Partition of fatty acids

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Abstract The partition ratios of radioactive fatty acids between *n*-heptane and a physiological buffer at 37°C were measured. The fatty acids included the saturated acids with an even number of carbons from 10 to 18 and the unsaturated acids oleic, linoleic, and linolenic. In addition, the partition ratios of decanoate, myristate, and palmitate were determined over a wide pH range. Any *single* plot of partition ratio vs. aqueous concentration of an acid gave a nearly straight line, a finding consistent with very little association in the aqueous phase. In the case of the acids with 16 and 18 carbon atoms, however, comparison of the constants calculated from these plots with the assumption of no aqueous phase association revealed several inconsistencies. These inconsistencies cannot be resolved completely by assuming the existence of fatty acid association in the aqueous solution. We believe that at least some of the deviations are due to the presence of trace quantities of radioactive impurities in the labeled fatty acids. For example, purification of a sample of supposedly pure [1-^{14}C]myristate by a series of solvent extractions increased the partition ratio by a factor of 1.5. Although all of the observations cannot be explained by this interpretation, we believe that our studies suggest that there is no appreciable association of fatty acids under the usual physiological conditions.

Supplementary key words association · albumin · dimerization · binding

The partition of long-chain fatty acids between *n*-heptane and an aqueous phosphate buffer was measured by Goodman (1, 2) in conjunction with his studies on fatty acid binding to serum albumin. His work was done at 23°C in a sodium phosphate solution of ionic strength 0.16, pH 7.45. The model employed by Goodman for the analysis of these data assumed the existence of fatty acid dimerization in the heptane phase but no association in the aqueous phase. Consistent deviations from this model were noted with the longer-chain saturated and unsaturated fatty acids when the fatty acid concentration was high. To account for these deviations, Mukerjee (3) proposed the existence of fatty acid anion dimerization in aqueous media; and quite recently, Smith and Tanford (4) suggested that other forms of association occur in the aqueous phase. Subsequent studies, however, indicate that the hypothesis of Mukerjee is untenable (5, 6). Moreover, in our opinion, the hypothesis of Smith and Tanford (4) does not satisfactorily explain all of the available partition data. In an attempt to gain further insight into this question, we have conducted an extensive series of fatty acid partition measurements. Although we employed the same general method as Goodman (1), our experiments were done at 37°C in a buffered salt solution of physiological ionic strength and composition. Several of the acids were studied over a wide pH range.

EXPERIMENTAL PROCEDURE

Materials

Unlabeled fatty acids were purchased from the Hormel Institute (Austin, Minn.). Radioactive fatty acids were supplied by New England Nuclear Corp. (Boston, Mass.) and Amersham/Searle Corp. (Arlington Heights, Ill.). Each long-chain radioactive fatty acid was dissolved in *n*-heptane and extracted into alkaline ethanol. The ethanol was acidified and fatty acids were reextracted into fresh heptane. Heptane (50 ml) containing the radioactive compound was then extracted with an equal volume of 0.01 N H_2SO_4 followed by extraction with an equal volume of 0.05 M Na_2HPO_4, pH 7.0. This final step was repeated five times in order to ensure removal of short-chain fatty acid impurities. The effects of this extraction on the partition of myristate is shown in **Table 1.** Similar results were obtained with other long-chain fatty acids. After purification of the isotopes, unlabeled carrier fatty acid (10–

Abbreviations: $\bar{\nu}$, the molar ratio of bound fatty acids to albumin.

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TABLE 1. Extraction of [1-^{14}C]myristate in heptane solution by aqueous buffer[a]

Number of Extractions	Radioactivity in the Aqueous Phase	
	Sample 1	Sample 2
	dpm	
1	1675	1665
2	1126	1148
3	994	1044
4	1219	lost
5	1131	1197

[a] 1 vol of heptane extracted with 2.5 vol of phosphate buffer, pH 7.4.

20 μeq/ml) was added to the heptane solution. Analysis of the purified compounds by thin-layer chromatography indicated that more than 99% of the radioactivity was present as fatty acid. Moreover, analysis of palmitate, myristate, and laurate by gas–liquid chromatography indicated that from 96.5 to 96.9% of the radioactivity actually was present in that particular acid. When samples of these labeled acids collected from the column effluent were rechromatographed, the radioactive purity was found to be only 97.5%. Therefore, we believe that the actual purity of labeled fatty acids was 99% or more.

Unless noted otherwise, a modified Krebs-Ringer phosphate buffer solution containing 0.122 M NaCl, 0.0049 M KCl, 0.0012 M $MgSO_4$, and 0.016 M sodium phosphate, pH 7.4, was used for all incubations.

