The partitioning of bilirubin between albumin and model and biological membranes and the differential partitioning of bilirubin between membranes with different lipid and protein compositions were measured. Partition coefficients were independent of the concentration of bilirubin in membranes up to at least 7 mol of bilirubin/mol of phospholipid. The avidity of albumin for bilirubin was greater than that of membranes, but the avidity of the latter for bilirubin depended on the composition of the membrane. Bilirubin partitioned preferentially into model membranes comprised of microsomal lipids > dioleoylphosphatidylcholine = plasma membrane lipids ≫ egg phosphatidylcholine = dimyristoylphosphatidylcholine. Partitioning into membranes was increased if these contained proteins, but the effect of proteins could not be attributed to specific binding to sites on proteins, as reflected by the temperature independence of partition coefficients. Differential partitioning of bilirubin into different membranes of pure lipids also was independent of temperature. Differences in the bulk phase fluidity of membranes does not appear to account for the preferential partitioning of bilirubin into some membranes. It appears that bilirubin partitions into elements of free volume of differing sizes in membranes with variable lipid compositions and that the size of these elements can be increased by adding proteins to membranes.

Interactions between small apolar or amphipathic molecules and lipid bilayers have been used as a tool to perturb membranes and thereby to study dynamic and thermodynamic properties as well as the influence of the lipid matrix on the function of integral membrane proteins (1–10). In addition, there have been many attempts to correlate the dynamic properties as well as the influence of the lipid matrix on the function of integral membrane proteins (1–10). Dill and co-workers (14, 15) have pointed out the theoretical objections to this approach. This will be true not only for completely apolar compounds but for amphipathic compounds for which trans-membrane movement is catalyzed. Thus, the rate of flip-flop in this case will be given by 

\[ \frac{\text{flip-flop}}{\text{time}} = k[E][S], \]

where \( E \) is the catalyst and \( S \) is the pool of compound in the membrane. It follows that knowledge of the solubility characteristics for apolar compounds in different membranes and the rate constants determining solubility provide a basis for predicting and quantitating the sites and rates of metabolism of apolar compounds from relatively simple chemical data. We have shown, for example, that this idea applies to predictions about the metabolism of fatty acids by perfused liver (21, 24). We examine in this paper the influence of membrane composition on the partitioning of bilirubin.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bilirubin purchased from Sigma or Fluka was purified according to the method of McDonagh and Assisi (27) and stored in the dark at −70 °C. Purity was maintained for at least 6 months under this condition. Bilirubin was added to all reaction mixtures as a solution in 10 mM NaOH. Solutions of bilirubin were made fresh and used within 60 min. Solutions of bilirubin and all reaction mixtures with bilirubin were kept in the dark as much as possible and were worked with in dim light. Bovine serum albumin was purchased from Calbiochem and used without further purification. Phospholipids were purchased from Avanti or Sigma and used without further purification. Male Wistar rats were obtained from Charles River Breeding Laboratories.

**Preparation of Vesicles**—Lipids dissolved in hexane or chloroform were added to a stainless steel cup and solvent-evaporated under a stream of argon. The lipids were dried further under a vacuum overnight and then suspended in 100 mM KCl, 1 mM ascorbate, and 10 mM Tris, pH 7.4. Unilamellar vesicles (ULVs)1 were prepared by

1 The abbreviations used are: ULVs, unilamellar lipid vesicles; DMPC, dimyristoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; egg PC, egg phosphatidylcholine.
continuous sonication for 20 min under a stream of argon. The temperature for sonication was 4 °C for vesicles of DOPC and egg PC and 37 °C for DMPC. The standard tip of a Heat Systems W225 sonicator, set for 25% of maximum power, was used. ULVs were centrifuged at 100,000 × g for 15 min, after completion of sonication, to remove multimolecular lipids and debris from the tip of the sonicator.

Plasma membranes extracted from male rats weighing 250-300 g by the method of Song et al. (28). The membranes were characterized by enzymatic markers and were typical of those described in Ref. 28. Liver microsomes were prepared as described previously (29). Total lipids were extracted from membrane fractions by the method of Kates (30) and used to make ULVs as above.

