Potentiometric Titration of Monomeric and Micellar Acylcarnitines

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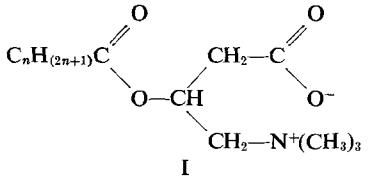
Abstract □ Carnitine (β-hydroxy-γ-trimethylammonium butyrate) enzymatically combines with fatty acids to facilitate their transport through mitochondrial membranes and thus their metabolism. In view of this fact, a study of the physical-chemical properties of some acylcarnitines was conducted. Potentiometric titrations were carried out at concentrations at which the acylcarnitines are monomeric and micellar. Unlike the monomeric species, the micellar acylcarnitines do not have a constant pK value but rather one that is dependent on the degree of protonation and hence the net positive charge of the micelle. The pK value for the zwitterionic micelle is about 4.85 and decreases linearly with β at low degrees of protonation. The pK of the zwitterionic micelle was taken as the intrinsic pK of a carboxyl group, and pK differences at any value of β were attributed to electrostatic interactions. This permitted calculation of the surface potential of the micelle. The dependence of the observed pK and the calculated surface potential on β and on ionic strength was shown to be qualitatively consistent with the Debye-Hückel theory for a spherical impenetrable particle.

Keyphrases □ Acylcarnitines, monomeric, micellar—potentiometric titration □ CMC—acylcarnitines □ Surface potential—acylcarnitine micelles □ Ionization, micelles—pK value effect □ Potentiometric titration—monomeric, micellar acylcarnitines

Although the coenzyme A derivatives of fatty acids are metabolized through β-oxidation in the mitochondrion, these activated fatty acids cannot enter the mitochondrion from the cytoplasm unless carnitine (β-hydroxy-γ-trimethylammonium butyrate) is present (1–3). At the mitochondrial membrane surface, fatty acyl coenzyme A derivatives are enzymatically combined with carnitine, producing free coenzyme A and fatty acylcarnitine (I).



I

The acylcarnitines pass through the mitochondrial membrane and react with intramitochondrial coenzyme A to form free carnitine and fatty acyl coenzyme A. The intramitochondrial acyl coenzyme A is metabolized *via* the β-oxidation cycle, and the carnitine can pass back across the membrane to react with another extramitochondrial acyl coenzyme A molecule. The fact that several compounds having structures similar to carnitine and acylcarnitine are not able to pass through the mitochondrial membrane suggests that the acylcarnitine structure is specifically required for fatty acid transport (4, 5).

Carnitine and its acylated derivatives (Table I) have three functional groups that can interact with each other and with groups on neighboring molecules. In the case of long-chain acylcarnitines, micelle formation also can occur. These interactions could affect the ionization behavior of such compounds and hence their ability to function in membrane transport. The present study was designed to determine the effects of structural modification and environmental conditions, *e.g.*, ionic strength, pH, and micelle formation, on the ionization behavior of these compounds, with a long-range view of understanding their transport-mediating properties.

EXPERIMENTAL

Materials—The various acylcarnitines used in this study were synthesized by reacting DL-carnitine HCl (Mann Research Laboratories, Inc.) with high purity (99+%) fatty acids (Applied Science Laboratories) according to the method of Ziegler *et al.* (6).

DL-Acetylcarnitine (Otsuka Pharmaceutical Co.), DL-γ-dimethylamino-β-hydroxy butyric acid HCl (norcarnitine) (Riker Laboratories), γ-amino butyric acid (GABA) (Nutritional Biochemicals Corp.), γ-butyrobetaine (GBB), and DL-γ-amino-β-hydroxy butyric acid HCl (Calbiochem) were used.

All physical constants and tests for purity agree well with literature values (6). Plots of surface tension *versus* concentration for the long-chain acylcarnitines show no minima, suggesting the absence of free fatty acid (7).

Determination of Critical Micelle Concentration—The determination of the critical micelle concentration (CMC) is necessary to evaluate the relative contribution of monomeric and micellar species to the titration behavior of the long-chain acylcarnitines. This was accomplished by determining the concentration at which the break in the log concentration *versus* surface tension plot occurs. The surface tension of solutions containing various concentrations of decyl-, lauryl-, myristyl-, and palmitylcarnitine was determined by the drop volume method as described by Weiner and Zografi (7). Table II lists the CMC values for acylcarnitines in their cationic and zwitterionic forms in the presence and absence of 0.20 *M* KCl.