Incubation and analysis

Incubations in specially constructed glass vials were performed as described previously (7). In most experiments, 15 flasks were prepared. The removable glass rods were inserted before the solutions were added to the flask, and then 1 ml of the buffer solution was added. Heptane containing the required amount of labeled fatty acid of known specific radioactivity was added next, and the total volume of heptane in each flask was made up to 1 ml. The range of fatty acid concentrations initially present in the heptane phases was from 1×10^{-5} to 1×10^{-2} M. When unsaturated fatty acids were used, the flasks were gassed with nitrogen; with saturated fatty acids, air served as the gas phase. The flasks were capped tightly and incubated with shaking in a constant-temperature water bath for 16–20 hr. After incubation, aliquots of the heptane and the aqueous phases from each flask were added to 15 ml of a toluene–Triton X-100 80:20 (v/v) scintillator solution containing 0.55% 2,5-diphenyloxazole and 0.01% 1,4-bis-2-(4-methyl-5-phenyloxazoyl)-benzene. A refrigerated Packard Tri-Carb 2425 liquid scintillation spectrometer was used for measurements of radioactivity. Corrections for quenching were made by monitoring with the external standard and, in certain cases, by addition of an internal standard. Each set of data was analyzed on an IBM 370 computer using a least squares model-fitting procedure in conjunction with an IBM 2250 graphic display unit and an interactive PL/1 program called MODELAIDE (8).

The Mechrolab 301 vapor pressure osmometer (similar to the osmometer now available from Coleman Co.) was used to measure the vapor pressure-lowering in heptane solutions of decanoic acid.

THEORY

The partition ratio (*P.R.*) is defined as the ratio of concentration of all fatty acid in the organic phase (C_o) to that in the aqueous phase (C_w). It is well established that in organic solvents dimerization of carboxylic acids occurs. Association of various species in the aqueous phase may also occur. To include all of these we write

$$P.R. = \frac{C_o}{C_w} = \frac{[HA]_o + 2[(HA)_2]_o}{[HA]_w + [A^-]_w + \sum_{m=0}\sum_{n=2} n[H_mA_n^{-(n-m)}]} \quad \text{Eq. 1}$$

where $[HA]_o$ and $2[(HA)_2]_o$ represent, respectively, the concentrations of the monomer and the dimer in the organic phase, and $[HA]_w$, $[A^-]_w$, and $\Sigma\Sigma n[H_mA_n^{-(n-m)}]$ represent the concentrations of the un-ionized acid, the anion, and the aggregates in the aqueous phase, respectively. By defining the dimerization constant in the organic phase as $D_o = [(HA)_2]/[(HA)]^2$ and the association constants K_{mn} in the aqueous phase in a similar way, we get

$$P.R. = \frac{[HA]_o + 2D_o[HA]_o^2}{[HA]_w + [A^-]_w + \sum_{m=0}\sum_{n=2} nK_{mn}[HA]^m[A^-]^{(n-m)}} \quad \text{Eq. 2}$$

In all of our experiments except those in Tables 3 and 4, $[HA]_w$ is negligible in comparison with $[A^-]_w$, so Eq. 2 becomes

$$P.R. = \frac{[HA]_o + 2D_o[HA]_o^2}{[A^-]_w} \times \frac{1}{1 + \sum_{m=0}\sum_{n=2} nK_{mn}[HA]^m[A^-]^{(n-m-1)}} \quad \text{Eq. 3}$$

At low concentrations where association in the aqueous phase must be negligible, the second fraction in Eq. 3 is nearly 1. We define the partition coefficient (*P.C.*) of the monomeric acid as $P.C. = [HA]_o/[HA]_w$ and the ionization constant (K) as $K = [H^+][A^-]_w/[HA]_w$. By substituting these relationships into the first fraction of Eq. 3,

we obtain, at low concentrations in basic solutions:

$$P.R. = \frac{P.C.}{10^{pH-pK}} + 2D_o\left(\frac{P.C.}{10^{pH-pK}}\right)^2[A^-]_w \qquad \text{Eq. 4}$$

so that a plot of $P.R.$ vs. $[A^-]_w$ gives a straight line with intercept $P.C./10^{pH-pK}$ and slope $2D_o(P.C./10^{pH-pK})^2$. For all of the long-chain acids, the apparent pK of ionization was taken as 4.8 (9), a value obtained by making an ionic strength correction of -0.1 unit to the intrinsic pK reported for fatty acids containing five to nine carbon atoms (10). At high concentrations, association in the aqueous phase would reduce the value of the second fraction in Eq. 3, so that the partition ratio would decrease below the values given by Eq. 4. Thus, in a plot of $P.R.$ vs. aqueous concentration, aqueous phase association would produce curves that would fall farther below the straight line of Eq. 4 as concentration increased.

