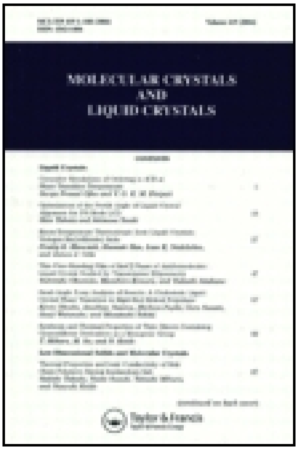

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GAS-SOLID REACTIONS IN BILAYER STRUCTURED SYSTEMS

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ABSTRACT: It is shown that various gases can diffuse through and react with amphiphilic solids having a "stacked bilayer" type of structure, such as, for example, fatty acids and fatty amides and lecithin. The rate of this diffusion and of any reaction which may take place is dependent on the length and efficiency of packing of the hydrocarbon chain of these molecules. It is relatively independent of the nature of the polar or hydrogen bonded part of the molecule. Some interesting parallels exist between the behavior of these solids and other bilayer systems.

INTRODUCTION

The molecules of many amphiphilic substances can be pictured as having a polar head group attached to one or more long hydrocarbon tails. These molecules usually tend to arrange themselves in layers, polar group alongside polar group and hydrocarbon tail alongside hydrocarbon tail, resulting in a lamellar structure in which the hydrophillic polar groups in a planar array, are attached to a layer consisting of the hydrophobic hydrocarbon tails. This tendency manifests itself in the characteristic behavior these substances have of forming monolayers on the surface of water. Two such layers back to back, with the polar groups facing outward, form what may be called a "bilayer", a structure, if one may draw the analogy, reminiscent of the lipid bilayer found in cell membranes. The crystal structure of many of these materials can be pictured as a stack of bilayers. In a way this is analogous to a stack of Langmuir-Blodgett layers in the head to head, tail to tail configuration. Substances such as fatty acids (1) and linear aliphatic amides (2,3,4,5,6) commonly fit this pattern. The same also seems to be true of the phospholipids, such as lecithin (7,8), which form such a major part of cell membranes, and also many surface active agents such as the di-n-alkyl sulphosuccinates (9).

These solids can be looked upon as essentially stacked bilayer systems. They have interesting properties and can easily be obtained in bulk with reasonable purity. The structures of many of them have been solved by diffraction techniques and are known in good detail. Certain parameters can be varied easily. For example, the linear aliphatic amides all seem to have very similar crystal structures (3). The major difference between them lies in the variation of one of the lattice parameters with the length of the aliphatic chain. This results from the variation of the thickness of the bilayer with aliphatic chain length and, therefore the lattice spacing orthogonal to the plane of the bilayers. The effect of lattice spacing on various properties can easily be studied in these systems by choosing compounds of varying chain length (10,11).

In our previously published work with amides having relatively short aliphatic chains (6,10,11), we demonstrated that a number of gases can diffuse readily into these solids and permeate their structure. This was done by generating free radicals within the solid using gamma radiation and observing their reaction with active gases which diffused into the crystal. In this sense the free radicals, which can be easily monitored by ESR, served as a tracer for the diffusion process. It was suggested that diffusion in these systems could be broken down into three distinct components. These were 1) rapid diffusion along grain boundaries, dislocations and similar defects, 2) diffusion parallel to the layers, most readily in the plane between the ends of the facing hydrocarbon tails, and 3) slow diffusion in a direction orthogonal to the layer planes. This was indicated, among other things, by the rapid decrease in reaction rate as hydrocarbon chain length was increased. The diffusion is therefore strongly anisotropic. Among the compounds we investigated, this pattern of behavior was seen among mono-amides having the "bilayer" type of structure. Diamides such as succinamide and adipamide, which yielded chemically similar radicals but which have a different type of crystal structure, yielded no evidence of diffusion or reaction of the radicals with the gas (6).