The CMC values shown for octylcarnitine were obtained more conveniently by determining the break in the pH *versus* log concentration plots (8). Values obtained in this manner for other acylcarnitines were in excellent agreement with the results of surface tension measurement.

Table I—Critical Micelle Concentrations of Cationic and Zwitterionic Acylcarnitines in Water and 0.20 *M* KCl at 25°

Compound	Critical Micelle Concentration, moles/l.			
	Cationic Form		Zwitterionic Form	
	H_2O	0.20 *M* KCl	H_2O	0.20 *M* KCl
Octylcarnitine	1.6×10^{-1}	1.0×10^{-1}	1.1×10^{-1}	9.5×10^{-2}
Decylcarnitine	1.5×10^{-2}	7.0×10^{-3}	1.1×10^{-2}	9.0×10^{-3}
Laurylcarnitine	1.5×10^{-3}	7.5×10^{-4}	1.2×10^{-3}	8.5×10^{-4}
Myristylcarnitine	1.5×10^{-4}	9.0×10^{-5}	1.0×10^{-4}	7.5×10^{-5}
Palmitylcarnitine	1.5×10^{-5}	8.5×10^{-6}	—	—

Potentiometric Titration—Potentiometric titrations were carried out using a Beckman 101900 research pH meter equipped with a Beckman 39167 general-purpose glass electrode and a Beckman 19168 silver–silver chloride reference electrode. The instrument was standardized with Beckman pH 4 buffer solution (pH 4.01 at 25°) and calibrated at pH 10.184 by an internal calibration device before and after each titration.

The solutions were prepared by dissolving the undissociated acid in doubly distilled CO_2-free water. The titrant was added in 0.01 mmole or smaller increments by means of an Agla micrometer syringe outfit (Burroughs Wellcome & Co.). The entire titration was run at constant temperature and under nitrogen atmosphere. The apparent ionization constant of zwitterionic compounds such as the acylcarnitines may be defined as:

$$K = \frac{(H^+)(R^\pm)}{(RH^+)} \qquad \text{(Eq. 1)}$$

where $(R^\pm)$ and (RH^+) are the concentrations of the zwitterionic and cationic species, respectively. The logarithmic form of Eq. 1 is, therefore,

$$pK = pH + \log\left(\frac{RH^+}{R^\pm}\right) \qquad \text{(Eq. 2)}$$

If β is defined as the fraction of the total acylcarnitine that is protonated or ionized, then

$$\beta = \frac{(RH^+)}{(R^\pm) + (RH^+)} \qquad \text{(Eq. 3)}$$

and Eq. 2 becomes

$$pK = pH + \log\frac{\beta}{1-\beta} \qquad \text{(Eq. 4)}$$

If the hydrogen- or hydroxyl-ion concentration is not negligible compared to the concentration of acylcarnitine, the condition of electrical neutrality must be considered and these equations must be modified according to a standard method described by Albert and Serjeant (9).

RESULTS

Titration of Monomers—The pK values of 0.01 *M* solutions of carnitine and those acylcarnitines that are monomeric at this concentration are given in Table II. The pK values of other compounds having structures similar to the carnitines are shown for comparison. As expected from Eq. 4, the values are independent of the degree of ionization, β, and of temperature in the range of 20–40°. The pK values obtained at concentrations as high as 0.10 *M* differed only slightly. The pK of acetylcarnitine is increased by 0.1 pK unit in the presence of 0.2 *M* LiCl, NaCl, KCl, KBr, or KI, indicating a relatively small ionic strength effect and no specific effect for these ions.

Effects of Concentration—The pK values of cationic decylcarnitine determined potentiometrically are shown in Fig. 1. It can be seen that the pK is constant below the CMC, indicating that the surfactant behaves as a normal electrolyte at these concentrations. Above the CMC (Fig. 1) the pK is dependent on concentration due to the changing ratio of monomeric to micellar surfactant. After this transition region the pK begins to become constant again, due to the diminishing ratio of the monomeric surfactant to the micellar surfactant. This type of concentration dependence was observed by Ekwall *et al.* for certain cholates (10) and by Tokiwa and Ohki for alkyl amine oxides (11).