Eqs. 1–4 were developed with the implicit assumption that the species in the organic phase were anhydrous. Actually, as Christian, Affsprung, and Taylor (11) have emphasized, the monomer and the dimer in the organic phase may be partially hydrated. Eqs. 1–4 may be used exactly as written, however, if we redefine the constants to include the hydrated species. Let us define equilibrium constants, K_1 and K_2, for the hydration of the monomer and the dimer, respectively. We may then show that Eqs. 1–4 remain unaltered (*a*) if we define D_o as an apparent constant equal to the anhydrous dimerization constant multiplied by $(1 + K_2[H_2O])/(1 + K_1[H_2O])^2$ and (*b*) if the partition coefficient ($P.C.$) is defined as the ratio of both hydrated and anhydrous monomer in the organic phase to monomeric acid in the aqueous phase, i.e., if it is equal to $(1 + K_1[H_2O])$ times a hypothetical anhydrous partition coefficient.

Infrared measurements in carbon tetrachloride show very little difference in dimerization constants among the acids from acetic to lauric (12). Likewise, dipole moment measurements in anhydrous benzene show that the dimerization constant for stearic acid is only about 20% greater than for butyric acid (13). Therefore, the best available evidence indicates that in anhydrous solvents the dimerization constants of the long-chain saturated acids do not vary with chain length.

If we assume that the hydration of the fatty acids in the organic phase is due to interaction between the water molecules and the carboxyl groups, then the hydration constants K_1 and K_2 should be independent of chain length. From this, one concludes that the apparent as well as the true organic phase dimerization constant should be the same for each of the saturated acids.

One assumption we make throughout is of ideality in the heptane phase. Support for this comes from our measurements with the Mechrolab osmometer that indicate that the vapor pressure of heptane in equilibrium with decanoic acid solutions as concentrated as 0.4 M can be adequately explained solely on the basis of dimer formation.

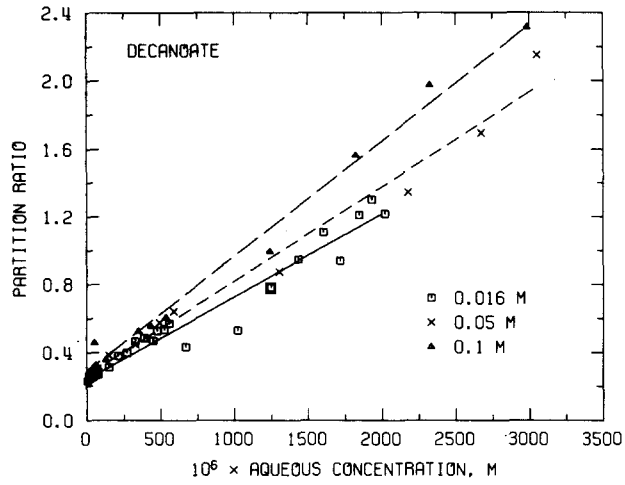

Fig. 1. Effect of buffer concentration on the partition of decanoate between *n*-heptane and phosphate buffers at pH 7.4, 37°C. The lines were drawn using the values from Table 2 in Eq. 4.

RESULTS AND DISCUSSION

Figs. 1–8 illustrate the experimental data points and the best-fitting partition ratio curve calculated from Eq. 4 for the fatty acids that we studied. The curves were drawn using the values of $P.C./10^{pH-pK}$ and D_o listed in **Table 2.** As seen with decanoate, changes in the phosphate buffer concentration from 0.016 to 0.1 M had very little effect on either the partition coefficient ($P.C.$) or the dimeriza-

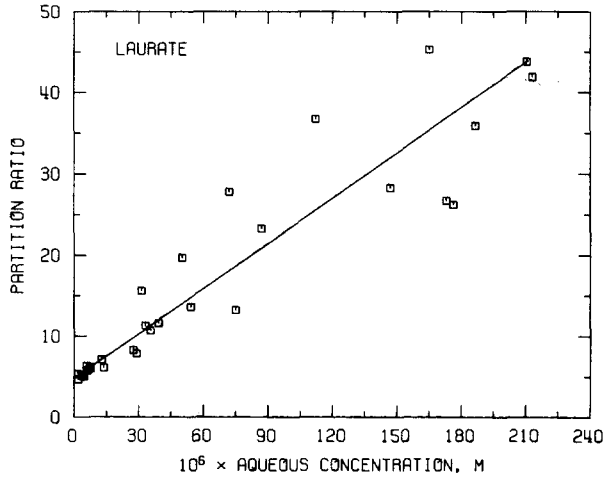

Fig. 2. Partition of laurate between *n*-heptane and 0.016 M phosphate buffer at pH 7.4, 37°C.