In this communication we principally describe work on lipids 18 carbons long. We consider the effect on the reaction rate of various factors such as the presence of cis and trans double bonds, the packing of the hydrocarbon chains in the lattice and other relevant parameters. The principal gases used were nitric oxide, nitrogen dioxide, oxygen and sulfur dioxide. However, since all these gases gave similar results, we will confine our discussion largely to the nitrogen oxides. In a subsequent communication, (13), we will describe some of the effects of the reaction on the properties of these substances when cast as monolayers on the surface of water. Finally, we shall attempt to point out some plausible parallels to the behavior of lipids in some other bilayer systems such as cell membranes and liposomes. It should be pointed out, however, that the results described here are preliminary in nature and should be considered simply as suggestive of trends. Our principal purpose here is to point out some of the potential that work with these systems offers.

EXPERIMENTAL

The stearamide, oleamide, palmitamide, elaidic acid and stearic acid were the purest obtainable and then recrystallized twice. Stearamide, stearic acid and elaidic acid were recrystallized, first from chloroform and then from methanol. Acetone was used for both recrystallizations of oleamide. Lecithin was first dissolved in ether and precipitated with acetone. Then it was *redissolved in chloroform and precipitated* with acetone. For most work the material was ground and sieved to obtain a particle size range between 44 and 88 micron.

For the electron spin resonance observations we used essentially the same technique as in our previous work (10,11,12). The sample was sealed under vacuum in a pyrex tube designed so that one end could be annealed while the sample was in the other end. The sample was then irradiated in a ^{60}Co source, usually to a dose of 10 megarad at liquid nitrogen temperature. After annealing the unoccupied end of the tube to remove any residual ESR signal in the pyrex, and warming the sample to 23C, the sample was shaken into the annealed end and placed in the ESR spectrometer. The spectrometer was then centered on the largest peak of the spectrum and monitored long enough to indicate the rate of radical decay under vacuum conditions. The relevant gas was then admitted and the change in amplitude was observed. The gases used were oxygen, nitric oxide, nitrogen dioxide and sulfur dioxide unless otherwise stated. The gas pressure was 260 nm unless otherwise stated. For the first few minutes of the radical decay, the ESR spectrometer remained centered on the largest peak of the spectrum. Afterwards the spectrum was scanned every five minutes to determine if its shape had changed. Since, in all cases examined, the spectrum remained constant in shape during the radical decay, the amplitude can be taken as proportional to the radical concentration. In some cases with nitric oxide and nitrogen dioxide the samples were not previously irradiated. For details see references (10,11,12).

RESULTS AND DISCUSSION

After irradiation by gamma rays, the concentration of free radicals in stearamide, an 18 carbon saturated amide with a linear aliphatic chain, exhibits a relatively slow rate of decay when under vacuum at room temperature. The rate markedly increases upon the addition of a gas capable of reacting with free radicals, such as nitric oxide, nitrogen dioxide, oxygen or sulfur dioxide. The decay in the presence of nitric oxide or oxygen is much more rapid than with sulfur dioxide, as would be expected. For brevity's sake, only the data for nitric oxide is shown (Figure 1A) and will be discussed. As previously demonstrated, (6,10,11,12), this enhanced decay is not simply a surface effect. Palmitamide, the 16 carbon analog of stearamide, shows larger decay rates as would be expected for a molecule with a shorter aliphatic chain, (Figure 1C). The decay rate of the 12 carbon amide is faster yet. Experience with compounds having very short chain lengths (10,11,12) shows that radical decay rates, and therefore, by implication the overall gas diffusion rates, decreases regularly with chain length.

A solid solution of two compounds of differing chain length would be expected to show some disorder in the plane that included the hydrocarbon chain ends from the two sides of the bilayer. This would tend to disrupt efficient packing of these chain ends. Figure 1B shows the data for a solid solution of 90% stearamide and 10% palmitamide. As can be seen (Figure 1B), the decay rate is considerably greater than in either compound alone. Similar rates are obtained if the concentrations of the two compounds are reversed. This suggests that disorder, at least within the bilayer in the region of the chain ends, increases the rate of reaction of the free radical with the gas and therefore, by implication, the gas diffusion rate. It also reinforces the hypothesis that diffusion in the plane that includes the chain ends is an important factor in the determination of the overall rate of diffusion.

