# The Possible Role of Quinone Methines in Phosphorylation Reactions in *Rhodospirillum rubrum* and Liver Mitochondria*

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ABSTRACT: The possible role of quinone methines in phosphorylation reactions coupled to electron transport in rat liver mitochondria and chromatophore particles from *Rhodospirillum rubrum* was investigated by determination of the exchange of H atoms of the ring C-methyl group of ubiquinone with solvent H atoms. Suspensions of chromatophore particles incubated in tritiated water under conditions of active photophosphorylation showed no significant uptake of tritium into ubiquinone-10. When chromatophore particles containing ubiquinone-10, labeled in the ring C-methyl group with tritium, were incubated in water under conditions favoring photophosphorylation, no decrease in the specific activity of the C-methyl group could be observed. When rat liver mitochondria in which the endogenous ubiquinone was labeled in the ring C-methyl group with tritium were incubated under conditions where P:O ratios between 2 and 3 were observed, no significant loss of tritium from the C-methyl group was detected. When rats were injected with a mixture of methionines labeled with $^{14}C$ and $^{3}H$ in the methyl group, the ratio of $^{3}H:^{14}C$ in ubiquinone extracted from liver mitochondria was the same as the original ratio in the methionine. These results provide evidence against the direct participation of ubiquinone methines in phosphorylation reactions. They do not, however, rule out the possibility that a small portion of the total mitochondrial or chromatophore ubiquinone in the form of methines may be involved in phosphorylation processes.

It is well established that quinones undergo oxidation and reduction in mitochondria, chromatophore fragments, chloroplasts, and in some bacteria (Hatefi, 1963; Wolstenholme and O'Connor, 1961; Vernon and Avron, 1965; Morton, 1965; Amesz, 1964). There is little agreement on whether quinones participate in the main routes of electron transport, or whether they lie on side paths of unknown significance. Except for the isolation of a naphthochromanyl phosphate from *Mycobacterium phlei* (Russell and Brodie, 1961; Asano *et al.*, 1962), there is no direct evidence that quinones are involved in the phosphorylation reactions which are coupled to electron transport. Model reactions, however, indicate that pyrophosphate can be generated during the oxidation of hydroquinone phosphates (Clark *et al.*, 1958; Wieland and Pattermann, 1958), (Scheme I, reaction 6), and several hypothetical schemes for oxidative phosphorylation have been based on this principle (Wessels, 1954; Harrison, 1958; Brodie and Ballantine, 1960; Todd, 1960; Dallam, 1961; Vilkas and Lederer, 1962; Lederer, 1964; Chmielewska, 1960). According to a mechanism of the same general type proposed by Erickson *et al.* (1963), oxidation of a 5-phosphomethyl derivative of the quinone activates the phosphate group for ATP[1] (adenosine triphosphate) formation. Gruber *et al.* (1963) have observed ATP formation linked to the oxidation of ubiquinol 6-phosphate in a mitochondrial preparation.

One of the theoretical difficulties associated with schemes of the type described is the problem of forming the hydroquinone phosphate or phosphomethyl derivative which is to be oxidized. Vilkas and Lederer (1962) and Erickson *et al.* (1963) suggested that this could be done by 1,4 addition of $P_i$ (inorganic phosphate) to a quinone methine (Scheme I, reaction 2), and Chmielewska (1960) proposed a 1,2 addition to a quinone methine. Evidence was obtained for the existence of quinone methines as intermediates in some chemical reactions of naphthoquinones (Wagner *et al.*, 1963; Mamont *et al.*, 1963). In support of the postulated biological role of quinone methines, it appeared to be significant that the naturally occurring quinones which have been implicated in electron transport are substituted in the quinone ring by a C-methyl group and an isoprenoid side chain, and thus, could structurally be suitable for the formation of quinone methines. However, recent studies (White, 1965), implicating a demethyl vitamin K in electron transport in *Hemophilus parainfluenzae*, weaken this argument. Scott (1965)

* From the Department of Biochemistry, Western Reserve University, School of Medicine, Cleveland, Ohio 44106. *Received October 26, 1965.* Supported by grants from the National Institutes of Health (National Heart Institute Grant No. HE-06304-04 and Research Training Grant 2 TIGM 35-06) and the Life Insurance Medical Research Fund.