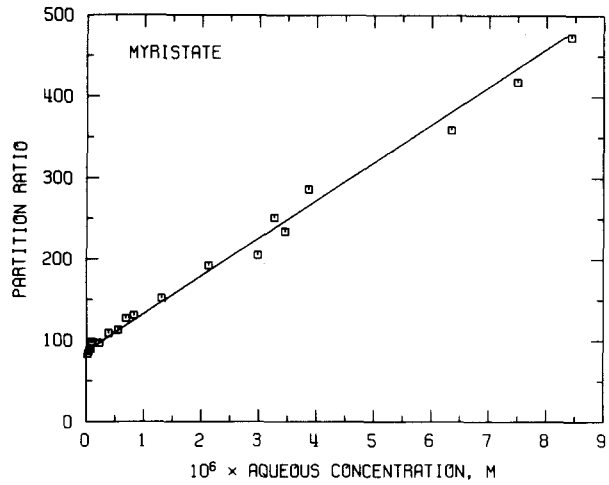

Fig. 3. Partition of myristate between *n*-heptane and 0.016 M phosphate buffer at pH 7.4, 37°C.

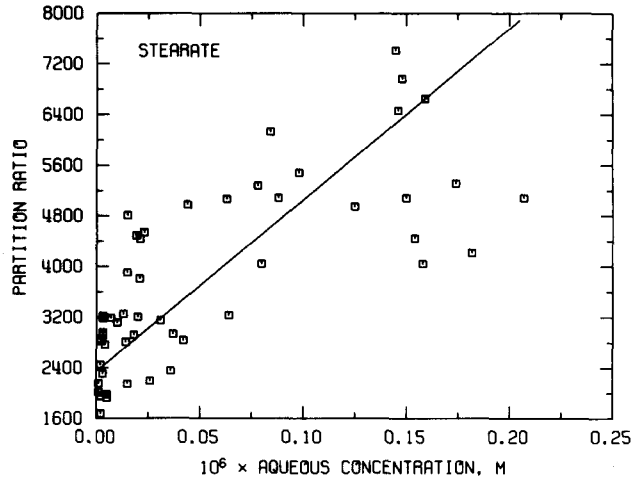

Fig. 5. Partition of stearate between *n*-heptane and 0.016 M phosphate buffer at pH 7.4, 37°C.

tion constant (D_o) in the organic phase. **Tables 3–5** show the effect of pH on the calculated values of the partition coefficients for decanoate, myristate, and palmitate. As shown for myristate and palmitate, the data obtained with phosphate, Tris, and borate buffers were quite similar.

The data for the 10- and 12-carbon acids follow partition theory very well. They fit Eq. 4 with the same value of *P.C.* throughout the pH range and with a value of D_o that is practically independent of chain length. Furthermore, the values of *P.C.* fall on an extension of the line given by the shorter acids in a plot of log *P.C.* vs. chain length (3, 4).

For myristic acid, Table 4 shows that the values of *P.C.* (calculated according to the method in Table 3) show a slight trend toward higher values with an increase in pH, and Table 2 shows that the value of D_o is smaller than for the shorter acids.

For the saturated and unsaturated acids containing 16 or 18 carbon atoms, there are great inconsistencies between the calculated constants and the predictions of partition theory, even though a plot of partition ratio vs. aqueous concentration obeys Eq. 4 over most of the concentration range (Figs. 4–8). In other words, if the acids with 16 or 18 carbons are studied at a single pH, the data are consistent with the assumption that there is little, if any, aqueous association. However, if all of the data are considered together, a number of inconsistencies appear:

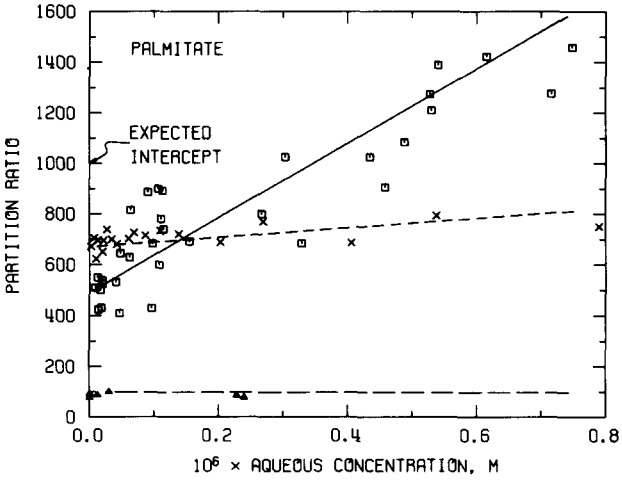

Fig. 4. Comparison of data by different investigators on the partition of palmitate between *n*-heptane and phosphate buffer. Squares represent our data at pH 7.4, 37°C. Crosses are Goodman's data (1) at 23°C, converted to pH 7.4. According to Eq. 4, the small "correction factor," $10^{7.45-7.4} = 1.12$, will convert his measurements at pH 7.45 to our pH of 7.4. Actually, Tables 4 and 5 and Fig. 4 themselves do not bear out Eq. 4 (if *P.C.* is considered constant), but over the small pH range used in the corrections, the errors should be negligible. Triangles represent data of Smith and Tanford (4) at 25°C, converted from pH 7.3 to pH 7.4. The expected intercept is that calculated by Eq.4 from Smith and Tanford's pH 9.4 or 10.4 data (see text).

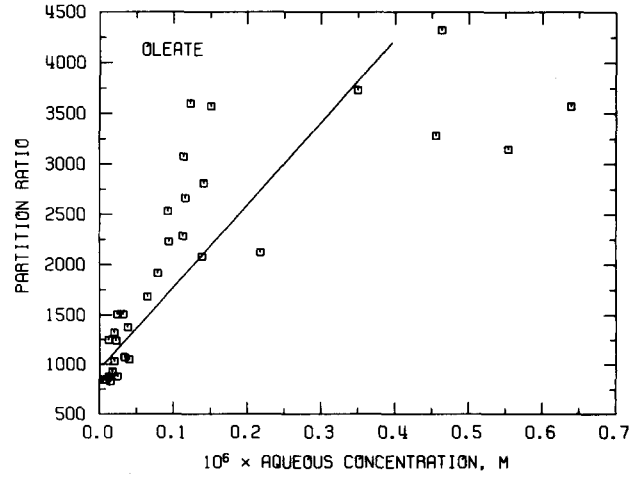

Fig. 6. Partition of oleate between *n*-heptane and 0.016 M phosphate buffer at pH 7.4, 37°C.

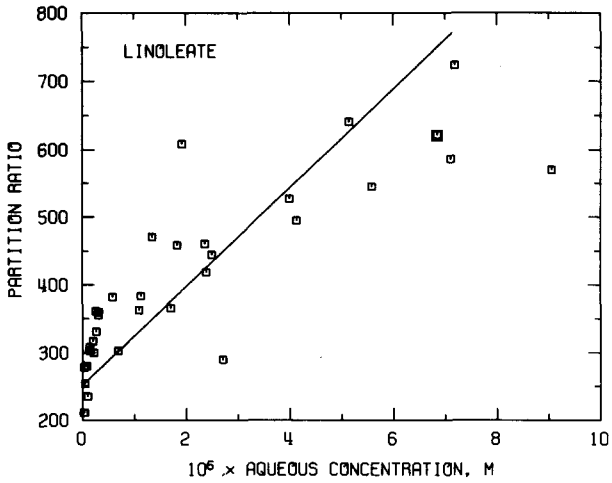

Fig. 7. Partition of linoleate between *n*-heptane and 0.016 M phosphate buffer at pH 7.4, 37°C.

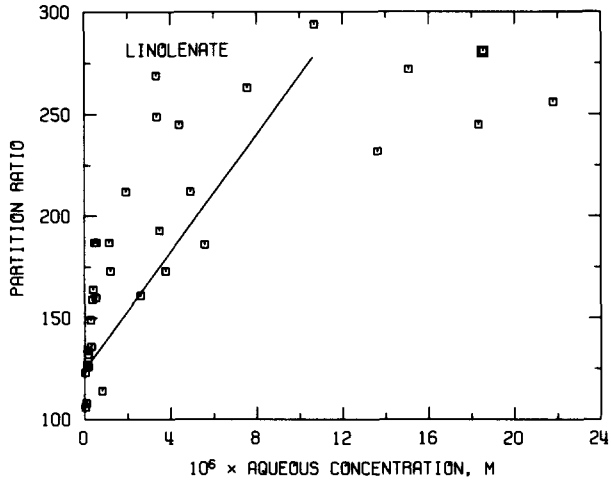

Fig. 8. Partition of linolenate between *n*-heptane and 0.016 M phosphate buffer at pH 7.4, 37°C.

