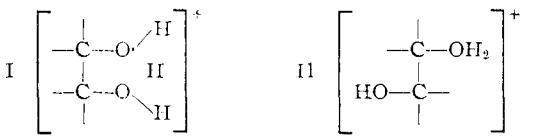
diate decrease with increasing methyl substitution from propylene glycol to trimethylethylene glycol, becoming quite small in the case of the latter compound. Buist[12] has presented evidence which indicates that for trimethylethylene glycol the rates of formation and disproportionation of the intermediate may be comparable. For pinacol,[3] where rotation is expected to be highly hindered, resulting in a more favorable *trans*-configuration for the hydroxyl groups, and for all of the cyclopentanediols studied in this work, no intermediate could be demonstrated from rate data, presumably because the formation of such an intermediate has become slow compared to its disproportionation.

Second, *trans*-1,2-dimethylcyclopentanediol-1,2, a glycol which from steric considerations might not be expected to form a cyclic intermediate at all, is not oxidized by periodate.

Third, *cis*-1,2-dimethylcyclopentanediol-1,2 is cleaved more slowly than *trans*-1-methylcyclopentanediol-1,2. This last observation is not surprising if the intermediate is considered to be cyclic; from models, it appears that two methyl groups for the *cis*-compound may offer more hindrance to the formation of an octahedrally coördinated complex with periodate ion than one methyl group for the *trans*-compound. Presumably, the optimum oxygen–oxygen distance for the formation of such a complex is intermediate to that for the *cis*- and the *trans*-compounds.

Finally, cyclic structures have been postulated for several stable complexes of glycols, notably those with boric acid and with sodium molybdate.

It is observed that methyl substitution increases the ratio k_{II}/k_2 at 0° for the *cis*-compounds of the cyclopentanediol series, from 170 for the unmethylated glycol to 625 for the dimethylated glycol; methyl substitution has little effect on the ratio for the *trans*-isomers. These facts suggest the observed hydrogen ion catalysis may involve a prior equilibrium to give a protonated glycol species, the latter reacting more rapidly with IO_4^- ion than does the glycol itself. *cis*-Glycols could form a protonated species such as (I) whereas *trans*-glycols would be limited to cations of type II.



The rate constants for the two paths, given in Tables I and II, together with the fact that *trans*-1,2-dimethylcyclopentanediol-1,2 does not cleave at any *p*H in the acid region, suggest that the two paths are substantially the same, differing only in the role of hydrogen ion, and that the same type intermediate is involved in both.

LARAMIE, WYOMING

[CONTRIBUTION FROM THE LABORATORY OF CELLULAR PHYSIOLOGY AND METABOLISM, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

The Distribution of Fatty Acids between *n*-Heptane and Aqueous Phosphate Buffer

BY DEWITT S. GOODMAN

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A study has been made of the distribution of eight fatty acids between *n*-heptane and aqueous phosphate buffer at *p*H 7.45, ionic strength 0.16 and 23°. The fatty acids studied included octanoic, decanoic, lauric, myristic, palmitic, stearic oleic and linoleic acids. C^{14}-Carboxyl labeled acids were used for assay, enabling accurate measurements to be made at extremely low concentrations and over a range of several orders of magnitude in each case. The data obtained with octanoic, decanoic, lauric and myristic acids are consistent with the assumption of a monomer–dimer equilibrium in the organic phase, without association in the aqueous phase. The association (dimerization) constants for these four fatty acids in heptane saturated with water have been calculated from these data and are, respectively, 5.8, 6.9, 7.0 and 9.3 × 10^3. These constants are compared with others available in the literature. With these 4 fatty acids the partition ratio for the monomeric forms in the two phases has been found to increase geometrically with chain length, the increase being approximately 17-fold for each two carbon increment. In contrast, the data obtained with the four highest fatty acids studied are markedly different and are not susceptible to quantitative interpretation at the present time.

In 1891 Nernst successfully accounted for the distribution of benzoic acid between water and benzene by assuming the existence of dimers in the benzene phase.[1] Since then the association of carboxylic acids has been the subject of numerous investigations by a variety of methods. Vapor density measurements by Fenton and Garner[2] and by Coolidge,[3] demonstrated the existence of fatty acid dimers in the gaseous state. X-Ray studies of the solid[4] and liquid[5] states have indicated that in these states fatty acid molecules are paired. Molecular weight and distribution measurements have shown that fatty acids are present as dimers when in solution.[6,7] Most of the quantitative studies available have dealt, however, with carboxylic acids other than the fatty acids.[8] Notable exceptions are the investigations of Pohl, Hobbs and