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‡ Research Career Awardee GM-K6-993 of the U. S. Public Health Service.

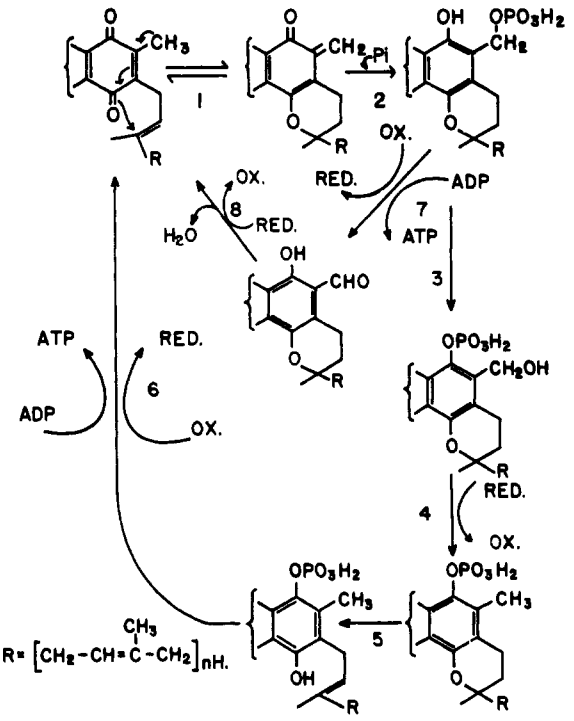
[1] Abbreviations: ATP, adenosine triphosphate; $P_i$, inorganic phosphate; NADH, reduced nicotinamide adenine dinucleotide; UQ, ubiquinone; UQ-*n*, ubiquinone-*n*, where *n* indicates the number of isoprenoid units in the side chain.

TABLE I: Chromatophore Experiments, Uptake of Tritium.[a]

| Expt | Protein (mg) | Conditions | $P_i$ Uptake ($\mu$moles) | Sp Act of $^3H_2O$ in Incubation (dpm/$\mu$mole) | UQ-10 Isolated ($\mu$moles) | UQ-10 (dpm) | UQ-10 (dpm/$\mu$mole) |
|---|---|---|---|---|---|---|---|
| 1 | 6.3 | Light | 30 | $2.0 \times 10^4$ | 0.0677 | 3.6 | 53 |
| | 6.3 | Dark | 0 | $2.0 \times 10^4$ | 0.0571 | 0 | 0 |
| 2 | 7.9 | Light | 30 | $6.0 \times 10^4$ | 0.0893 | 0 | 0 |

[a] Conditions as described in the text.

SCHEME I: Some Mechanisms Involving Quinone Methines in the Formation of Quinol Phosphates [Vilkas and Lederer (1962), reactions 1–6; Erikson *et al.* (1963), reactions 1, 2, 7, 8].




has shown that the formation of quinone methines in alkaline methanol[2] does not require an isoprenoid side chain; furthermore, the formation of a hydroquinone phosphate from a chromanyl phosphate is not a facile reaction, and this conversion is a step in the mechanism of Vilkas and Lederer (1962). Finally, Szarkowska and Klingenberg (1963) have reported that succinate reduces UQ (ubiquinone) in the absence of $P_i$, in tightly coupled rat heart mitochondria. In the schemes of Vilkas and Lederer (1962), Chmielewska (1960), and Erickson *et al.* (1963), phosphorylated derivatives of the quinone are involved in the oxidation–reduction reactions.

In spite of these possible objections, the idea that quinone methines participate in oxidative phosphorylation remains of general interest. It appeared possible to obtain direct evidence for the biological importance of quinone methines by determining whether the hydrogen atoms of the ubiquinone C-methyl group exchange with those of the solvent during oxidative phosphorylation. We were encouraged to investigate this point by a report of Vishniac (1963), that two substances which appeared to be quinones incorporated $^3H$ when chloroplast fragments were suspended in $^3H_2O$ and illuminated.