Stearic acid, the 18 carbon saturated fatty acid has

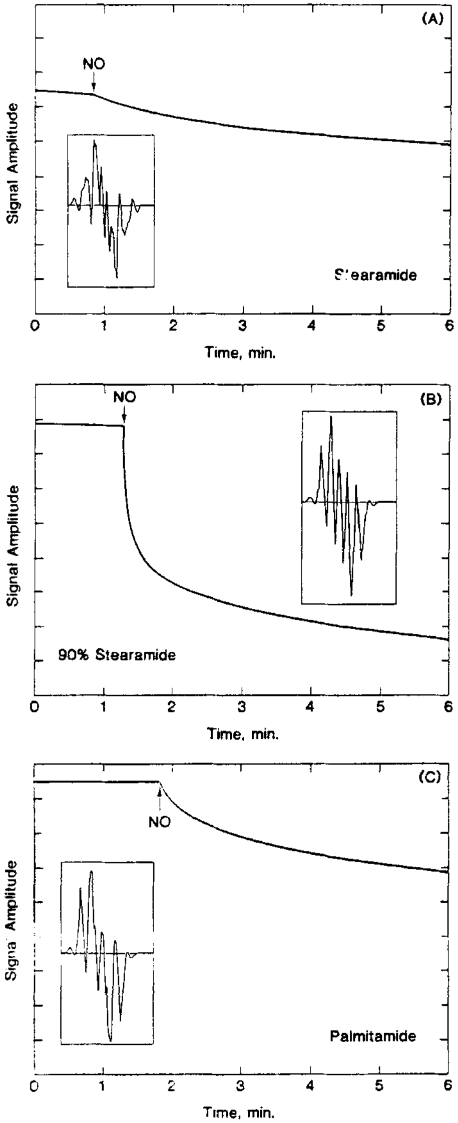

Fig. 1 Decay of ESR spectra upon admission NO. A: stearamide, B: 90% stearamide, 10% palmitamide, C: Palmitamide.

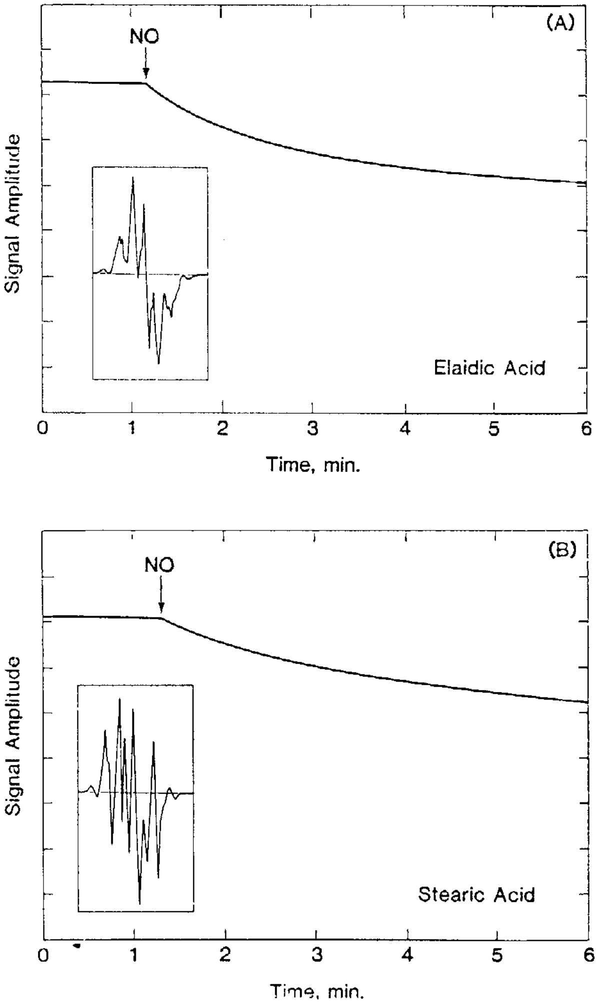

Fig. 2 Decay of ESR spectra upon admission of NO.
A: Elaidic acid, B: stearic acid.