(*a*) The *P.C.* for these acids, calculated in the same way as for decanoic and myristic acids, shows a large increase as the pH increases (Table 5). A comparison of Arvidsson, Green, and Laurell's (14) *P.C.* for palmitic acid at pH 8.5 with that of Goodman (1) at pH 7.45 shows the same phenomenon. The pH dependence of *P.C.* calculated from the data of Smith and Tanford (4) is even greater.

(*b*) The value of D_o, the apparent heptane phase dimerization constant, shows large variations among the acids (Table 2). It should be pointed out that the calculated D_o is sensitive to the value obtained for the intercept as well as the slope (Figs. 1–8), but, even considering this, the differences seem to be beyond experimental error.

(*c*) When calculated from experiments at pH values below 9 or 10, the ratio of *P.C.* for palmitate to that for myristate or the ratio of *P.C.* for stearate to that for palmitate is much less than the factor observed among shorter acids (1, 4) (Table 2).

(*d*) When data for the binding of palmitate or oleate to serum albumin are analyzed using the assumption that there is very little fatty acid association in the aqueous phase (i.e., by the direct application of points from, for example, Figs. 4 and 6), the binding curves ($\bar{\nu}$ vs. unbound fatty acid concentration) show large variations with pH (7).

The partition coefficient discrepancy (*a*) can be resolved by assuming that the longer-chain acids have higher pK values than the short-chain acids. For example, the palmitate data could be resolved by using a pK value of about 8.5. Nevertheless, we believe that the pK values of all of the fatty acids actually are very nearly the same. The available reports fall into two categories: mixed solvent titrations showing no significant differences among fatty acid pK values (9, 15–17) and measurements made in pure aqueous solutions that give higher pK values for the longer acids (18–20). We believe that the latter experiments can be explained by association of acid forms at low pH. If the aggregates do not ionize easily, then the acid

TABLE 2. Partition and dimerization constants[a]

Fatty Acid	$P.C./10^{pH-pK}$	D_o
Decanoate	0.243	4.14×10^3
Decanoate[b]	0.268	3.86×10^3
Decanoate[c]	0.293	3.94×10^3
Laurate	4.70	4.20×10^3
Myristate	86.3	3.12×10^3
Palmitate	489	3.09×10^3
Stearate	2340	2.47×10^3
Oleate	953	4.50×10^3
Linoleate	252	5.74×10^2
Linolenate	124	4.68×10^2

[a] Incubations were at 37°C with a 0.016 M phosphate buffer and pH 7.4 unless otherwise noted.
[b] 0.05 M phosphate buffer.
[c] 0.1 M phosphate buffer.

TABLE 3. Effect of pH on partition of decanoate[a,b]

pH	Buffer	Partition Ratio	*P.C.*	Concn in Aqueous Phase
				M
1.5	0.05 M HCl	110	96	1.89×10^{-7}
5	0.05 M phosphate	43	97	4.95×10^{-7}
6	0.05 M phosphate	5.5	83	3.05×10^{-6}
7	0.05 M phosphate	0.064	97	1.17×10^{-5}
7.85	0.05 M phosphate	0.074	82	1.85×10^{-5}

[a] Each value is the mean of two determinations.
[b] For Tables 3, 4, and 5 it is assumed that the aqueous phase concentrations are so low that no aqueous phase association occurs, allowing us to use Eq. 1 with only the first two terms in the denominator. Then, using the definitions of D_o, pK, and *P.C.*, we get

$$P.C. = \frac{[HA]_o}{[HA]_w} = \frac{-1 + \sqrt{1 + 8D_oC_o}}{4D_o} \times \frac{1 + 10^{pH-pK}}{C_w}$$

TABLE 4. Effect of pH on partition of myristate[a]

pH	Buffer	Partition Ratio	*P.C.*	Concn in Aqueous Phase
				M
5	0.05 M phosphate	7807	1.91×10^4	1.25×10^{-9}
5	0.05 M phosphate	7894	1.97×10^4	9.52×10^{-10}
5	0.05 M phosphate	13660	3.33×10^4	7.78×10^{-9}
6	0.05 M phosphate	1772	2.81×10^4	5.90×10^{-9}
6	0.05 M phosphate	988	1.58×10^4	5.64×10^{-9}
7	0.05 M phosphate	175	2.66×10^4	3.24×10^{-8}
7	0.05 M phosphate	219	3.30×10^4	4.63×10^{-8}
7	0.05 M Tris	286	4.29×10^4	3.74×10^{-8}
8	0.05 M phosphate	21.7	3.32×10^4	2.36×10^{-7}
8	0.05 M Tris	37.6	5.62×10^4	2.81×10^{-7}
8	0.05 M borate	23.2	3.48×10^4	4.42×10^{-7}
8.95	0.05 M borate	2.96	4.09×10^4	1.37×10^{-6}
8.95	0.05 M borate	2.64	3.58×10^4	2.75×10^{-6}