Measurement of Equilibrium Constants—The partition coefficient (Kw) for the distribution of bilirubin between two different populations of membranes is given as the ratio of the mole fractions of bilirubin (moles of bilirubin/mol of phospholipid) bound to each membrane in the pair. Kw for the distribution of bilirubin between a membrane and albumin is the ratio moles of bilirubin/mol of albumin to moles of bilirubin/mol of phospholipid.

The distribution of bilirubin between albumin and plasma membranes or microsomes was determined as follows. Albumin, 0.25 ml of a 0.2 mM solution, was mixed with phospholipids containing 10% phospholipid phosphorous in the Tissuette mentioned above. Bilirubin in 10 mM NaOH was added to achieve ratios of bilirubin to albumin (mole/mol) of 0.02-0.5. After treatment at 37 °C for 10 min, the mixture was centrifuged at 39,000 rpm for 60 min, at the same temperature, in a No. 40 Beckman rotor. This was sufficient to precipitate the membranes, which was validated by measurement of phospholipid phosphorous in the supernatant in each experiment. Bilirubin was measured in the separated components by extraction into chloroform/methanol (2/1, v/v) followed by determination of optical density at 454 nm. The molar extinction coefficient of bilirubin at 28 °C was 99,300. The mole fraction of bilirubin in the lipid portions of the membranes was less than 7% in all experiments. The bilirubin in the aqueous phase of the experimental mixtures was less than 1% of the total bilirubin in the system. Partition coefficients were independent of the amounts of bilirubin, membranes, and albumin under the above conditions.

Equilibrium constants for the distribution of bilirubin between ULVs, and either plasma or microsomal membranes were measured as follows. ULVs were mixed with membranes in the buffer above. The ratio of lipid phosphorous in the two populations of membranes were varied from 0.1 to 10 (mole/mol). Bilirubin was added in 10 mM NaOH. Each equilibrium constant was determined over a range of concentrations of bilirubin in phospholipids (0.05 to 7 mol %) to insure that partitioning was independent of the concentration of bilirubin in the membranes in each experiment. ULVs were separated from membranes by centrifugation at 39,000 rpm for 60 min at the same temperature in the Tissuette above. Measurements of phospholipid phosphorous in control experiments showed that the membranes sedimented to the bottom of the centrifuge tubes, but the ULVs remained suspended under these conditions of centrifugation. In experiments in which partitioning was measured as a function of temperature, a No. 40 Beckman rotor was equilibrated in the chamber of an L-70 Beckman centrifuge. The temperature of the rotor was checked by the temperature of water added to one compartment and adjusted to the temperature at which ULVs and cell membranes were mixed. These mixtures, plus bilirubin, were transferred from water baths to the rotor and ULVs and membranes separated as above. The rotor was opened, after centrifugation, without removing it from the chamber and the supernatants aspirated rapidly as each tube was removed from its compartment.

Preparation of Large Vesicles—Large ULVs of egg PC were prepared by freezing (at -70 °C) small ones made by sonication and then thawing them at room temperature (31-33). Light scattering by the frozen-thawed vesicles was increased markedly as compared with the ULVs prepared by sonication. The distribution of bilirubin between small and large vesicles was measured by mixing the two populations in buffer and adding bilirubin as above. Separation of the populations of vesicles was achieved by centrifugation at 39,000 rpm for 60 min, as above.

Preparation of Lipid-Protein Complexes of Egg PC and Bacteriorhodopsin—Bacteriorhodopsin was prepared as in Scotto and Zakim (34) and inserted into ULVs of egg PC by cosonication. The resulting protein-liposomes had a lipid to protein ratio of 40/1 (mole/mol). The proteoliposomes were separated from protein-free ULVs by centrifugation at 100,000 × g for 60 min.

Protein was measured by the method of Lowry et al. (35) and phospholipid phosphorous by a modification of the Fiske Subbarow method (36).

RESULTS

The Partitioning of Bilirubin between Albumin and Membranes or between Membrane Vesicles with Differing Compositions—Usually, the distribution of apolar compounds between different types of lipid bilayers is inferred by comparing distributions of a given compound between water and different types of bilayers. It was technically difficult to do this accurately with bilirubin because of its high avidity for membranes and the resulting low concentration of bilirubin in water. In addition, it is difficult to separate ULVs completely from water; small errors in this separation would lead to large errors in partition coefficients for bilirubin between ULVs and water. For these reasons, the distribution of bilirubin was measured directly between membranes and albumin or between membranes and ULVs, which are systems that can be separated into components. The amounts of bilirubin in water in all the experiments reported were less than 1% of the total bilirubin in the experimental systems and were ignored in making the calculations of the distributions of bilirubin reported below. A single membrane/water partition coefficient was measured for liver microsomes. K was for this system was at 37 °C. Other membrane/water or ULV/water partition coefficients can be calculated from this value and the data in Table I.

