# Effect of Synthetic Detergents on the Swelling and the ATPase of Mitochondria Isolated from Rat Liver*

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ABSTRACT

A study was made of the effects of various types of detergents on the swelling of isolated mitochondria and on mitochondrial ATPases which are activated by Mg or DNP respectively. The rate of swelling was measured in the Beckman spectrophotometer by following the decrease in turbidity of dilute suspensions of these organelles. It was found that non-ionic detergents containing a nonyl phenoxy side chain or anionic detergents caused swelling of the mitochondria and activation of Mg-ATPase. On the other hand, cationic detergents promoted the clumping of mitochondria and did not activate Mg-ATPase. DNP-ATPase was inhibited by all of the detergents tested. It would appear from these observations that the inhibition of DNP-ATPase is not related to a gross change in the morphology of the organelles; in contrast, the activation of Mg-ATPase definitely is correlated with swelling of the isolated mitochondria. These data also suggest that the ionic detergents combine with charged sites on the protein moiety of the lipoprotein in the mitochondrial surface, whereas the non-ionic detergents form inclusion compounds with the lipide moiety, thereby altering the mitochondrial structure and permeability.

## INTRODUCTION

Surface active agents are known to cause swelling of mitochondria isolated from rat liver (1, 2), to activate Mg-ATPase[1] (3, 4), and to inactivate DNP-ATPase[1] (3, 4) of these organelles. A variety of synthetic detergents of known structure are available, and a systematic study of the effects of these substances on these processes might reveal what chemical groups are responsible for the effects observed, and furthermore might provide a clue as to the mechanism of action of these surface active compounds. In this connection the non-ionic detergents are of particular interest since, although these surface active agents apparently do not react directly with protein (5, 6), some of them interact with biological systems to cause such diverse effects as the release of enzymes from cell particulates (7, 8), the hemolysis of red cells (9), or sustained hyperlipemia and hypercholesterolemia (10–12).

Aging or freezing of the mitochondria or the presence of calcium also activates Mg-ATPase and inhibits DNP-ATPase (3, 14, 17), and these conditions promote as well the swelling of these organelles (18–20). It would thus be of interest to determine whether a similar correlation could be made in the case of the surface active agents. Therefore, a study was made of the effects of different types of detergents on the activities of Mg-ATPase and DNP-ATPase and on the swelling of the isolated mitochondria. The latter was estimated by determining the decrease in the turbidity of mitochondria suspended in solutions of sucrose containing various detergents (18, 21). The rate of decrease in turbidity was assumed to be a measure of the rate of swelling of the mitochondria (18, 21). In addition, these results were checked by observation of the mitochondria under

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[1] The following abbreviations will be used: Mg-ATPase, an ATPase activated by Mg; DNP-ATPase, an ATPase activated by DNP; ATP, adenosinetriphosphate; DNP, dinitrophenol; tris, (tris) hydroxymethylaminomethane.

TABLE I

*Properties of Detergents Used*

| Detergent trade name | Chemical name or description | Type | Source |
|---|---|---|---|
| Dupanol | Sodium dodecyl sulfate | Anionic | Dupont Chemical Co., Wilmington, Delaware |
| BLS 704 | Sodium cetyl sulfate | Anionic | Dupont Chemical Co., Wilmington, Delaware |
| Lantomuse 3 | Sodium dodecyl benzene sulfonate | Anionic | Monsanto Chemical Co., St. Louis, Missouri |
| Lantomuse 4 | Sodium decyl benzene sulfonate | Anionic | Monsanto Chemical Co., St. Louis, Missouri |
| Cetab | Cetyl trimethyl ammonium chloride | Cationic | Fairfield Laboratories, Plainfield, New Jersey |
| Octab | Octadecyl dimethyl benzyl ammonium chloride | Cationic | Fairfield Laboratories, Plainfield, New Jersey |
| Cetyl pyridinium chloride | Cetyl pyridinium chloride | Cationic | Merck and Co., Rahway, New Jersey |
| Tween 80 | Polyoxyethylene mono-oleate | Non-ionic | Atlas Powder Co., Wilmington, Delaware |
| Tween 20 | Polyoxyethylene sorbitan monolaurate | Non-ionic | Atlas Powder Co., Wilmington, Delaware |
| Pluronic L-62 | Polyoxyethylene derivatives | Non-ionic | Wyandotte Chemicals, Wyandotte, Michigan |
| Igepal CO 630 | Nonyl phenoxy polyethylene oxide ethanol | Non-ionic | Antara Chemicals, New York, N. Y. |
| Antaron F-C-34 | Complex fatty amido amphoteric compound cationic below pH 4.5, anionic above pH 8.5 | Non-ionic | Antara Chemicals, New York, N. Y. |
| Anatarox GI00 | Alkyl polyoxyethylene glycol amine | Non-ionic | Antara Chemicals, New York, N. Y. |

the phase microscope. These procedures are capable of detecting gross changes in the morphology of the organelles, but fine changes in morphology would be overlooked.