## Experimental Section

*Methods.* Chromatophores were prepared from the photosynthetic bacterium *Rhodospirillum rubrum* (Geller and Lipmann, 1960), and stored at −4° in ethylene glycol–0.2 M glycylglycine, pH 7.4 (40:60). From a suspension containing 15.8 mg of protein/ml, 0.5 ml was added to a solution (adjusted to pH 7.0 with KOH) containing 2 $\mu$moles of sodium succinate, 40 $\mu$moles of glucose, 50 $\mu$moles of glycylglycine, 30 $\mu$moles of $KH_2PO_4$, 10 $\mu$moles of $MgCl_2$, 5 $\mu$moles of NaADP, hexokinase (29 units), $^3H_2O$ as indicated in Table I, and $H_2O$ to a final volume of 2.0 ml. The suspension was shaken anaerobically in the light (Rudney, 1961) for 1 hr at 30°. Control flasks were incubated in the dark. Following the incubation, the flasks were placed in a boiling-water bath for 1 min, then diluted with cold water, and centrifuged. $P_i$ uptake was measured in the supernatant solutions (Fisk and SubbaRow, 1925). The precipitates were lyophilized and extracted with petroleum ether, and UQ-10 was isolated by silica gel thin layer chromatography (Parson and Rudney, 1965a). The 95% methanol extraction of the petroleum ether solutions, used previously (Parson and Rudney, 1965b), was unnecessary when the initial extraction was performed with petroleum ether, and was omitted in order to avoid exposing the UQ-10 to hydrophilic solvents.

To demonstrate that the methods used for isolation of

[2] Evidence for the formation of quinone methines in alkaline methanol solution was based on detection of hydrogen exchange by nuclear magnetic resonance spectroscopy. However, recent results reported by (Lapidot *et al.*, 1965) cast doubt on Scott's interpretation of his observations, and in fact they show that hydrogen exchange does not occur in methyl quinones under alkaline conditions.

TABLE II: Chromatophore Experiments, UQ-10 Prelabeled with Tritium.[a]

| Expt | Protein (mg) | Conditions | $P_i$ Uptake ($\mu$moles) | UQ-10 Isolated ($\mu$mole) | UQ-10 (cpm) | UQ-10 (cpm/$\mu$mole) | Acetate (cpm) | Acetate % of Total (cpm) |
|---|---|---|---|---|---|---|---|---|
| 1 | 21 | Light | 71.4 | 0.330 | $2.97 \times 10^5$ | $9.01 \times 10^5$ | ... | ... |
| | 21 | Dark | 1 5 | 0.339 | $2.97 \times 10^5$ | $8.75 \times 10^5$ | ... | ... |
| 2 | 28 | Boiled zero time | 0 | 0.434 | $3.12 \times 10^5$ | $7.19 \times 10^5$ | $2.91 \times 10^4$ | 9.32 |
| | 28 | Light | 75 | 0.414 | $2.98 \times 10^5$ | $7.19 \times 10^5$ | $2.80 \times 10^4$ | 9.40 |
| 3 | 40 | Boiled zero time | 0 | 0.456 | $3.62 \times 10^5$ | $7.94 \times 10^5$ | $7.27 \times 10^4$ | 20.1 |
| | 40 | Light | 72 | 0.450 | $3.50 \times 10^5$ | $7.78 \times 10^5$ | $7.13 \times 10^4$ | 21.6 |
| | 40 | Dark | 2 | 0.409 | $3.29 \times 10^5$ | $8.05 \times 10^5$ | $7.13 \times 10^4$ | 21.6 |

[a] Conditions as described in the text.

the UQ-10 did not cause complete loss of $^3H$ from the C-methyl group, endogenous UQ-10 was prelabeled by incubating suspensions of *R. rubrum* cells with L-methionine-methyl-$^3H$. The 2-hr incubations were performed anaerobically in the light, as described previously (Parson and Rudney, 1965b). Chromatophores were then prepared from the tritiated cells, and were used without storage. Portions of the chromatophores were boiled and lyophilized directly; others were incubated as described above, except in unlabeled $H_2O$ and in larger quantities.