The data in Table I show that bilirubin partitions preferentially into albumin when this protein is mixed with liver microsomes or plasma membranes and when the mole fraction of bilirubin bound to albumin is less than 0.5. Of interest is that the partitioning of bilirubin in this system favors the membrane phases by a factor of 10-30-fold more than reported values for the partitioning of long chain fatty acids between albumin and these same membranes (19, 25) even though the values of Kw for the complexes albumin-bilirubin and albumin-fatty acids are nearly identical at 37 °C (37, 38). Lipid bilayers, therefore, must have a greater avidity for bilirubin than for fatty acids.

TABLE I

| System                          | Kw          |
|--------------------------------|-------------|
| Albumin/microsomes              | 10.6 ± 0.2 (3) |
| Albumin/plasma membranes        | 14.8 ± 0.2 (5) |
| Albumin/egg PC                  | 815 (calculated) |
| DOPC/microsomes                 | 0.014       |
| Egg PC/microsomes               | 0.013 ± 0.001 (4) |
| DOPC/microsomes                 | 0.021 ± 0.03 (4) |
| Microsomal lipids/microsomes    | 0.05 ± 0.05 (3) |
| Plasma membrane/plasma membrane| 0.33 ± 0.11 (5) |
| Microsomal lipids/plasma membrane| 2.5 (calculated)  |
| Lipids                          |             |
| Egg PC/microsomal lipids        | 0.027 ± 0.002 (5) |

The distributions of bilirubin between the indicated pairs of different phases were determined at 37 °C. The distributions represent equilibrium values. Kw is given as the ratio of mole fraction of bilirubin/albumin or bilirubin/phospholipid in each pair of phases. In experiments with albumin, the mole fraction of bilirubin bound to albumin was always less than 0.5. The mole fractions of bilirubin in lipid phases were in the range 0.5-5%. The lipid phases were comprised of ULVs of pure DOPC or egg PC or the whole lipid extracts from microsomes or plasma membranes. Values of Kw are the mean for the number of experiments in parentheses. Each experiment was the mean value for partitioning over a 10-fold range of final concentrations of bilirubin in the lipid phases.
The data in Table I also show that the extent to which bilirubin transfers from albumin into membranes depends on the nature of the membrane. Two aspects of membrane composition are important in this regard, the lipid composition of the membranes and the presence of proteins in the membranes. Partitioning of bilirubin into biological membranes was far greater than for the model membranes. This effect was not due simply to the lipid composition of the biological membranes versus the model membranes, however. Thus, bilirubin partitioned selectively into microsomes as compared with ULVs comprised of the lipids extracted from microsomes. The same was true for plasma membranes, that is bilirubin partitioned selectively into plasma membranes as compared with ULVs made from lipids extracted from plasma membranes.

The influence of lipid composition on the partitioning of bilirubin can be seen from the data in Table I showing that bilirubin partitioned preferentially into ULVs comprised of microsomal lipids > DOPC = plasma membrane lipids > egg PC = DMPC. The differences in partitioning of bilirubin between various lipid bilayers were quite large in a biological context. One can calculate from the data in Table I that the $K_{eq}$ for partitioning of bilirubin between ULVs of DOPC and egg PC was 18. For partitioning between ULVs of microsomal lipids and egg PC or DOPC, $K_{eq}$ values were 38 and 3.3, respectively.

The results in Table I were independent of the interval between mixing (from 10 to 90 min) the different pairs of membranes and albumin or membranes and ULVs and separation by centrifugation or the order of addition of components. Also, results were identical if bilirubin was cosonicated with lipids to form ULVs in the presence of bilirubin. The data in Table I, therefore, reflect distributions of bilirubin that were at true equilibrium between components in the experimental systems. This includes an equilibrium distribution of bilirubin in both halves of the membranes. Direct measurements of the rate constants for the movement of bilirubin between separated membranes and across membranes, which will be reported separately, verified that equilibrium was achieved in the time for mixing the components in Table I.