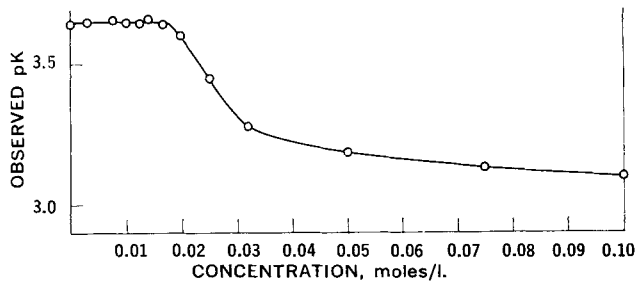


Figure 1—*Effect of micelle formation on the observed pK of decylcarnitine (β = 0.5).*

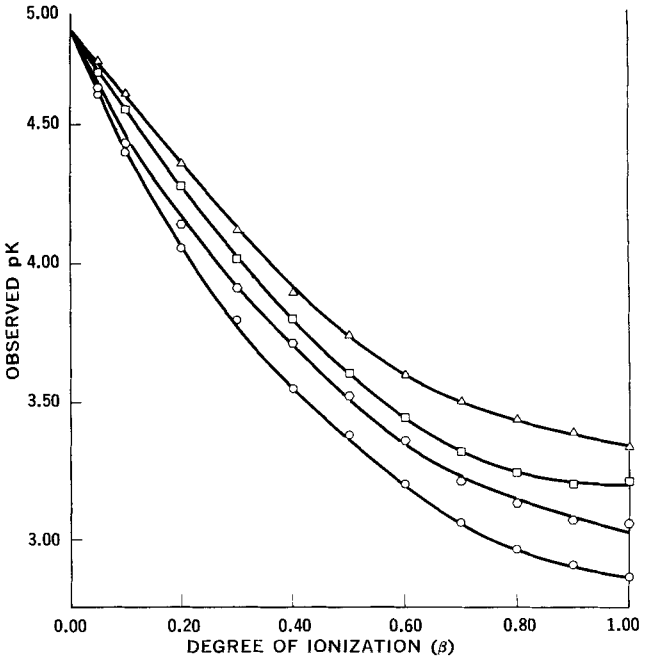


Figure 2—*Titration curves (observed pK* versus β*) for laurylcarnitine at several concentrations of added KCl. Key:* ○, *no salt;* ○, *0.05* M; □, *0.10* M; *and* △, *0.20* M.

Titration of Micelles—At concentrations significantly greater than the CMC (where the contribution of the monomer to the titration curve is negligible) the apparent ionization constant, pK, at any value of β is not independent of β, as is usually the case for monomers. These values, however, are independent of temperature. Figures 2–4 show curves of pK *versus* β for lauryl-, myristyl-, and palmitylcarnitine at several concentrations of added KCl.[1] Values of pK appear to decrease linearly at lower degrees of ionization and extrapolate to a pK at $\beta = 0$ of 4.85 ± 0.03. The change in pK with β is less pronounced at high salt concentrations. Figure 5 contains the titration curves of myristylcarnitine in 0.2 *M* LiCl, NaCl, KCl, KBr, and KI, and indicates that the curves for the three chlorides are identical while those for titration in KBr and KI show significantly higher pK values for each β.

DISCUSSION

The pK values of butyric acid, pentanoic acid, hexanoic acid, and most other aliphatic acids are all about 4.83 ± 0.03. This value may therefore be taken as the intrinsic pK or pG for an aliphatic carboxylic acid. The difference in pK between each compound listed in Table II and 4.83 is proportional to the work required to bring a proton from infinity to the site of the carboxylate group on the molecule. Thus, if the acid also has a positively charged group, work must be done to bring a proton to the molecule, and the pK decreases. If the acid molecule contains a negatively charged group, free energy is lost by the process of bringing a proton to the molecule and the pK increases. The magnitude of this effect is given by the expression (12)

$$pK - pG = 0.434\,\frac{z\epsilon}{D_E RkT} \qquad \text{(Eq. 5)}$$

[1] These curves are also independent of the concentration of acylcarnitine present as long as the ionic strength is not changed. Because of the high CMC of decylcarnitine, titration curves which are free of contributions from monomers present could not be obtained, but significant differences in pK below and above the CMC were noted.