(1) W. Nernst, *Z. physik. Chem.*, **8**, 110 (1891).
(2) T. M. Fenton and W. E. Garner, *J. Chem. Soc.*, 694 (1930).
(3) A. S. Coolidge, THIS JOURNAL, **50**, 2166 (1928).
(4) A. Muller, *Proc. Roy. Soc. (London)*, **154A**, 624 (1936).
(5) R. M. Morrow, *Phys. Rev.*, **31**, 10 (1928).
(6) M. Trautz and R. Moschel, *Z. anorg. Chem.*, **155**, 13 (1926).
(7) W. Herz and H. Fischer, *Ber.*, **38**, 1138 (1905).
(8) The term "fatty acid" is herein used to refer to the straight or branched chain aliphatic carboxylic acids.

Gross,[9-10] and of Maryott, Hobbs and Gross[11] who determined the monomer–dimer equilibrium constants for several fatty acids in benzene solution by measuring the changes in dielectric constants with concentration for dilute solutions. These workers also studied the association of formic and acetic acids in heptane, but found the association constants to be too large for precise evaluation by their method.

In the present study, the distribution of eight fatty acids between *n*-heptane and phosphate buffer, *p*H 7.45, ionic strength 0.16, has been studied. The use of C^{14}-labeled fatty acids has permitted measurements at extremely low concentrations. The data obtained with the fatty acids of chain length 14C and below are consistent with the assumption of a monomer–dimer equilibrium in the organic phase, without association in the aqueous phase. The data obtained with both saturated and unsaturated fatty acids of chain lengths 16C and 18C are, however, inconsistent with these assumptions and are not susceptible to quantitative interpretation at the present time.

Experimental

Materials. (a) **Non-radioactive Fatty Acids.**—Octanoic (caprylic) and decanoic (capric) acids were Eastman Kodak chemicals. Lauric and myristic acids were Fisher reagent grade chemicals; their melting points were: lauric 41–43° (lit. 44°), myristic 52–53° (lit. 53–54°). Palmitic, stearic, oleic and linoleic acids were supplied by the Hormel Institute. The melting points were: palmitic 63–64° (lit. 63–64°), stearic 69° (lit. 69°). The oleic acid was reported to have an iodine value (Wijs) of 89.8 (theoretical 89.87), and the linoleic acid of 180.5 (theoretical 181.4). Analysis of these latter two acids by infrared spectrometry[12,13] revealed no detectable *trans* double bonds in either. All acids were used without further purification.

(b) **C^{14}-Labeled Fatty Acids.**—The radioactive fatty acids were all labeled with C^{14} in the carboxyl group, and, with the exception of oleic acid-1-C^{14}, were all supplied by the Nuclear Instrument and Chemical Corporation of Chicago. The oleic acid-1-C^{14} was supplied by Tracerlab, Inc. The specific activity of all the fatty acids except oleic and linoleic was close to 2.5 mC./m*M*; that of linoleic acid was 4.46 and of oleic 0.427. The linoleic acid-1-C^{14} had been prepared by the method of Howton, *et al.*,[14] and can be expected to have contained about 15% *trans* double bonds. Analyses of purity were conducted on three of the labeled fatty acids. Countercurrent distribution of palmitic and linoleic acids by the method of Ahrens and Craig,[15] using a 50 tube system, revealed, in each case, the presence of 2–4% of the radioactivity in a peak of much greater polarity than the main body of the material. This peak was presumed, in each case, to represent a short chain fatty acid impurity. The rest of the radioactivity distributed itself in a single symmetrical peak. In the case of linoleic acid, this main symmetrical peak was identical in location with the peak of unlabeled Hormel linoleic acid (*vide supra*) run simultaneously. Vapor phase chromatography of the oleic acid-1-C^{14} [16] revealed it to contain 89.6% oleic acid, with 6.5% of the material as either stearic or longer chain fatty acids, 2.1% as palmitoleic acid, 1.6% as 15C and 14C saturated fatty acids and 0.2% as a 12C acid. Analysis of the peaks other than oleic acid indicated the impurities to have the same C^{14} specific activity as the oleic acid. The labeled palmitic, stearic, oleic and linoleic acids were partially purified before use by dissolving each in a 1:1 mixture of isooctane–glacial acetic acid, adding $^1/_{10}$th volume H_2O to achieve separation into two phases and discarding the acetic acid phase. This procedure has been shown to remove most of the short-chain fatty acids from a mixture of short and long-chain fatty acids[17] and was performed to remove the small amounts of short chain fatty acid impurities present in each of the samples. The remaining labeled fatty acids were used without further purification.