All the ULVs in Table I were prepared by sonication. Partitioning experiments were carried out usually within a few hours of preparation of the ULVs. Possibly, packing defects induced by sonication (31, 39, 40) could have affected results. This was excluded by comparing partitioning between microsomes and freshly prepared ULVs or ULVs that were kept above the phase transition temperature for as long as several days. Partitioning in ULVs of a given lipid composition was independent of the interval between preparation of the ULVs by sonication and use in a partition experiment (data not shown). The sizes of lipid vesicles also did not appear to influence the partitioning of bilirubin. The $K_{eq}$ for partitioning of bilirubin between ULVs prepared by sonication (small ULVs with a diameter of about 250 Å) versus ULVs that were frozen and thawed to induce fusion (to sizes of about 950 Å), was 1.22 ± 0.20.

The Effect of Protein on the Partitioning of Bilirubin into Lipid Bilayers—The data in Table I lead to the questions of why proteins in membranes and/or certain lipid compositions enhanced the partitioning of bilirubin. The presence of specific binding proteins is the obvious explanation for the binding of bilirubin to microsomes and plasma membranes in preference to ULVs derived from the lipids of these membranes. Liver microsomes contain a specific UDP-glucuronyltransferase that catalyzes the glucuronidation of bilirubin (41). Plasma membranes from liver are alleged to contain a specific binding protein for bilirubin (42-44). The abundance of the UDP-glucuronosyltransferase in microsomes that is specific for conjugating bilirubin is quite low (45) making it unlikely that binding sites on this enzyme could be the reason for the preferential partitioning of bilirubin into microsomes as compared with microsomal lipids. The abundance of the putative bilirubin-binding protein in plasma membrane from liver is unclear. Most important, however, is that the details of a typical experiment in Table I do not support the idea that liver microsomes and plasma membranes bind bilirubin in preference to ULVs containing only the lipids of these membranes, because the intact membranes have proteins with specific binding sites for bilirubin. For example, the amount of bilirubin bound per mg of protein of plasma membrane for the membrane pair plasma membrane lipids/plasma membranes was 30 nmol when the total amount of bilirubin in the plasma membrane was 5 mol % (moles of bilirubin/mol of phospholipid). If one assumes that an average molecule of protein in the plasma membrane is 50,000 Da, then 1 mg of membrane contains about 20 nmol of protein. Specific binding of bilirubin to proteins in the plasma membranes would depend, therefore, on at least one bilirubin binding site for each molecule of protein in the plasma membranes or the presence of a large number of sites for bilirubin on a small population of proteins. Neither of these possibilities is reasonable. The same analysis applies to results in Table I for microsomes.

A direct method for deciding whether bilirubin interacted with specific binding sites in liver microsomes and plasma membranes is to study the thermodynamics of binding. For example, if the preferential binding of bilirubin to microsomes versus ULVs comprised only of lipids reflected the shift of bilirubin from nonspecific interactions with the polar interior of ULVs to specific binding sites on microsomal proteins, then there should be a negative enthalpy change and a smaller negative entropy change associated with the transfer of bilirubin from the ULVs to microsomes. $K_{eq}$ values for the partitioning of bilirubin between microsomes and ULVs of egg PC were determined, therefore, over the temperature range of 10-40 °C. A van't Hoff plot for these data shows (Fig. 1A) that the preferential partitioning of bilirubin into microsomes versus ULVs of egg PC was driven by a positive entropy for the transfer of bilirubin from the latter to the former type of membrane. There were no differences in enthalpy for bilirubin in protein-containing membranes or vesicles of egg PC. The data in Fig. 1A, hence, are incompatible with the idea that bilirubin bound to sites on proteins in microsomes, except in the case that only a very small population of molecules was bound at specific sites. Shown in Fig. 1B is a similar experiment for the membrane pair egg PC/plasma membranes. The data in Fig. 1B indicate also that only very small amounts of bilirubin, if any at all, bound to specific sites on proteins in the plasma membranes. Certainly, the preferential partitioning of bilirubin into microsomes or plasma membranes as compared with ULVs of lipids from these membranes cannot be attributed to this kind of mechanism.