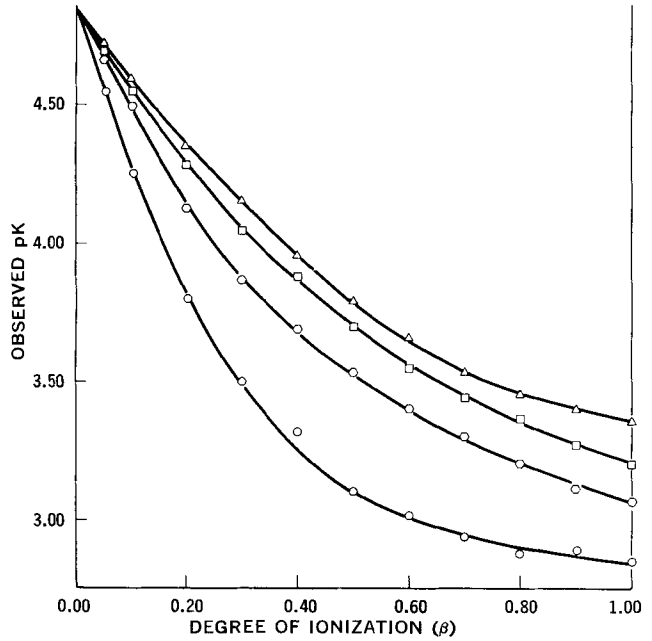


Figure 3—*Titration curves (observed pK* versus *β) for myristylcarnitine at several concentrations of added KCl. Key:* ○, *no salt;* ◯, *0.05* M; □, *0.10* M; *and* △, *0.20* M.

where ϵ is the charge on an electron or carboxylate group; z is the charge of the substituent; R is the distance between the substituent and the carboxyl group; and D_E is the effective dielectric constant separating the substituent and the carboxyl group.

From the data in Table II, it can be seen that carnitine and the acylcarnitines have pK values which are significantly lower than 4.83. These low values are primarily due to the electrostatic interaction between the positively charged nitrogen and the carboxyl group as already discussed. In addition to the above ion–ion interactions, ion–dipole, hydrogen-bonding, and steric interactions involving other groups on the molecule can contribute to the change in the ionization constant of a carboxylic acid. For instance, both the β-hydroxy and β-acyl substituted derivatives have lower pK

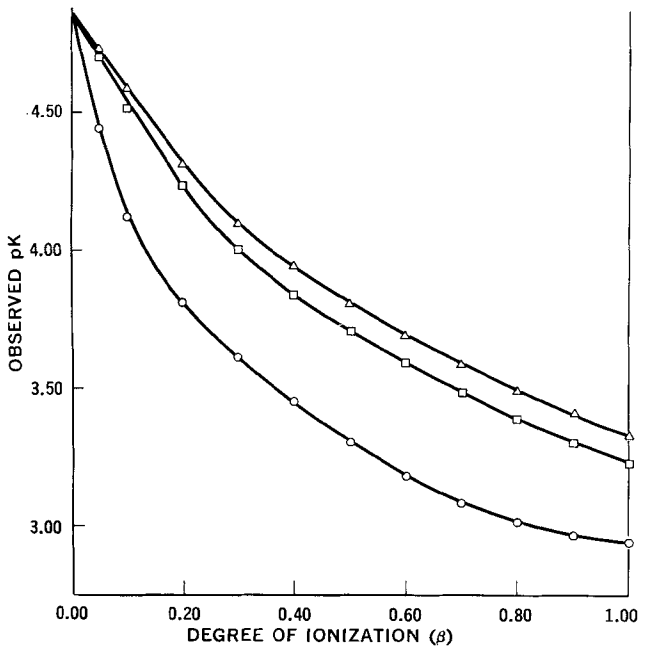


Figure 4—*Titration curves (observed pK* versus *β) for palmitylcarnitine at several concentrations of added KCl. Key:* ○, *no salt;* □, *0.10* M; *and* △, *0.20* M.