a layer structure and chain length analogous to that of stearamide. In both compounds the hydrocarbon chains are linear and can therefore pack efficiently. However, the acid is less extensively hydrogen bonded than the amide since each amide group can engage in twice as many hydrogen bonds as the carboxyl group. Consistent with this, the melting point of stearic acid (71.5C) is about thirty eight degrees lower than that of stearamide (109C), (ref. 14). Insofar as melting point is a rough indicator of lattice energies, one would expect gas diffusion to be more rapid in stearic acid than in stearamide. Nevertheless, as Figure 2A shows, the radical decay rate and hence the gas diffusion rate in the acid is similar to that in stearamide. This suggests that diffusion across the hydrogen bonded plane may not be an important factor in the overall rate of diffusion.

Elaidic acid differs from stearic acid in having a trans double bond at the ninth carbon in the middle of the hydrocarbon chain. As will be shown subsequently, the double bond can react rapidly with nitrogen oxides provided that it is accessible to the gas. Since the double bond has a trans configuration, the molecule still should, with relatively minor perturbation, be capable of adopting an approximately linear conformation similar to stearamide and stearic acid. Thus the aliphatic chains may be expected to pack similarly for all three substances in the crystalline state. Figure 2B shows that in elaidic acid the radical decay rates in the presence of gas is only somewhat greater than in stearic acid despite the presence of a reactive double bond.

The situation is markedly different when a cis double bond is present. The cis bond introduces a distinct kink into the molecule. This would be expected to distort the strictly linear conformation of the aliphatic chains much more strongly than a trans double bond does. Thus there is more likely to be strong interference with efficient packing of the aliphatic chains. Oleamide is the 18 carbon amide with a cis double bond at the ninth carbon, the same position as the double bond in elaidic acid. It presumably also has a stacked bilayer structure. As is shown in Figure 3A, the rate of gas diffusion and therefore reaction increases enormously in this compound. Figure 3B, which presents the results obtained at a lower gas pressure, shows the effect more clearly. It is interesting to note that oleamide has twice the number of hydrogen bonds per molecule as elaidic acid. Its melting