(c) **Other Materials.**—*n*-Heptane was a reagent grade product of Matheson, Coleman and Bell; it was purified by distillation, the fraction distilling between 98.0 and 99.0° being collected and used.[18] NaH_2PO_4 and Na_2HPO_4 were Merck and Company analytic reagents. Phosphate buffer was prepared as a stock solution of ten times the desired ionic strength; dilution of this stock solution 1 in 10 resulted in a solution of *p*H 7.45 and ionic strength 0.160. *p*-Dioxane and naphthalene were Eastman Kodak chemicals. The dioxane was purified before use by freezing and thawing 3 times, the first 10% thawing being discarded each time. Diphenyloxazole (DPO) was a product of Pilot Chemicals, Inc.

Preparation of Solutions of Fatty Acid Salts.—A relatively concentrated solution of the sodium salt of each unlabeled fatty acid was prepared by weighing out a sample of the fatty acid, dissolving this in absolute ethanol, adding an amount of NaOH solution just sufficient to neutralize the fatty acid, evaporating the ethanol and then dissolving the fatty acid salt in a measured volume of freshly boiled distilled H_2O. Other solutions of the fatty acid salts were made from the initial ones by dilution. The concentration of at least one solution of each fatty acid salt was verified by acidification of an aliquot, extraction into isoöctane and titration with dilute NaOH, using a microburet and nile blue in absolute ethanol as indicator.[17,19] In all cases the concentration as determined by titration agreed within 3% with the expected concentration.

Solutions of the sodium salts of the C^{14}-labeled fatty acids were prepared in each case by evaporating a portion of an isoöctane solution of the fatty acid to dryness, adding a slight excess of NaOH solution and dissolving in boiled H_2O.[20] The amount of NaOH required was determined from the concentration of radioactivity in the isoöctane solution and the specific activity of the fatty acid. The final concentration in moles/liter was determined from the concentration of radioactivity and the specific activity, using calibration standards available in this Laboratory. With the exception of sodium oleate-1-C^{14}, the solutions of the labeled fatty acid salts were considerably more dilute than the solutions of the unlabeled fatty acid salts.

Equilibration of the Two Phases.—In each study equilibration was conducted in a series of 60-cc. separatory funnels, the stems of which had been cut off prior to use. To each funnel were added 4 cc. of the stock concentrated phosphate buffer, 0 to 1 cc. of the labeled fatty acid salt solution, 0 to 35 cc. of one or another of the unlabeled fatty acid salt solutions and enough distilled H_2O to make a total volume of 40 cc. The total quantity of fatty acid in the funnels ranged over several orders of magnitude in each study. In the studies of the longer-chain fatty acids (14C and above) the fatty acid salts were insoluble in the funnels containing the largest amounts of material, before the addition of heptane. To each funnel 2 cc. of *n*-heptane was then added and the funnels shaken for 8–10 hr. in a Burrell wrist-action shaker. Three to four hours were found adequate for full equilibration. After this the funnels were centrifuged for 15 minutes at 500 r.p.m. and the lower phases drawn off and separately collected. The funnels were again centrifuged to ensure that no tiny droplets of aqueous phase remained in the heptane phase. In order to eliminate errors due to adsorption of fatty acids to glassware, all glassware (collecting

(9) H. A. Pohl, M. E. Hobbs and P. M. Gross, *Ann. N. Y. Acad. Sci.*, **40**, 389 (1940).

(10) H. A. Pohl, M. E. Hobbs and P. M. Gross, *J. Chem. Phys.*, **9**, 408 (1941).

(11) A. A. Maryott, M. E. Hobbs and P. M. Gross, THIS JOURNAL, **71**, 1671 (1949).

(12) J. E. Jackson, R. F. Paschke, W. Tolberg, H. M. Boyd and D. M. Wheeler, *J. Am. Oil Chem. Soc.*, **29**, 229 (1952).

(13) G. D. Shreve, M. R. Heether, H. B. Knight and D. Swern, *Anal. Chem.*, **22**, 1498 (1950.)

(14) D. R. Howton, R. H. Davis and J. C. Nevenzel, THIS JOURNAL, **76**, 4970 (1954).

(15) E. H. Ahrens, Jr., and L. D. Craig, *J. Biol. Chem.*, **195**, 299 (1952).

(16) Kindly performed by Drs. E. H. Ahrens, Jr., and W. Insull of the Rockefeller Institute for Medical Research.

(17) R. S. Gordon, Jr., *J. Clin. Inv.*, **36**, 810 (1957), and unpublished observations.