Table II—pK Values of Carnitine and Some Related Compounds

Compound	Structure $XCH_2CH(Y)CH_2COOH$ X	Y	pK
Butyric acid	H	H	4.83
Pentanoic acid	CH_3	H	4.80[a]
Hexanoic acid	C_2H_5	H	4.85[a]
GBB	$(CH_3)_3N^+$	H	4.02
GABA	H_3N^+	H	4.01
Carnitine HCl	$(CH_3)_3N^+$	OH	3.80
Norcarnitine HCl	$(CH_3)_2HN^+$	OH	3.81
β-Hydroxy-γ-ammonium butyric acid	H_3N^+	OH	3.80
Acetylcarnitine HCl	$(CH_3)_3N^+$	$C_2H_3O_2$	3.60
Butylcarnitine HCl	$(CH_3)_3N^+$	$C_4H_7O_2$	3.56
Octylcarnitine HCl	$(CH_3)_3N^+$	$C_8H_{15}O_2$	3.60
Decylcarnitine HCl	$(CH_3)_3N^+$	$C_{10}H_{19}O_2$	3.65

[a] Literature values (10).

values than compounds having the same structure except for these β-substituents. The pK values of the four acylcarnitines are nearly the same, indicating that the size of the acyl group has little or no effect on the electrostatic interactions of these compounds. The reason for the difference in pK values between carnitine and its esters (about 0.2 pK unit) is unclear. However, it is likely that the ester carbonyl interacts with the carboxyl group and facilitates dissociation of a proton.

The structures of GBB and GABA are identical except for the substituents on the quaternary nitrogen and, likewise, the structures of carnitine, norcarnitine, and γ-amino-β-hydroxy butyric acid differ only in the number of methyl groups on the nitrogen. The fact that the first two compounds have the same pK value and the remaining three pK values are the same indicates that the electrostatic interaction is independent of the minimum distance with which the quaternary nitrogen and the carboxyl group can approach each other. This interpretation is in agreement with the results of other workers (12, 13), who determined that the carboxyl proton and the quaternary nitrogen of GABA are from 4 to 6 Å apart, *i.e.*, that the molecule is almost fully extended. In view of this the cyclic conformation proposed for acetylcarnitine by Fellman and Fujita (14) seems unlikely.

In the titration of a carboxyl group on a positively charged surface, such as the surface of an acylcarnitine micelle, the observed pK at a particular degree of ionization is dependent upon the surface potential of the micelle, ψ. This relationship is expressed by an equation analogous to Eq. 5, known as the general potentiometric equation for polyelectrolytes (12, 15–18). This equation has been shown to be valid for micelles by Tokiwa and Ohki (19, 20):

$$\mathrm{pK} = \mathrm{pG} + 0.434\,\frac{\epsilon\psi}{kT} \qquad \text{(Eq. 6)}$$

As in Eq. 5 the term pG is related to the work of protonating the carboxyl group in an uncharged environment, *i.e.*, when $\beta = 0$, and the last term in the equation is related to the work required to bring the proton to the micelle surface from infinity. Equation 6 can be rearranged so that ψ is expressed in terms of the difference between the observed and intrinsic pK values:

$$\psi = 2.303\,\frac{kT}{\epsilon}\,(\mathrm{pK} - \mathrm{pG}) \qquad \text{(Eq. 7)}$$

This method of determining the surface potential of a micelle is more convenient than the electrophoretic method (21–23) since it is rapid and requires no equipment other than a pH meter. Since it gives the potential at the plane of the carboxyl groups, it has theoretical advantages over the electrophoretic method in that the value is independent of the thickness of the hydration layer. This method is not limited to the present system, but can be applied to any titratable group solubilized at a micelle surface including those of the surfactant itself.

The values of ψ obtained by applying Eq. 7 to the data shown in Fig. 3 are plotted in Fig. 6. The surface potentials of lauryl-, myristyl-, and palmitylcarnitine at $\beta = 0.1$, 0.5, and 1.0 and at