Membrane proteins might have some type of nonspecific effect on the properties of the lipids in bilayers, which enhances the capacity of the bilayers to sequester bilirubin as compared with protein-free bilayers. If this were so, any integral membrane protein might enhance the partitioning of bilirubin into ULVs containing the protein. This idea was tested by inserting bacteriorhodopsin into ULVs of egg PC at a ratio of lipid to protein of 40/1 (mole/mol), which is close
Interactions of Bilirubin with Model and Biological Membranes

The partitioning of bilirubin between different pairs of membranes as a function of temperature. Microsomes (A) or plasma membranes (B) were mixed with ULVs of egg PC in ratios of 0.1 to 10 at each of the indicated temperatures. Bilirubin was added as a solution in 10 mM NaOH. The total amount of bilirubin in each experiment was from 0.5 to 5 mol/mol phospholipid in the mixture. Final pH was 7.4. ULVs were separated from biological membranes by centrifugation at the same temperature as the mixing part of each experiment. Bilirubin in each population of membranes was extracted into chloroform/methanol (2/1, v/v) and measured spectrophotometrically, as under "Experimental Procedures." Each point is the average of four to six determinations of the partition coefficient at different ratios of membrane/ULVs and different amounts of bilirubin.

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Data presented above clarify this issue for physiologic concentrations of bilirubin. Thus, as shown in Figs. 1 and 2, bilirubin partitions selectively into different membranes because of entropic factors only. A simple physical interpretation of this result is that the free volume accessible to bilirubin in some types of membranes is larger than in others and that bilirubin distributes between membranes with different free volumes until the concentrations of bilirubin in these volumes are equal in different pairs of membranes. Bilirubin appears not to interact, therefore, with the polar region of membranes. It also does not appear to interact with the apolar regions of membranes but to localize there because of voids in the packing of bilayers, which is consistent with its limited solubility in nonpolar solvents (46). The lack of solvation of bilirubin by either the polar or apolar parts of a membrane was observed up to concentrations of about 5 mol/mol phospholipid. Based on the data in Table I for the equilibrium distribution of bilirubin between plasma membranes and albumin, one can calculate that the concentration of bilirubin in liver plasma membranes is about 0.25 mol % (mole/mmol phospholipid) at physiologic concentrations of bilirubin in blood. Bilirubin IXa hence will partition into liver plasma membranes with the same $K_{eq}$ as given in Table I at concentrations in blood at least about 20-fold greater than normal. Interactions between bilirubin and membranes may be different at higher concentrations of bilirubin (49).

The partitioning of bilirubin between ULVs of DOPC and egg PC. The data for the direct comparison are difficult to obtain, however, because ULVs of egg PC and DOPC cannot be separated from each other in the absence of incorporating molecules that allow precipitation of only one population of ULVs, e.g., lectin-induced precipitation of ULVs containing a suitable glycolipid (48). This technical problem can be avoided because $K_{eq}$ values for the partitioning of bilirubin between DOPC and egg PC can be calculated from the expression:

$$K_{eq} = \frac{K_{eq} \text{DOPC/microsomes}}{K_{eq} \text{egg PC/microsomes}}.$$  

Since the partitioning of bilirubin between DOPC and microsomes was independent of temperature (Fig. 2), and the partitioning between egg PC and microsomes also was independent of temperature (Fig. 1A), then the partitioning of bilirubin between ULVs of DOPC and egg PC also must be independent of temperature.

**DISCUSSION**

Interactions between Bilirubin and Membranes—The chemical structure of bilirubin suggests that it will behave like a polar molecule. The polar groups are involved, however, in internal H bonds in the physiologic conformer of bilirubin IXa, which has only limited solubility in water at neutral pH (46). Since the physiologic isomer of bilirubin also has limited solubility in organic solvents (46), it has been unclear whether the interaction between bilirubin and membranes is polar or nonpolar. Data supporting both conclusions have been published (47, 48). The data presented above clarify this issue for physiologic concentrations of bilirubin. Thus, as shown in Figs. 1 and 2, bilirubin partitions selectively into different membranes because of entropic factors only. A simple physical interpretation of this result is that the free volume accessible to bilirubin in some types of membranes is larger than in others and that bilirubin distributes between membranes with different free volumes until the concentrations of bilirubin in these volumes are equal in different pairs of membranes. Bilirubin appears not to interact, therefore, with the polar region of membranes. It also does not appear to interact with the apolar regions of membranes but to localize there because of voids in the packing of bilayers, which is consistent with its limited solubility in nonpolar solvents (46). The lack of solvation of bilirubin by either the polar or apolar parts of a membrane was observed up to concentrations of about 5 mol/mol phospholipid. Based on the data in Table I for the equilibrium distribution of bilirubin between plasma membranes and albumin, one can calculate that the concentration of bilirubin in liver plasma membranes is about 0.25 mol % (mole/mmol phospholipid) at physiologic concentrations of bilirubin in blood. Bilirubin IXa hence will partition into liver plasma membranes with the same $K_{eq}$ as given in Table I at concentrations in blood at least about 20-fold greater than normal. Interactions between bilirubin and membranes may be different at higher concentrations of bilirubin (49).