Because the $^3H$ in the UQ-10 was expected to be distributed among the two methoxyl groups and in the C-methyl group (Parson and Rudney, 1965b), the UQ-10 was degraded by the Kuhn–Roth oxidation. To approximately 0.1 mg of UQ-10, was added 0.35 ml of concentrated $H_2SO_4$, followed by 0.61 ml of 5 N $CrO_3$. The solutions were heated at 130° in sealed tubes for 30 min. Acetic acid was steam distilled from the reaction mixture, the distillate was brought to pH 8.0 and taken to dryness, and the residue was redissolved in $H_2O$ and evaporated. This procedure was repeated twice to remove $^3H_2O$. The acetate, derived in part from the C-methyl group and the ring carbon to which it was bound, contained 10–20% of the total $^3H$ found in the UQ-10 (Table II). The figures given for the yields of radioactivity in the acetate indicate the actual amounts of $^3H$ or $^{14}C$ measured.

In the Kuhn–Roth degradation, acetate arises also from the isoprenoid side chain, but it seemed unlikely that appreciable $^3H$ would have been incorporated into the side chain during the brief incubation with methionine-methyl-$^3H$. In a separate experiment, cell suspensions were incubated with L-methionine-methyl-$^{14}C$, and UQ-10 was isolated and degraded by ozonolysis (Parson and Rudney, 1965a). The levulinaldehyde bis-dinitrophenylhydrazone obtained from the isoprenoid side chain contained less than 0.5% of the total $^{14}C$ in the UQ-10, indicating that most of the radioactivity recovered in acetate in the Kuhn–Roth degradation came from the C-methyl group of the ring.

In another experiment, cell suspensions were incubated with a mixture of L-methionine-methyl-$^3H$ and L-methionine-methyl-$^{14}C$, the UQ-10 isolated from the cells was degraded by the Kuhn–Roth method, and the ratio of $^3H$ to $^{14}C$ in the acetate was compared with that in the UQ-10. Jauréguiberry *et al.* (1965) have shown that in several species the biosynthesis of C-methyl groups from methionine-methyl-$^2H_3$ involves loss of one of the three $^2H$ atoms; accordingly, the $^3H/^{14}C$ ratio in the acetate should be less than that of the UQ-10. However, because an isotope effect may alter the rate of loss of $^3H$ relative to that of H, the theoretical ratio cannot be calculated. The $^3H/^{14}C$ ratio in the UQ-10 was 5.85; that found in the acetate was 5.04. The acetate contained 19.0% of the $^{14}C$ in the UQ-10 and 17.1% of the $^3H$. Apparently, the isolation procedures and the Kuhn–Roth degradation can be used without causing extensive loss of $^3H$ from the C-methyl group. No labilization of $^3H$ during the isolation had been anticipated, but it had not been clear in advance whether the Kuhn–Roth oxidation would prove feasible, in view of the studies of Erickson *et al.* (1963) on vitamin $K_{1(20)}$. Concentrated sulfuric acid converts vitamin $K_{1(20)}$ to products which Erickson *et al.* (1963) view as arising from a carbonium ion, in which one of the protons of the C-methyl group has been lost. Part of the UQ may undergo similar side reactions during the degradation, since the yield of $^3H$ and $^{14}C$ in acetate is less than one might expect. Assuming that the same pool of methionine labels the two methoxyl groups and the C-methyl group, and that exchange reactions do not enrich the methoxyl groups relative to the C-methyl, one would expect to find 33% of the $^{14}C$ in the C-methyl group.