(18) Kindly performed by Dr. J. Bragdon's laboratory.

(19) Kindly performed by Miss A. Cherkes.

(20) This was not done in the case of octanoic acid, which was supplied as sodium octanoate-1-C^{14}.

flasks and pipets) with which the aqueous phase had contact after equilibration was rinsed with 2 or 3 aliquots of the aqueous phase immediately before use. All experiments were conducted in an air-conditioned laboratory at 23 ± 1°.

Analyses after Equilibration.—The concentration of radioactivity in each upper and lower phase after equilibration was measured with a Packard Tri-Carb Liquid Scintillation Spectrometer. One cc. of the solution to be measured was added to a vial containing 15 cc. of a scintillation solution, the latter consisting of 50 g. of naphthalene and 7 g. of DPO dissolved in a liter of *p*-dioxane.[21,22] Measurements were conducted with the vials and counting unit at room temperature. The absolute efficiency of this counting system was 30–35%; the back-ground varied between 10 and 14 c.p.m. The solutions were counted long enough so that the standard error was less than 2%.[23] After subtracting the background radiation, each measurement was corrected for the quenching resulting from the added heptane or phosphate buffer. A final correction then was applied to correct for small systematic changes in efficiency which occurred during each series of measurements, by use of a reference standard solution of palmitic acid-1-C^{14}.

Preliminary Calculations.—After determining the number of counts per minute (c.p.m.) per cc. for each heptane and aqueous phase, the total number of c.p.m. in each phase and in each system (*i.e.*, each sep. funnel) was calculated. The recovery of c.p.m. was within 5% of the expected recovery for each two-phase system. The total concentration of fatty acid molecules (or ions) in each heptane and aqueous phase was then calculated from these values, together with the known volumes and the known amounts of fatty acid added to each system. Finally, the partition ratio, defined as the total concentration of fatty acid molecules in heptane divided by the total concentration in phosphate buffer, was determined for each point by dividing the c.p.m. per cc. in heptane by the c.p.m. per cc. in phosphate buffer.

Results and Discussion

The results are shown in Figs. 1 through 6, in which, for each fatty acid studied, the partition

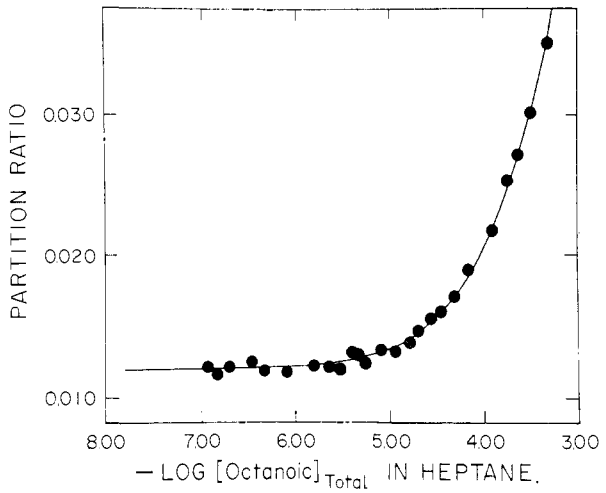

Fig. 1.

ratio (*vide supra*) is plotted against the negative logarithm of the total fatty acid concentration in the heptane phase. Inspection of the figures reveals that they appear to fall into two distinct groups. In the first group, consisting of the studies with fatty acids of chain length 14C and below (Figs. 1 through 4), the partition ratio increases

(21) M. Furst, H. Kallman and F. H. Brown, *Nucleonics*, **13**, 58 (1955).

(22) W. H. Langham, W. J. Eversole, F. N. Hayes and T. T. Trujillo, *J. Lab. Clin. Med.*, **47**, 819 (1956).

(23) D. Steinberg and S. Udenfriend, Article 20 in "Methods in Enzymology," Vol. IV, Academic Press, Inc., New York, N. Y., 1957.

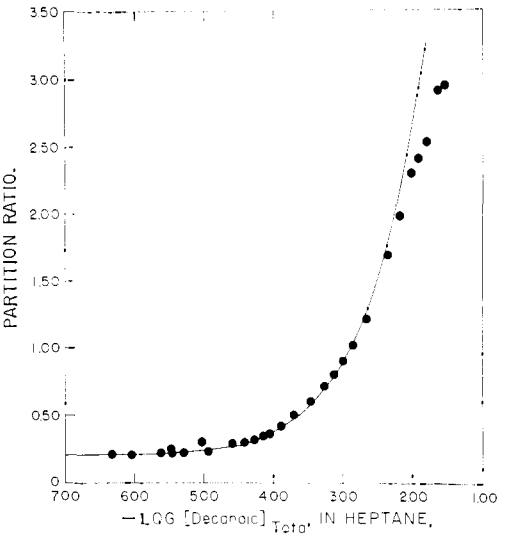

Fig. 2.