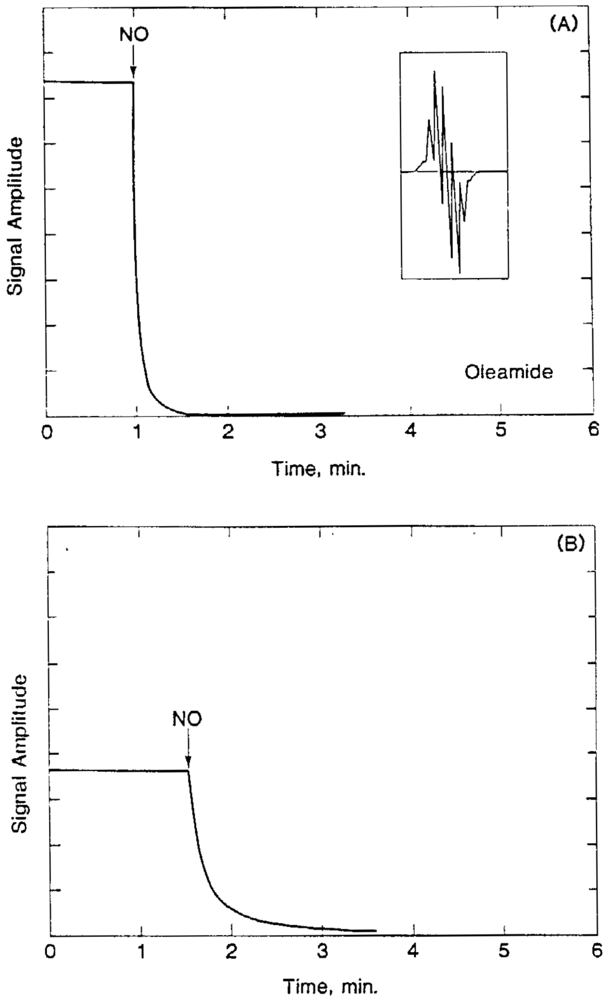

Fig. 3 Decay of oleamide spectra upon admission of NO. A: 260 mm NO, B: 65 mm NO.

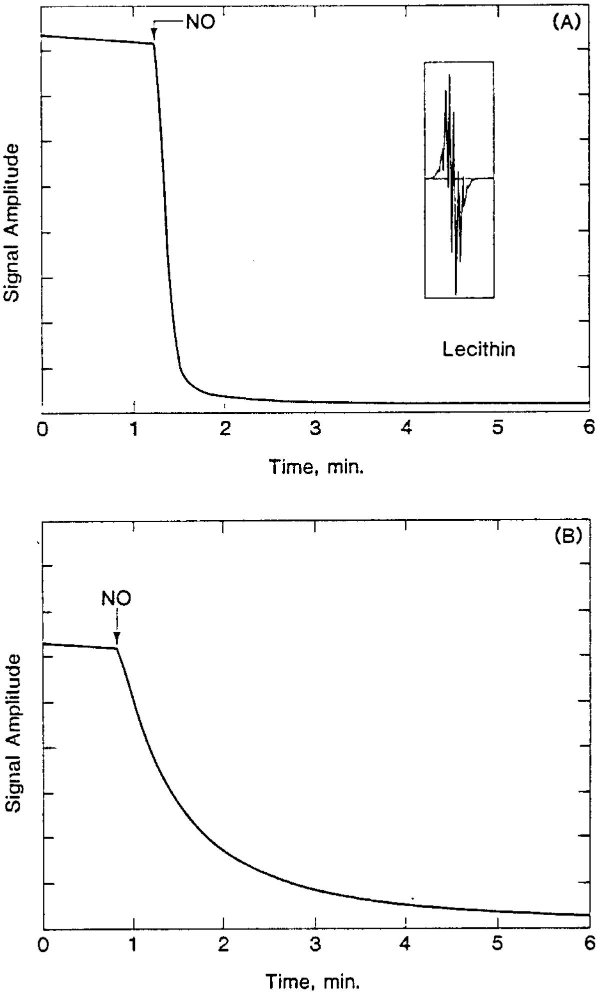

Fig. 4 Decay of lecithin spectra upon admission of NO.
A: 260mm NO, B: 65 mm NO.

point (76C) is therefore greater than that of elaidic acid (45C), (ref. 14). This is similar to the difference in melting points between stearic acid and stearamide. Insofar as melting point and hydrogen bonding is a reflection of the lattice energy in these systems, one would expect its lattice energy to be greater than that of elaidic acid. Yet the diffusion of a foreign gas into it is apparently more than an order of magnitude more rapid. This effect, so different from what might have been expected a priori, suggests that it is the efficiency of the packing of the hydrocarbon chains in these systems, rather than the overall lattice energy, that is the major factor in determining the diffusion rate and therefore the reaction rate in these compounds. Again, this suggests that diffusion across the hydrocarbon bonded plane is negligible.