[a] Each value is the mean of two determinations.

would appear to have a high pK. Moreover, the titrations that appeared to give high pK values were run at much higher concentrations than were employed in our partition ratio experiments. Another reason why we do not accept the possibility of high pK values is that the limiting values of *P.C.* for the longer acids found at high pH will fit the log *P.C.* vs. chain length plot only if the pK is the same as that of the shorter acids.

Because of the inconsistencies (*a–d*) noted above, the aqueous phase association of fatty acids containing 16 or 18 carbons must be considered.

Association in the aqueous phase

Mukerjee (3) attempted to explain the deviations of Goodman's data[3] from Eq. 4 on the basis of dimerization of long-chain fatty acid anions. Mukerjee, Mysels, and Dulin (21) had originally proposed anion dimerization to explain conductivity data at low concentrations of sodium lauryl sulfate. More recent conductivity (5, 22) and potentiometric studies (6), however, showed no evidence of anion dimerization below the critical micelle concentration. The pH dependence of the partition ratio observed by Smith and Tanford (4) and ourselves also is incompatible with anion dimerization. At a given organic phase concentration, the anion concentration increases by about a factor of 10 for a unit increase in pH, so that anion dimerization and therefore deviations from Eq. 4 should also increase greatly with increase in pH if Mukerjee's hypothesis were correct.

On the other hand, association involving the un-ionized acid cannot be ruled out. In fact, the following argument indicates that a great deal of association of the un-ionized long-chain acids occurs at low pH. A partition ratio in saturated solutions of the long-chain acids may be calculated from the published solubilities in water (23) and hexane (24). For decanoic and longer acids, partition ratios calculated from these data are considerably smaller than those calculated from the partition coefficient and the heptane phase dimerization constant. This indicates the presence of association or micelles in the aqueous phase and also that there is much more association as the chain length of the fatty acid increases. One perplexing aspect of these deductions is that the association appears to decrease as the temperature increases, whereas hydrophobic bonding would predict the opposite.

Another possible associated species is the combination of the acid with the anion. Eagland and Franks (25) have calculated acid–anion association constants for lauric, myristic, and palmitic acids. Their association constants decrease as temperature increases, and therefore if one accepts hydrophobic bonding as the main attractive force, one encounters the same thermodynamic dilemma noted above. Refitting our data to a partition model with a term for acid–anion association did not significantly improve

TABLE 5. Effect of pH on partition of palmitate[a]

pH	Buffer	Partition Ratio	*P.C.*	Concn in Aqueous Phase
				M
7.5	0.05 M phosphate	651	3.08×10^5	1.52×10^{-8}
8	0.05 M phosphate	290	4.33×10^5	3.54×10^{-8}
8	0.05 M borate	296	4.34×10^5	3.69×10^{-8}
9	0.05 M borate	49.6	7.43×10^5	2.02×10^{-7}
9.5	0.05 M borate	16.9	8.06×10^5	5.08×10^{-7}

[a] Each value is the mean of two determinations.

[3] At the highest concentrations in Goodman's (1) graphs, the partition ratios are too small partly because of insufficient buffering. For example, our calculations indicate that with 0.1 M fatty acid in the heptane phase, his partition ratio should be increased by a factor of 1.8 to conform to the pH of the points at low concentration. These corrections account for about half of the deviations of Goodman's data from Eq. 4.

the sum of squares, and it introduced even larger variations in the value of D_o among the acids.

To account for discrepancy *c* above, Smith and Tanford (4) proposed that at pH values below 9 or 10 there is so much association of palmitate and stearate in the aqueous phase that only at high pH can a valid *P.C.* be determined. While Smith and Tanford's hypothesis gives a satisfactory dependence of *P.C.* on the chain length (i.e., it eliminates discrepancy *c*), it does not resolve the other three inconsistencies. For example, the intercepts of the curves in Smith and Tanford's plot for palmitate (their Fig. 2) show the same kind of behavior with change in pH that we found (Table 5). Although their partition ratio at infinite dilution shows the expected 10-fold increase predicted by Eq. 4 when the pH is changed from 10.4 to 9.4, it increases only by another factor of 10 on decreasing the pH to 7.3, whereas an invariant partition coefficient would predict an increase by a factor of 126 (i.e., $10^{9.4-7.3}$). We found it impossible to choose any combination of aqueous association constants to fit both the precipitous drop from the expected intercept to the first data point in Fig. 4 and the nearly horizontal slope of the experimental points. Therefore, we must conclude that the variation in partition ratios with pH cannot be accounted for by the straightforward application of Smith and Tanford's (4) hypothesis.