Rat liver mitochondria were isolated in 0.38 M sucrose essentially as described by Schneider (1964), and were used without delay. One milliliter of a suspension of mitochondria (46 mg of protein) in 0.38 M sucrose was added to 3.0 ml of a solution containing 10 $\mu$moles of NaADP, 160 $\mu$moles of glucose, 80 $\mu$moles of $K_2HPO_4$, 40 $\mu$moles of $MgCl_2$, 40 $\mu$moles of potassium pyruvate, 4 $\mu$moles of sodium succinate, 120 $\mu$moles of N-acetyl-histidine, hexokinase (29 units), KOH (105 $\mu$moles) to

TABLE III: Mitochondria Experiments, Uptake of Tritium.[a]

| Expt | Protein (mg) | Conditions | $P_i$ Uptake ($\mu$moles) | Sp Act of $^3H_2O$ in Incubation (dpm/$\mu$mole) | UQ-9 Isolated ($\mu$mole) | UQ-9 (dpm) | UQ-9 (dpm/$\mu$mole) |
|---|---|---|---|---|---|---|---|
| 1 | 46 | Air, 60 min | 78 | $1.0 \times 10^5$ | 0.110 | 38.6 | 351 |
| 2 | 43 | Air, 30 min | 70 | $6.25 \times 10^4$ | 0.0549 | 41.3 | 750 |
| | | $N_2$, 30 min | 0 | $6.25 \times 10^4$ | 0.0568 | 23.5 | 415 |

[a] Conditions as described in the text.

TABLE IV: Mitochondria Experiments, UQ-9 Prelabeled with Tritium.[a]

| Expt | Protein (mg) | Conditions | $P_i$ Uptake ($\mu$moles) | UQ-9 Isolated ($\mu$mole) | UQ-9 (dpm) | UQ-9 (dpm/$\mu$mole) | Acetate (dpm) | Acetate % of Total dpm |
|---|---|---|---|---|---|---|---|---|
| 1 | 42 | Boiled, zero time | 0 | 0.100 | 1462 | $1.46 \times 10^4$ | 80 | 6 |
| | | Air, 30 min | 94 | 0.0832 | 1220 | $1.47 \times 10^4$ | | |

[a] Conditions as described in the text.

pH 7.4, and $^3H_2O$ as indicated in Table III. The suspensions were shaken in a Warburg apparatus at 30° with air as the gas phase, then boiled for 1 min, diluted with cold water, and centrifuged. $P_i$ was measured in the supernatant solutions; the precipitates were lyophilized, and UQ-9 was isolated by the procedures described above. P/O ratios, measured between the 10th and 20th min of the incubations, were between 2 and 3. Control flasks incubated under $N_2$ showed no significant $P_i$ uptake.

## Results

When suspensions of *R. rubrum* chromatophore particles were illuminated in $^3H_2O$, the amount of $P_i$ taken up was some 400 times the UQ-10 content. If the UQ-10 C-methyl group were to equilibrate with the solvent, and if there were no isotope effect on the equilibrium constant, the specific activity of the UQ-10 would have been 3/2 that of the $^3H_2O$. However, no significant $^3H$ was found in the UQ-10 (Table I).

Suspensions of washed cells were incubated with L-methionine-methyl-$^3H$, and chromatophores were prepared from the resultant tritiated cells. The UQ-10 isolated from the chromatophores was radioactive, and Kuhn–Roth oxidation released 10–20% of the $^3H$ as acetic acid (Table II). If the chromatophores were incubated in $H_2O$ under conditions favoring photophosphorylation, there was no decrease in either the specific activity of the UQ-10 or the fraction of the total $^3H$ released in the acetic acid (Table II). Control experiments described in the Methods section indicated that most of the $^3H$ obtained in the acetate originated in the C-methyl group of the UQ-10 ring.

When rat liver mitochondria were incubated in $^3H_2O$, a trace of $^3H$ appeared in the UQ-9 (Table III). The UQ-9 specific activities were on the order of 1% of that of the solvent, but the measured amounts of $^3H$ in the UQ-9 were so small that they were considered insignificant. Thus, in expt 1, a total of 10 mc of $^3H$ was present in 4.0 ml during the incubation, but the UQ-9 contained a total of only 10 cpm above background. Possibly, these traces of $^3H$ were due to a contaminant, rather than to the UQ-9.