The partitioning of bilirubin between water and membranes reflects the balance between changes in the enthalpy and entropy for the transfer of bilirubin between phases. In the experiments above, however, we considered only the partitioning of bilirubin between membrane phases and neglected partitioning between membranes and water. Consideration of the bilirubin in the water will have essentially no effect on results, because the amount of bilirubin in aqueous solution, in all experiments, was very small. Moreover, the amounts in water do not affect conclusions about the relative solubility of bilirubin in different membranes.

**The Basis for the Preferential Partitioning of Bilirubin into ULVs of DOPC Versus ULVs of Egg PC**—As shown in Fig. 2 is a van't Hoff plot for the partitioning of bilirubin between vesicles of DOPC and microsomes. This experiment was done to assess the mechanism for the differential partitioning of bilirubin between ULVs of DOPC and egg PC. The data for the direct comparison are difficult to obtain, however, because ULVs of egg PC and DOPC cannot be separated from each other in the absence of incorporating molecules that allow precipitation of only one population of ULVs, e.g., lectin-induced precipitation of ULVs containing a suitable glycolipid (48). This technical problem can be avoided because $K_{eq}$ values for the partitioning of bilirubin between DOPC and egg PC can be calculated from the expression:

$$K_{eq} = \frac{K_{eq} \text{DOPC/microsomes}}{K_{eq} \text{egg PC/microsomes}}.$$
Different Membranes—There is an apparent correlation between preferential partitioning of bilirubin and membrane structure. Bilirubin partitioned selectively into ULVs of DOPC versus egg PC and into vesicles with protein versus protein-free vesicles of the same lipid composition. Because of double bonds in both acyl chains in bilayers of DOPC, such bilayers might have more free volume than bilayers of egg PC. Also, the mismatch in packing between acyl chains and the helices of integral membrane proteins is likely to increase the free volume in the membrane as compared with a membrane composed only of lipids. On the other hand, there was no selective partitioning of bilirubin between ULVs of egg PC and DMPC, and the difference in the partitioning of bilirubin between ULVs of egg PC and DOPC appears to be too large to be accounted for by the small differences in the viscosities of bilayers of egg PC and DOPC, as reflected by the phase transition temperatures for these lipids. It is attractive to suggest that it is easier to create space for bilirubin in less viscous membranes, but this type of mechanism should have a temperature dependence associated with it, especially as bilirubin shifted between membranes with different temperature dependences for changes in viscosity. Any change of this type must be small, however. Also, this kind of mechanism is not consistent with the data for the equal partitioning of bilirubin into membranes of egg PC and DMPC. Resolution of these uncertainties about the mechanism for the differential partitioning of bilirubin in membranes with differing lipid compositions will come from studies of the exact location of bilirubin within the apolar region.

The data for the partitioning of bilirubin between albumin and membranes suggest that bilirubin has considerable freedom of motion in the membrane, that is, it is not oriented by the membrane. Thus, although bilirubin and long chain fatty acids bind to albumin with nearly the same avidity (37, 38), bilirubin partitions into membranes from albumin, about 30-fold more than fatty acids (24). The carboxyl group of a fatty acid will be at the membrane-water interface thereby limiting the rotational freedom of the fatty acid. There are no groups on bilirubin IXα that will keep the molecule at the interface, so bilirubin as compared with fatty acids will partition more extensively from albumin into membranes because the entropy change accompanying transfer from albumin to membranes will be greater for bilirubin than for long chain fatty acids. This line of reasoning also supports the idea that bilirubin is embedded into the apolar region of membranes.

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