We may make another application of Smith and Tanford's hypothesis by using it to interpret the data on the binding of fatty acids to albumin (7). Let us assume (*a*) that Smith and Tanford's hypothesis that the partition coefficient determined at high pH is valid at lower pH values, (*b*) that only the anion is bound to albumin, and (*c*) that the heptane phase dimerization constant is independent of chain length so that D_o for laurate may be used for palmitate. **Fig. 9** shows that these assumptions lead to a disagreement in the binding curves for palmitate in the region of pH 8 to 10. It does not seem possible to account for this discrepancy by loss of positive charge at the albumin binding site, since the pK of the imidazole group is about 7, and the pK of the ϵ-amino or phenolic hydroxyl group is about 10.

In trying to decide whether the data on the long-chain acids can be explained by association in the aqueous phase, one should keep in mind that for palmitate and stearate there are large differences between the partition ratios observed by different investigators under nearly similar conditions. For example, Smith and Tanford's (4) partition ratios for palmitate are less than one-sixth as large as Goodman's (1) or ours. Even a single investigator may encounter unexplained irreproducibility. Smith and Tanford mention an experiment with palmitate at pH 8.3 that was entirely inconsistent with the others. Especially in our earlier work, we have often found partition ratios in a series of experiments that differed by a factor of about

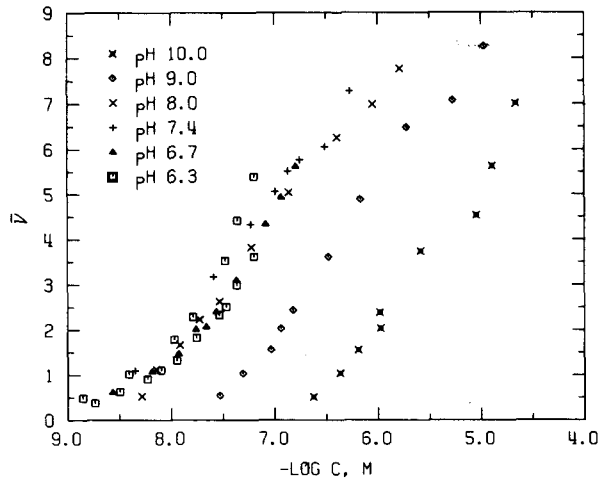

Fig. 9. Calculation of the binding of [1-^{14}C]palmitate to bovine serum albumin at 37°C from the data of Spector, John, and Fletcher (7). C is the concentration of unbound anion in the aqueous phase calculated from the heptane phase concentration alone on the assumption that the partition coefficient determined at high pH (8.06×10^5) is valid throughout the pH range and that the dimerization constant of palmitate in the heptane phase is the same as that for laurate (4.2×10^3 l/m).

2 from those found in a similar series run a month or so later. We feel that a large portion of the discrepancies can be ascribed to radioactive impurities (presumably shorter-chain fatty acids), for Table 1 shows that a partition ratio can be increased by a factor of at least 1.5 by successive purifications. This factor is, however, nowhere near as large as the factor (13 for Smith and Tanford's data; 4 for ours) by which the experimentally determined intercept in Fig. 4 differs from the intercept calculated from data at a higher pH. However, it may be noted that all of the deviations from Eq. 4 may be considered as measuring more fatty acid in the aqueous phase than would be calculated from the heptane phase concentration, so that radioactive impurities or contamination from the heptane phase would give a change in the same direction as aqueous association.

In conclusion, we believe that the hypothesis of no association in the aqueous phase has as few serious objections to it as those that assume the presence of aqueous association. We realize that this interpretation is beset with several difficulties when considering the 16- and 18-carbon acids. However, none of the hypotheses involving association that have been advanced so far is capable of explaining all of the data obtained with these acids.

This work was aided by research grants from the National Heart and Lung Institute (HL 14781) and the American Heart Association (71-895). The latter grant was supported in part by the Iowa Heart Association.

Manuscript received 27 August 1973 and in revised form 25 January 1974; accepted 3 April 1974.

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