Mitochondrial UQ-9 was prelabeled with $^3H$ by injecting two rats intraperitoneally with L-methionine-methyl-$^3H$ (250 $\mu$c/rat). Two hours later, the rats were killed, and their liver mitochondria were isolated. The specific activity of the mitochondrial UQ-9 was the same, whether or not the mitochondria were incubated in $H_2O$ under conditions favoring oxidative phosphorylation (Table IV). Because the amounts of $^3H$ in these samples were small, the two samples were pooled for Kuhn–Roth oxidation. The acetate had 6% of the total $^3H$.

In another experiment, two rats were injected with a mixture of L-methionine-methyl-$^3H$ and L-methionine-methyl-$^{14}C$ (250 $\mu$c of $^3H$ and 25 $\mu$c of $^{14}C$ per rat). These animals were killed 24 hr later, liver mitochondria were isolated, and UQ-9 was extracted immediately. Within experimental error, the $^3H/^{14}C$ ratio in the UQ-9 (12,500 dpm of $^3H$/1119 dpm of $^{14}C$, $^3H/^{14}C$ = 11.2) was the same as that of the methionine which had been injected (10.0). Kuhn–Roth oxidation yielded acetate containing 10.0% of the $^3H$ and 8.94% of the $^{14}C$; the $^3H/^{14}C$ ratio was 12.5. Thus, there was no evidence that $^3H$ had been lost from the C-methyl group during the 24-hr period before the rats were killed.

## Discussion

These experiments give no indication of exchange of the H atoms of the UQ C-methyl group with those of the solvent during oxidative phosphorylation or photophosphorylation, and this result must be viewed as evidence against the idea that quinone methines participate in these processes. It is clear, however, that a negative result is less significant than a positive one might be, because of several arguments that are difficult to dismiss. First, only a small portion of the ubiquinone might be involved in phosphorylation. One or two per cent of the UQ could exchange protons without significantly affecting the accuracy in the present experiments. The conclusion that not all of the UQ is involved in the major path of electron transport has been expressed previously, based on the observations that the mitochondrial UQ concentration exceeds that of the other respiratory carriers (except pyridine nucleotide) (Chance and Hagihara, 1963) and that the rates of oxidation and reduction of endogenous UQ are less than the rates of succinate and NADH (reduced nicotinamide adenine dinucleotide) oxidation (Chance and Redfearn, 1961; Redfearn and Pumphrey, 1960). Another possible argument is that the proton lost in formation of a quinone methine might be held in some pool which is not in equilibrium with the solvent.

In addition to the schemes which invoke quinone methines, several mechanisms which have been proposed for oxidative phosphorylation involve conversion of a quinone to a chromanyl derivative (Todd, 1960; Dallam, 1961; Vilkas and Lederer, 1962; Chmielewska, 1960; Gruber *et al.*, 1963; Erickson *et al.*, 1963; Slater, 1960). These mechanisms require addition and removal of a proton at the $\beta$ carbon of the isoprenoid side chain of the quinone, and in chemical systems this would result in an incorporation of $^3H$ from the solvent into the quinone (Scheme I, reactions 5 and 8). One would not necessarily expect to observe such an incorporation in an enzymatic system, however, since the two H atoms on the $\beta$ carbon are sterically distinct. While this manuscript was in preparation, Gutnick and Brodie (1965) reported that a phosphate-dependent incorporation of tritium into added naphthoquinone occurred during oxidative phosphorylation in *Mycobacterium phlei* extracts. The location of the tritium was not determined. Thus, the role of quinone methines in reactions involving naphthoquinones remains to be ascertained. The present experiments bear only on schemes involving ubiquinone, and which demand an exchange at the C-methyl group, where the three H atoms are sterically indistinguishable.[3]

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[3] In agreement with these results, A. F. Brodie (personal communication, 1965) has found that tritium from the solvent was not incorporated into benzoquinones in beef heart mitochondria with succinate as electron donor, during electron transport or oxidative phosphorylation.