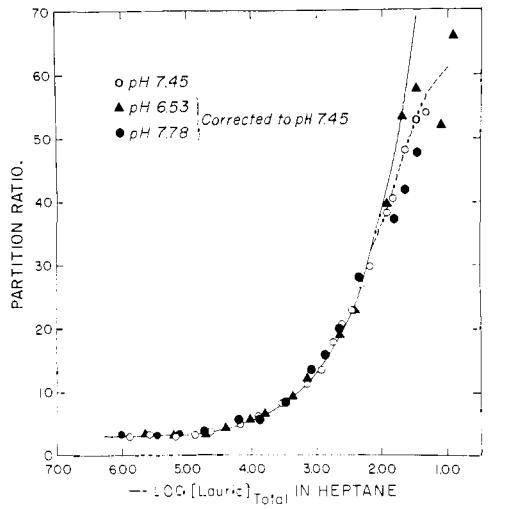

Fig. 3.

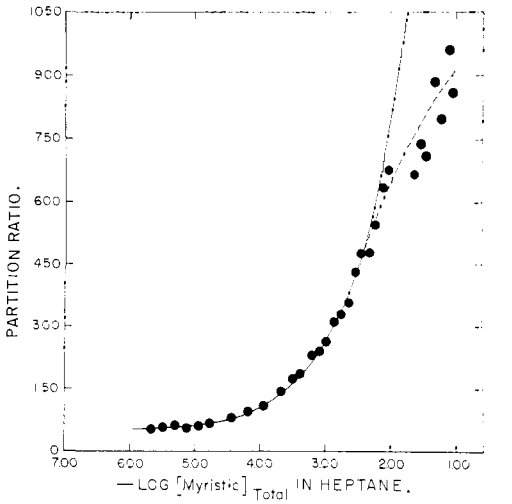

Fig. 4.

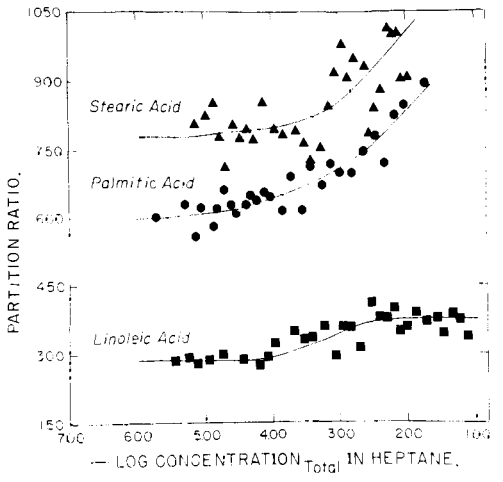

Fig. 5.

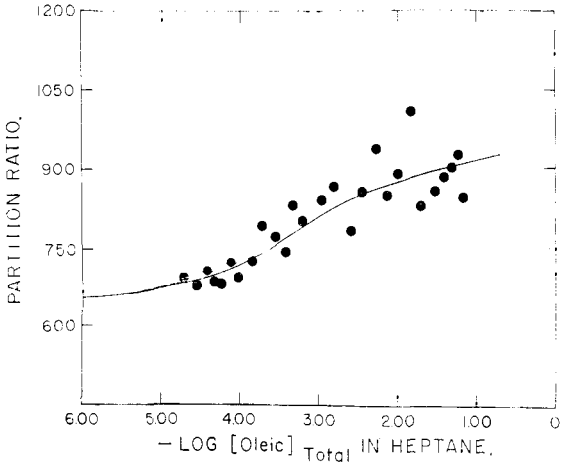

Fig. 6.

markedly with increasing concentration and, in fact, increases more than 10-fold over the range studied for the 10C, 12C and 14C acids. In the studies with fatty acids of chain length 16C and 18C, however, the partition ratio increases less than 1.5-fold over a comparable range of concentration (Figs. 5 and 6). It should also be noted that although the experimental points in Figs. 5 and 6 appear to scatter much more widely than those in Figs. 1 through 4, this scatter is really not very great when one considers it relative to the size of the partition ratio in dilute solutions.[24]

Theoretical Formulation.—Let us assume that a fatty acid HA distributes itself between heptane and aqueous phosphate buffer according to the following equilibria.[25] (1) In the heptane phase there is a monomer–dimer equilibrium, $2\,HA_h = (HA)_{2,h}$ with an association constant, k_d. (2) In the aqueous phase there is an ionization equilibrium, $A_a^- + H^+ = HA_a$, with an acid association constant k_{HA}. (3) Only the undissociated monomer distributes itself between the two phases, and the ratio of its activities in the two phases (heptane/aqueous) is a constant, K_p, at all concentrations.