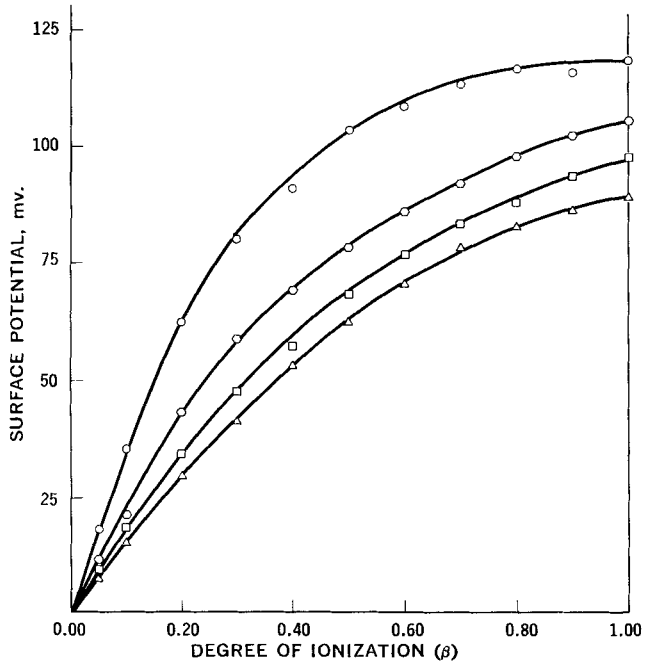


Figure 5—*Plots of surface potential* versus β *for myristylcarnitine at several concentrations of added KCl. Key:* ○, *no salt;* ○, *0.05* M; □, *0.10* M; *and* △, *0.20* M.

several concentrations of added KCl are shown in Table III for comparison. It can be seen from this table that the surface potentials at a given degree of ionization and, ionic strength show no dependence on the length of the aliphatic chain, suggesting that the surface-charge density and, therefore, the surface packing in the micelle is the same for these three compounds.

In order to interpret quantitatively the effect of β and ionic strength on the surface potential and hence the pK, it is necessary to express ψ in terms of measurable parameters such as micelle radius, b, aggregation number, n, and the net charge on the micelle at a given degree of ionization, Z. Unfortunately, this cannot be done analytically for a small spherical particle such as a micelle but it can be approximated; one such approximation is that of Debye-Hückel (12):

$$\psi = \frac{2kT}{\epsilon} wZ \qquad \text{(Eq. 8)}$$

where w, the electrostatic factor, is given by

$$w = \frac{\epsilon^2}{2DkT}\left[\frac{1}{b} - \left(\frac{\kappa}{1+\kappa a}\right)\right] \qquad \text{(Eq. 9)}$$

where a is b plus the average radius of the electrolyte ions,[2] D is the dielectric constant at the micelle surface, and κ is the inverse Debye radius which is given by

$$\kappa = \left(\frac{4\pi\epsilon^2 I}{DkT}\right)^{1/2} \qquad \text{(Eq. 10)}$$

where I is the ionic strength of the solution.

This approximation is valid for impenetrable spherical particles at low surface potential and at low concentrations of 1:1 electrolytes. It is assumed further that the net charge on the particle is spread evenly over the surface. Although this is not the correct physical situation, it has been shown (18, 24, 25) that under these conditions a more rigorous fixed charge model gives almost the same results as a spread charge model. This would be especially true for a micelle in which the individual charges are free to arrange themselves so that they are evenly spaced, *i.e.*, in their lowest energy state. The ability of the cationic and anionic groups of acylcarnitines to arrange themselves in such a manner seems very likely in view of the titration behavior of the monomeric species, which suggested that these groups were far enough apart to interact with neighboring molecules.

[2] Since the distance between charges on the micelle surface is considerably greater than the radius of the electrolyte ions, and since the micelle surface is probably not smooth, it might be more correct to let $a = b$, *i.e.*, to assume that the electrolyte ions behave as point charges.

Table III—Surface Potentials for Acylcarnitines at Several Degrees of Ionization (β) and Several Concentrations of Added Electrolyte

Concentration Added KCl, moles/l.	Surface Potential, mv. Lauryl-carnitine	Myristyl-carnitine	Palmityl-carnitine
	β = **0.10**		
0.00	26.6	29.6	42.6
0.05	24.2	21.9	—
0.10	17.2	17.7	20.1
0.20	14.2	14.8	15.4
	β = **0.50**		
0.00	87.1	102	91.7
0.05	73.7	77.0	—
0.10	68.0	66.8	68.0
0.20	62.2	62.7	62.2
	β = **1.00**		
0.00	117	117	113
0.05	104	104	—
0.10	97.2	97.2	96.0
0.20	89.0	88.7	90.6