The phospholipids such as lecithin and its analogs are widely distributed in nature. These lipids are among the principal constituents of the bilayer structure of cell membranes. One of its principal differences from the compounds mentioned above in that the phospholipids generally have two hydrocarbon chains attached to a large polar group. In the solid state, lecithins whose structures have been determined by diffraction have been found to have the expected bilayer type of structure (7,8). With the principal exception of the lung surfactant, usually in natural lecithins one of the hydrocarbon chains is saturated while the other has one or more cis double bonds. The close packing of the hydrocarbon chains should be disrupted similarly to oleamide. Figure 4 shows that the rate of radical decay in vegetable lecithin is about as the same order of magnitude as that of oleamide. Egg lecithin shows similar results.

These results suggest that in these and in similarly structured compounds, the rate of gas diffusion and reaction is largely governed by the packing of the hydrocarbon chains. The compounds with shorter chain lengths show more rapid radical decay in the presence of gas than compounds with longer chain lengths, probably due to a shorter diffusion path length in the hydrocarbon region of the bilayer. Diffusion across the more strongly interacting hydrogen bonded layer seems to be severely limited, if it takes place at all. This is further evidenced by an interesting observation. At room temperature, the ESR spectra of the linear amides seems to indicate that the free radicals generated by radiation are on the carbon alpha to the amide group (10,15,16). This appears to be true for amides at least up to 9 to 12 carbons long, though amides longer than that show evidence of more than one kind of radical being present. If the gas could diffuse through the hydrogen bonded layer, the shortest path length to reach the radical would be through this layer. Since the alpha radicals in the various compounds are all approximately the same short distance from this layer, the observed rate of decay would not be so sensitive to the hydrocarbon chain length. This is another indication that diffusion across the hydrogen bonded layer is negligible in these systems.

These observations suggest that we can arrange these systems according to their effect in increasing diffusion rate in the following order:

linear saturated chains < trans unsaturation
< mixed chain lengths < cis unsaturation

long chain length < short chain length

This is the order that would be expected on the basis of increasing diffusion path length with increasing aliphatic chain length and on hypothesis that increased interference with the close packing of the hydrocarbon chains and therefore increased "free space" within the lattice facilitates diffusion. As will be noted below, this order shows an interesting parallel to other bilayer structured systems such as liposomes and membranes.

An interesting observation was made with the compounds containing double bonds. After extensive decay of the initial free radical electron spin resonance spectrum in the presence of nitric oxide or nitrogen dioxide, a new three peak spectrum appears, (Figure 5). In oleamide this new spectrum can be detected within a few minutes and grows in size over several hours. This same spectrum appears at a similar rate in lecithin. In elaidic acid the new spectrum appears much more slowly and does not grow to the same extent. In the saturated compounds, it does not appear at all. Its much slower rate of appearance in elaidic acid again suggests that much slower rate of diffusion in trans unsaturated than in cis unsaturated compounds.

This new spectrum is probably due to an iminoxy radical (11). It suggests that the nitric oxide adds to