If one then makes the approximation that $HA_a + A_a^- \cong A_a^-$,[26] from the definition of the partition ratio (P.R.),[28] it can be shown easily that

$$\text{P.R.} = K_p k_{HA}[H^+] + 2k_d(K_p k_{HA}[H^+])^2[A^-]_a \quad (1)$$

Furthermore, since $[H^+]$ = constant = $10^{-7.45}$ in these experiments, we define $K_p' = K_p k_{HA}[H^+]$ and write equation 1 as

$$\text{P.R.} = K'_p + 2k_d K'^2_p[A^-]_a \quad (1a)$$

The partition ratio is hence a linear function of the fatty acid concentration in the aqueous phase. We can also relate the partition ratio to the total fatty acid concentration in the heptane phase, by means of the definition of the partition ratio and the use of $[A^-]_a$ as an approximation for $([HA]_a + [A^-]_a)$. Substituting in equation 1a and using the quadratic formula this gives

$$\text{P.R.} = \frac{K'_p + K'_p\sqrt{1 + 8k_d[HA]_{total,h}}}{2} \quad (2)$$

where $[HA]_{total,h}$ is the total concentration of fatty acid molecules in the heptane phase, (*i.e.*, $[HA]_{total,h} = [HA]_h + 2[(HA)_2]_h = \text{P.R.} \times [A^-]_a$).

Comparison of Data with Theory.—The experimental data for octanoic, decanoic, lauric and myristic acids were found to conform to equations of the form of equations 1a and 2 over most of the concentration ranges studied. By suitably plotting the data, values of k_d and K_p' were found for each fatty acid which resulted in curves of equations 1a and 2 which closely fitted the experimental data. These values are listed in Table I. The solid curves drawn in Figs. 1 through 4 are the theoretical curves constructed from equation 2, using the values of the constants given in Table I. It is evident from inspection of these figures that the theoretical equation precisely describes the experimental data over a wide range of concentration and of partition ratios. It is felt that such close agreement between the theoretical curves and the experimental data indicates that the equilibria formulated in the preceding section are those which actually exist in the system.

TABLE I

THE DIMERIZATION (ASSOCIATION) AND PARTITION CONSTANTS OF FOUR FATTY ACIDS IN THE SYSTEM: HEPTANE, PHOSPHATE BUFFER *p*H 7.45, Γ/2 0.16, 23°

Acid	k_d	K'_p
Octanoic	5.8×10^3	0.012
Decanoic	6.9×10^3	0.21
Lauric	7.0×10^3	3.0
Myristic	9.3×10^3	54

(24) In other words, the relative scale for the partition ratio is greatly expanded in Figs. 5 and 6, as *cf.* Figs. 1 through 4, and this greatly emphasizes the scatter of experimental points.

(25) In this formulation the subscript h refers to the heptane phase and the subscript a to the aqueous phase.

(26) The values of the acid *p*K for the fatty acids acetic through caprylic vary between 4.76 and 4.90.[27] Data are not available for the higher fatty acids, but it can be assumed safely that their *p*K's are not much greater than 5.0. These experiments were at *p*H 7.45. At this *p*H, therefore, less than 1% of the fatty acid in the aqueous phase will be present as the undissociated acid, and one can reasonably say that $HA_a + A_a^- = A_a^-$.

(27) "The Handbook of Chemistry," 9th Ed., N. A. Lange, editor, Handbook Publishers, Inc., Sandusky, Ohio, 1956. The solubility of heptane in water is reported to be less than one-tenth the solubility of benzene in water. It can be assumed that the solubility of water in heptane is relatively equally low.

(28) $\text{P. R.} = \frac{[HA]_h + 2[(HA)_2]_h}{[HA]_a + [A^-]_a}$

At the upper limits of the concentrations studied the experimental data appear to deviate from the theoretical curves, and the apparent true curves in this region are drawn in dotted lines in Figs. 3 and 4 These deviations are such that more fatty acid is present in the aqueous phase than would be expected from equations 1a and 2. *A priori* it would seem most likely that these deviations are the result of association between fatty acid anions in the aqueous phase at these higher concentrations. There is no direct evidence bearing on this matter but should this hypothesis prove correct, studies of this nature would provide data on the lower limit of association between fatty acids and their anions in aqueous solutions.