If the micelle has an aggregation number of n and a degree of ionization of β, the net charge is given by

$$Z = n\beta \qquad \text{(Eq. 11)}$$

and Eqs. 6 and 8 become, respectively,

$$\text{pK} = \text{pG} + 0.868\, wn\beta \qquad \text{(Eq. 12)}$$

and

$$\psi = \frac{2kT}{\epsilon} wn\beta \qquad \text{(Eq. 13)}$$

From Eqs. 12 and 13, it can be seen that at $\beta = 0$, pK must equal pG, and ψ must become zero. Indeed the value of the experimentally

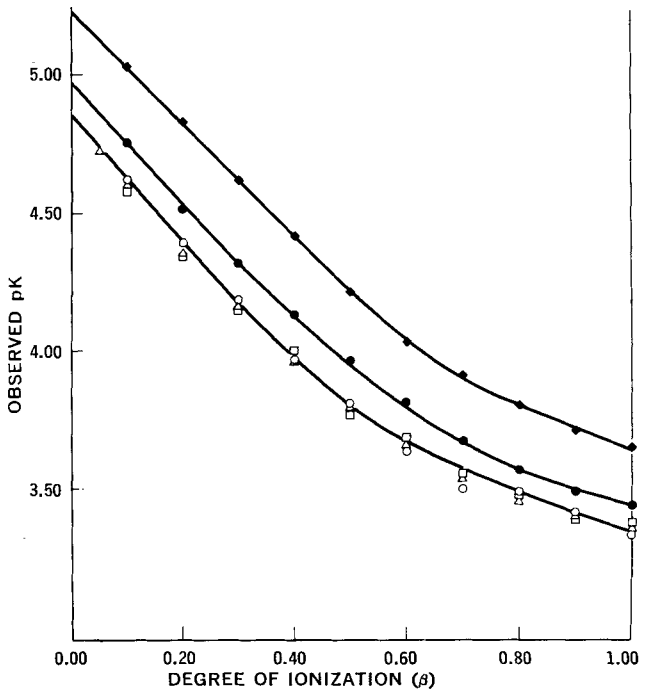


Figure 6—*Effects of specific monovalent ions on the titration curves of myristylcarnitine. Key:* △, *0.20* M *KCl;* ○, *0.20* M *LiCl;* □, *0.20* M *NaCl;* ●, *0.20* M *KBr; and* ◆, *0.20* M *KI.*

determined pG is in excellent agreement with the literature value for aliphatic carboxylic acids. The apparent linearity in the pK *versus* β with ψ *versus* β curves (Figs. 1–4) at low β is expected from Eqs. 12 and 13. The deviation of all of the curves from linearity at high β is probably due to a change in n or w with increasing charge, or more likely to the failure of the Debye-Hückel approximation at high potential (26). Further investigations of the relationship between w, n, and β are now being carried out using other mathematical treatments and experimental measurements of the micelle size (27).

The fact that the pG values of all of the acylcarnitines are independent of concentration of surfactant and added KCl (Figs. 2–4) indicates that neither K^+ nor Cl^- are bound to the zwitterionic micelle. Figure 5 shows that Li^+ and Na^+ also are not bound to the neutral micelle but that Br^- and, to an even greater extent, I^- appear to be bound (28). This order of ion binding to cationic micelles has been noted previously (29). The fact that Cl^-, Br^-, and I^- do not exhibit different effects on the titration of the monomeric acetylcarnitine suggests that these specific ion effects are, indeed, dependent on the presence of the micellar surface.

CONCLUSIONS

The pK values of micellar surfactants such as the acylcarnitines are not constant but change with β, the degree of ionization of the micelle. The difference between the pK value observed at a particular value of β and the intrinsic pK for a carboxyl group has been utilized to determine the surface potential ψ at that value of β.

A model utilizing the Debye-Hückel approximation has been shown to be qualitatively consistent with experimental results.

The ionization constants and surface potentials of lauryl-, myristyl-, and palmitylcarnitine micelles at any given value of β are independent of chain length but are highly dependent on ionic strength. Specific ion effects are observed for Br^- and I^- but not for Li^+, Na^+, K^+, or Cl^-.

The significant effect of surface charges on the ionization equilibria of this particular micellar system suggests the general importance of these considerations for understanding kinetic and adsorption processes involving charged species at charged interfaces such as micelles, monolayers, bilayers, and biological membranes.

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