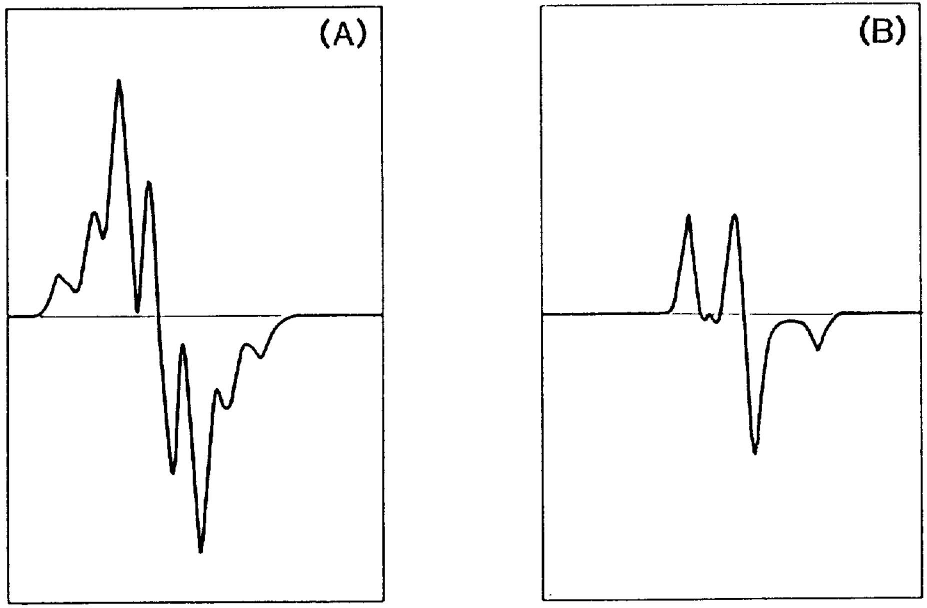

Fig. 5 ESR spectra of oleamide.
A: Irradiated oleamide before admission of NO,
B: unirradiated oleamide exposed to 20 mm NO.

the double bond even when free radicals are not initially present. Consistent with this, it was found that, in unsaturated compounds, the identical spectrum will slowly appear even in the absence of prior irradiation. Frequently a fourth peak would also appear between the two low field peaks in both the irradiated and unirradiated samples, (Figure 5). It seems to be due to the presence of a second radical. We believe this radical is probably an oxy or peroxy radical as suggested by its growth in the presence of oxygen. Preliminary analysis gave chemical evidence for peroxide formation upon extended exposure of oleamide to nitrogen dioxide. It is known that nitrogen dioxide can cause oxidation of hydrocarbons producing carboxylic acids, water and some carbonyl compounds. Oxygen, when present, enhances these reactions. In unsaturated lipids, nitrogen dioxide and oxygen together can lead to the production of peroxides and nitrous acid (17,18). It is interesting to note that lipo-peroxides have been found in the lungs and cell membranes of animals exposed to the nitrogen oxides (19).

The presence of the three peak spectrum suggests that nitrogen oxides can add to the double bond even in the absence of free radicals. As will be seen, this is supported by film balance work, (13). Since the nitrogen oxides used contain unpaired electrons, a free radical spectrum should appear upon addition of the gas. Figure 56 shows the ESR spectrum obtained upon addition of nitrogen dioxide to unirradiated oleamide. The peroxy radical peak is also seen here. Nitric oxide yields a similar spectrum. As is suggested by Figure 56, the spectrum grows with time fairly rapidly. The same effect is seen with lecithin. Elaidic acid develops a similar spectrum, but much more slowly and with smaller amplitude as would be expected.

CONCLUSIONS

The above results, and those previously obtained (6,10,11,12), indicate that gases whose molecular size is not too large are capable of diffusing into these "bilayer structured" crystalline solids. The principle factors governing the diffusion rates in these systems apparently are the size and packing efficiency of the hydrocarbon chains of the molecules. Molecules with shorter hydrocarbon groups show more rapid radical decay than longer molecules, probably due to the shorter diffusion path length in the hydrocarbon part of the bilayer. Molecules with lower packing efficiency of the hydrocarbon groups, and therefore more "free volume" within the crystal, permit much more rapid gas diffusion than molecules which can pack more efficiently. These results are apparently not sensitive to the nature of the hydrogen bonded layer and suggest that the hydrogen bonded layer, at least in these systems, acts as a barrier to gas diffusion. It is known that nitric oxide and nitrogen dioxide will react with alkenes (17,18). We have shown that these gases are capable of diffusing into the solids. It is therefore no surprise that they will react in the solid state with molecules containing double bonds even in the absence of free radicals. The rate of this reaction also seems to be diffusion controlled and therefore dependent upon packing efficiency.

As will be indicated in a subsequent communication (13), in situations where the double bond is easily accessible to the gas, the trans compound reacts as readily as the cis compound. The effect of the conformation of the double bonds, cis or trans, on reaction rate in these solids, therefore seems to depend on their effect on packing efficiency and, by implication, on diffusion rates. This suggests that, at least in these cases, decay rates of free radicals in irradiated solids of this type, are a rough indicator of the packing efficiency and the accessibility of the free volume within the crystal. Consistent with this, we found that larger, heavier molecules, such as sulfur dioxide and ethylene, react much more slowly with the radicals than the smaller molecules.