A further test of the correctness of the theoretical formulations herein described was performed by conducting experiments with lauric acid at two additional *p*H's, namely, 6.57 and 7.78. Phosphate buffers of identical ionic strength (0.160) were used in each case. The results of these experiments were then corrected to the results which would be expected at *p*H 7.45 if equation 1 actually applied to the system. These "corrected" data are plotted in Fig. 3 along with the data actually obtained at *p*H 7.45. It is evident that the data obtained at the three *p*H's coincide when "corrected" according to equation 1, despite the fact that the correction factors range over more than one order of magnitude.

In contrast to these results with the fatty acids of chain length 8C to 14C, the data obtained with the longer chain fatty acids (palmitic, stearic, oleic and linoleic) in no way conform to equations of the form of equations 1a and 2. Inspection of Figs. 5 and 6 reveals that despite the wide range of concentration studied, the partition ratios in these studies increase less than 1.5-fold. The solid lines in Figs. 5 and 6 were arbitrarily drawn to indicate the apparent change in partition ratio with concentration and have no other particular meaning. The reason for such different behavior by this group of fatty acids, as compared to the other, shorter fatty acids, is not clear. Part of the explanation probably involves the formation of association complexes in the aqueous phase at considerably lower concentrations than in the case of the shorter fatty acids. Association complexes in the aqueous phase certainly existed at the higher concentrations studied, for with palmitic, stearic and oleic acids the fatty acid became insoluble in the system at the highest concentrations studied (those just above the points plotted in Figs. 5 and 6). In order for association in the aqueous phase to be the entire explanation, however, one would have to postulate that such association occurs at concentrations below 10^{-6} molar, and this does not seem likely. Another possible factor could be the lining up of fatty acid molecules or anions at the heptane–water interface. This factor is hard to evaluate, but it seems difficult to believe that it could result in such unusual behavior of palmitic acid as compared to myristic acid. Errors due to the extremely low mutual solubility of the two solvents are not responsible, for partial acidification of one of the oleic acid solutions resulted in a partition ratio several times greater than those observed in this study. Nor are errors due to adsorption to glassware involved, for these were avoided as already described. One can only say, therefore, that fatty acids of chain length 16C and above behave differently from those of chain length 14C and below and that this difference is not susceptible to interpretation at the present time.

Discussion

It may be of interest to consider the relationship of fatty acid chain length to K'_p. K'_p is, of course, the ratio of the monomeric concentration in heptane to the monomeric anion concentration in water, at *p*H 7.45, ionic strength 0.16 and 23°.[29] Analysis of the values of K'_p listed in Table I reveals that the ratio of the values of K'_p for each fatty acid and its next higher homolog is approximately constant for the four fatty acids listed, and in fact, a plot of fatty acid chain length against log K'_p gives a straight line over the area studied. One may therefore conclude that for the even-numbered, normal, saturated fatty acids, an increment in chain length of two carbon atoms increases K'_p some 17-fold. This of course does not apply to fatty acids of chain length 16C and above.

One other factor requires consideration. This is the effect of the finite solubility of water in heptane upon the dimerization of fatty acids in heptane. The magnitude of this effect cannot be evaluated with information presently available, but it is felt that this effect is small in these studies. In the first place, the solubility of water in heptane is exceedingly low. Thus, most of the information concerning this effect deals with studies of the distribution of carboxylic acids between water and benzene,[30] and the mutual solubility of benzene and water is at least 10 times greater than that of heptane and water.[27] Secondly, this factor tends to be smaller the lower the solubility of a substance in water. Most of the fatty acids studied have extremely low solubilities in water. Finally, the data herein reported can be precisely described by the equations formulated, neglecting consideration of hydration. This effect of hydration should not be disregarded, however, and it must be remembered that the association constants for dimer formation reported here (k_d's) are for fatty acids in the solvent heptane saturated with water.

Further inspection of Table I reveals that the value of k_d increases as fatty acid chain length increases. This fact also was observed by Pohl, Hobbs and Gross[9,10] and by Maryott, Hobbs and Gross[11] in their studies of the dimerization of fatty acids in anhydrous benzene. In Table II are listed the values of k_d reported by these investigators, corrected to units of moles/liter. It should be noted that the values of k_d in Table I, for the solvent heptane saturated with water, are more than one order of magnitude greater than the values for anhydrous benzene listed in Table II.[31] It

(29) Strictly speaking, the activity of the fatty acid anions should be used, rather than their concentrations. Not doing so introduces a small error of equal magnitude in all the studies.