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# On the Conformation of Caseins. Optical Rotatory Properties*

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ABSTRACT: The optical rotatory properties of the whole casein of cow's milk and the three major casein components, $\alpha_{s1}$-, $\beta$-, and $\kappa$-casein, have been examined in both aqueous and organic solvents. Analysis of the dispersion data by means of the Moffitt–Yang, Drude, and Shechter and Blout equations and calibration constants, together with the analysis of the trough values of the 233-m$\mu$ Cotton effects, indicate very low $\alpha$-helix content and a low degree of structural organization of the major casein components. Around neutral pH, the caseins, including whole casein, are characterized by low Moffitt $b_0$ parameters ranging from approximately $-30$ to $+30$, and very low $a_0$ values ranging $-510$ to $-780$; the Drude $\lambda_c$ values range from 211 to 223 m$\mu$. The Shechter and Blout $A(\alpha,\rho)_{193}$ and $A(\alpha,\rho)_{225}$ parameters are found to vary from $-180$ to $-590$ and $-265$ to $-410$, respectively, and the mean residue rotations at 233 m$\mu$ range from about $-2800$ to $-3800$. These findings tend to rule out $\alpha$-helical organization as a basic feature of casein aggregates or micelles in aqueous media. It is also found that an appreciable degree of $\alpha$-helix formation can be produced by dissolving the caseins in structure-forming solvents such as acidic methanol or 2-chloroethanol.

In the past decade considerable effort has been directed toward the isolation and characterization of the caseins and various casein components of milk (see the reviews of Lindqvist, 1963; Timasheff, 1964; Gordon and Whittier, 1965). However, because of the complex nature of the stoichiometry of casein–casein interactions in the neutral pH region, relatively little work has been done concerning the conformation of the caseins of their native state. The light scattering studies of Krescheck *et al.* (1964) and Krescheck (1965) have suggested that $\alpha_{s1}$- and $\kappa$-casein in neutral solutions exist in the form of random coils. Payens and van Markwijk (1963) have concluded on the basis of ultracentrifugal and viscosity studies on $\beta$-casein that this casein component in neutral salt solutions behaves hydrodynamically as a rod-like aggregate or a coiled polymeric aggregate. Recent studies of Swaisgood and Timasheff (to be published), in this laboratory, have suggested that $\alpha_{s1}$-casein in the neutral and slightly alkaline pH region also exists in the form of aggregates, having average molecular weights which correspond to trimers to pentamers.

Earlier reports of the sodium D rotations and Drude constants of some of the casein components (Hipp *et al.*, 1952; Jirgensons, 1958a,b; Herskovits, 1964; Herskovits and Mescanti, 1965), together with the present study, have suggested that the caseins possess a random conformation similar to the disorganized structure of denatured globular proteins. In addition to a detailed study of the optical rotatory properties of the caseins in aqueous media, the present work also reports the properties of the caseins in 8 M urea and 2-chloroethanol, and in the case of $\alpha_{s1}$-casein, in a number of other organic solvents.[1]

## Experimental Section

*Materials.* Whole casein and the genetic variants of $\alpha_{s1}$-casein and $\beta$-casein A were generously provided by Dr. M. P. Thompson. The preparation of these proteins from the milk of single homozygous cows has been described by Thompson and Kiddy (1964) and Thompson and Pepper (1964). The B variant of $\beta$-casein

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[1] While this paper was in preparation Kresheck (1965) reported the rotatory dispersion parameters of pooled whole casein and $\beta$-casein. While the author's (Table I) Drude and Moffitt parameters are somewhat different from the Kresheck's (*i.e.*, $\lambda_c$ = 233 and 212 m$\mu$, $a_0 = -455$ and $-530$, and $b_0 = -97$ and $-53$ for whole casein and $\beta$-casein in pH 6.5, $\Gamma/2$ = 0.1 phosphate buffer, respectively), their conclusions are essentially the same; namely, that the caseins are largely devoid of $\alpha$-helical organization.