These considerations all point to the order of increasing effect in promoting diffusion rate which we noted above and which, for emphasis, we repeat here.

long chain length < short chain length
linear saturated chains < trans unsaturation < mixed chain lengths < cis unsaturation

It is interesting to compare these results with the behavior of other bilayer systems. Several workers have reported an order similar to the one given above, for passive diffusion in cell membranes and liposomes. For example, McElhaney et al. (20), found that, after growth in media supplemented with various fatty acids, the permeability to isotonic glycerol of Acholeplasma laidlawii cells, and liposomes made from their membranes, increases in the following order:

elaidic < oleic < linoleic

Linoleic acid is an 18 carbon acid with two cis double bonds and would be expected to perturb packing efficiency even more than oleic acid. The rate of glycerol diffusion into liposomes made from various lecithins was found to increase in the order

distearoyl < stearoyl-oleoyl < dioleoyl < dilinoleoyl

It was also found that the rate increases on mixing long and short chain fatty acids (21). The data reported in ref. 22 suggest that beta glucoside transport in E. coli auxotrophs is greater, both above and below the transition temperatures, when supplemented with oleic acid rather than elaidic acid. The results also suggested that a bulky side group, such as a bromine atom in the middle of the hydrocarbon chain, can be as effective as cis unsaturation (22). From the considerations given above, the side group could be expected to interfere with efficient packing of the hydrocarbon chains similarly to cis unsaturation. De Kruyf, et al. (23), determined the equilibrium eryhtritol flux through the membranes of Acholeplasma laidlawii cells grown on various fatty acids. The order was:

stearic acid < elaidic acid < oleic acid < linoleic acid

Silbert, et al. (24), found that Escherichia coli mutants deficient in unsaturated fatty acid biosynthesis, do equally well when grown with a fatty acid having a methyl side chain as when grown with oleic acid. These workers concluded that unsaturated fatty acids serve to control the packing of the fatty acid chains in the membrane bilayer. These results are all consistent with the pattern of behavior we have found in our work and suggest that there may exist some useful analogies between gas diffusion in these "bilayer structured" amphiphilic solids and passive transport through the bilayer of cell membranes.

There is another point that is of interest. The gases we picked for our work were chosen because their reaction with the free radicals could be easily monitored by ESR spectroscopy. We have also shown that nitric oxide and nitrogen dioxide will diffuse into and react, even without prior irradiation, if double bonds are present. As mentioned above, we have found some evidence for the formation of oxygenated radicals and peroxides in these systems. It is known that nitrogen dioxide can cause oxidation of hydrocarbons producing carboxylic acids, water and carbonyl compounds and that oxygen, when present, enhances these reaction (17). It is interesting to note that lipo-peroxides were reported to have been found in the lungs and cell membranes of animals exposed to nitrogen oxides (19). It has been suggested, (25), that lipo-peroxides, resulting from exposure to oxidants such as ozone and nitrogen dioxide, may be a cause of impaired bacterial uptake by alveolar macrophages due to an effect on the cell membranes. This, may be another interesting parallel to the behavior noted in our work. These results suggest that the "bilayer structured" systems we have studied may, under some circumstances, serve as useful, easily obtained, bulk models for both chemical and physical processes occurring in other bilayer systems such as cell membranes and liposomes. It should be kept in mind, however, that the analogy, like every other analogy, is not exact. Cell membranes are in a more fluid state than these solids. They consist of mixtures of lipids, extensively penetrated by proteins. However, if these solids are considered as a sort of extreme or "limiting case" for lipid behavior, they may perhaps serve as useful analogs.

The results reported above must be considered as preliminary in nature and are presented here merely to indicate the potential usefulness of these systems.

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