(30) E. N. Lassettre, *Chem. Revs.*, **20**, 259 (1937).

(31) It is assumed that the acids of chain lengths between butyric and stearic have association constants in benzene between the values for butyric and stearic listed in Table II.

TABLE II

REPORTED VALUES OF THE DIMERIZATION (ASSOCIATION) CONSTANTS OF FATTY ACIDS IN ANHYDROUS ORGANIC SOLVENTS, 30°[a]

Acid	Solvent	k_d[b]
Formic	Benzene	1.3×10^2
Acetic	Benzene	3.7×10^2
Propionic	Benzene	3.8×10^2
Butyric	Benzene	4.3×10^2
Stearic	Benzene	5.2×10^2
Formic	Heptane	2×10^4
Acetic	Heptane	4×10^4

[a] Data of Pohl, Hobbs and Gross[9,10] and Maryott, Hobbs and Gross.[11] [b] Calculated from their reported dissociation constants, after correcting their constants (given in units of moles/molar volume of solvent) to units of moles/liter.

should also be noted that the estimated values of k_d for formic and acetic acids in anhydrous heptane (Table II) are greater than the values obtained in this study (Table I).[32] Part of this difference may reside in the fact that in the present studies the solvent is heptane saturated with water, compared to anhydrous heptane (*vide supra*). Also relevant is the fact that the constants reported by Pohl, Hobbs and Gross for heptane are admittedly only estimates, since they were unable to make sufficiently precise measurements in solutions of sufficiently low concentration in heptane.

In conclusion, the magnitude of the values of k_d observed in these studies deserves re-emphasis. In heptane solution dimerization is significant at concentrations well below 10^{-4} molar, and the dimer is the stable and predominant form over a wide range of concentration. The studies of Pauling and Brockway[33] and others have shown that the configuration of such dimers involves a symmetrical coplanar structure of the two carboxyl groups, connected by two hydrogen bonds. The magnitude of k_d herein observed is a testimony to the great stability of this structure.

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(32) Reasoning from the values for k_d for benzene given in Table II, one would expect k_d in heptane for the acids longer than acetic (*e.g.*, those in Table I) to be greater than k_d for acetic and formic acids in heptane.

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The Interaction of Human Serum Albumin with Long-chain Fatty Acid Anions

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The interaction of human serum albumin with six long-chain fatty acid anions has been studied by the technique of partition analysis, in which varying quantities of each fatty acid were equilibrated between two phases. The phases employed were *n*-heptane and an aqueous solution of serum albumin in phosphate buffer, *p*H 7.45, ionic strength 0.16, at 23°. The concentration of unbound fatty acid in each aqueous phase was determined from the concentration in heptane, using the results of identical distribution experiments performed in the absence of albumin. The fatty acids studied included lauric, myristic, palmitic, stearic, oleic and linoleic acids. The human serum albumin was a special preparation with a fatty acid content of 0.1 mole/mole or less. The data obtained have been analyzed in terms of three classes of binding sites and the apparent association constants determined. The first class consists of 2 sites, the second of 5, and the third of a larger number, arbitrarily taken to be 20. The values of k_1' for the different fatty acids are: laurate 1.6×10^6; myristate 4.0×10^6; palmitate 6.0×10^7; stearate 8.0×10^7; oleate 1.1×10^8; linoleate 1.3×10^7. The relationship between the apparent and the intrinsic association constants is discussed in detail. The possible structural specificity of the different classes of binding sites is also discussed, as are some of the implications for metabolic studies.

It has long been known that serum albumin interacts strongly with fatty acid anions. In 1941 Kendall found that crystalline albumin was always associated with a small amount of free fatty acid that could not be removed by repeated crystallization.[1] Since then the nature and effects of this interaction have been the subjects of numerous investigations. Thus, fatty acid anions have been shown to stabilize serum albumin against denaturation by urea, guanidine and heat[2,3] and to compete effectively with organic dyes for binding sites on the albumin molecule.[4,5] Electrophoretic studies have demonstrated an increase in the mobility of serum albumin in the presence of fatty acid salts.[6,7] Furthermore, Davis and Dubos observed that serum albumin acts as a protective growth factor for tubercle bacilli by binding traces of fatty acids in the medium and that serum albumin could protect sheep erythrocytes against hemolysis by oleic acid.[8] Most of the available studies have, however, been indirect, qualitative or at best semi-quantitative. An outstanding exception to this statement is the study by Teresi and Luck, in which the binding of several short-chain fatty acid anions (8C and less in length) to bovine serum albumin was quantitatively studied by the method of equilibrium dialysis.[9] This investigation suffered, however,

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