The compounds tested included anionic and cationic detergents with either aromatic or alkyl side chains and various non-ionic detergents of the polyoxyethylene type, including those containing sorbitan mono-oleate or monolaurate groups (tween 80 or 20), amine groups (anatarox G100), and nonyl phenoxy groups (igepal). The swelling of isolated mitochondria is known to be influenced by the pH of the medium, the concentration of sucrose, and the presence of calcium ions (18–20). Therefore in the present experiment the effect of the various detergents was determined at pH values ranging from 6.2 to 8.0 and in the presence or absence of the complexing agent versene (18). Also the tests were carried out in 0.44 M sucrose, since at this concentration possible effects of the osmotic pressure of the solution are minimized (18).

## *Experimental*

*Sources of Chemicals:*

The chemicals used in these studies and their sources are given in the following list: Crystalline sodium ATP[1] (Pabst), sodium versenate, analytical grade (Bersworth Chemicals), sucrose, analytical reagent (Mallinckrodt), "tris" (Eastman Kodak); inorganic chemicals, analytical reagent (Bakers). Glass-redistilled water was used to make up all reagents and to rinse all glassware. The properties and chemical nature of the detergents used are listed in Table I.

The detergent igepal was titrated with alkali to test for the presence of acidic groups. A 1 per cent solution of this detergent had a pH of 6.7 (the pH of distilled water), and 0.02 ml. of 0.1 N alkali brought 25 ml. of the solution to the end point with phenolphthalein. Evidently acidic or basic groups were not present. The detergent was further purified by passage through the acid form of a cation ion exchange resin (1R-100, Rohm and Haas, analytical grade) and then through the chloride form of an anion exchange resin (1R4B, Rohm and Haas, analytical grade). 25 ml. of 1 per cent solution required 0.03 ml. to bring to the phenolphthalein end point after passage through the sodium salt of the resin, and the pH was 6.7 after passage through the chloride form of the resin. Evidently this non-ionic detergent was not contaminated with acidic or basic groups.

*Assay Systems:*

The experiments on the effect of the detergents on the stability of mitochondria were conducted in 3.3 ml. of 0.44 M sucrose plus $6.7 \times 10^{-3}$ M buffer as described in the legends of the various figures. From 0.025 to 0.03 ml. of mitochondrial suspension was added to the incubation mixture at 28°, and the change in optical density of the suspension with time was noted in the Beckman spectrophotometer at 520 mμ. The temperature of the incubation mixture did not change during a 10 minute period, and rose to 30° after about 30 minutes in the cell compartment of the spectrophotometer. Usually the first reading was taken within 15 seconds after the motochondria had been added to the incubation mixture.

Mg-ATPase or DNP-ATPase was assayed as previously described (15), and the conditions for these experiments are given with Table II.

*Preparation of the Mitochondria:*

The mitochondria were prepared in 0.44 M sucrose, as previously described (22), from the livers of rats weighing 180 to 190 gm. The final suspension of mitochondria was made up so that 1 ml. was equivalent to 0.5 gm. (wet weight) of liver or about 20 mg. dry weight. The concentration of mitochondria was adjusted so that the optical density of the control was about 0.400. Each preparation of mitochondria was examined under the phase microscope, and the effect of the detergents on the gross structure of these organelles also was noted by this means.

TABLE II

*Effect of Detergents on the Activities of Mg-ATPase and DNP-ATPase of Mitochondria from the Liver of the Rat*

0.05 ml. of mitochondrial suspension was used per test. The conditions for the assay of ATPase are described in reference 15.

| Detergent | Concentration of detergent | Condition of mitochondria | Activator present | | | |
|---|---|---|---|---|---|---|
| | | | None μM $PO_4$ | Versene μM $PO_4$ | $MgCl_2$ μM $PO_4$ | DNP μM $PO_4$ |
| None | — | Fresh | 0.15 | 0.05 | 0.2 | 2.9 |
| Dodecyl sulfate | $1 \times 10^{-4}$ M | Fresh | 0.15 | 0.05 | 0.5 | 2.6 |
| | $2.5 \times 10^{-4}$ M | Fresh | 0.40 | 0.30 | 1.7 | 1.9 |
| | $5.0 \times 10^{-4}$ M | Fresh | 0.70 | 0.20 | 3.2 | 1.0 |
| | $1 \times 10^{-3}$ M | Fresh | 0.40 | 0.10 | 3.4 | 0.6 |
| Tween 80 | 4 mg. per cent | Fresh | 0.50 | 0.30 | 0.60 | 1.90 |
| | 12 mg. per cent | Fresh | 0.40 | 0.20 | 0.50 | 0.70 |
| | 24 mg. per cent | Fresh | 0.40 | 0.20 | 0.70 | 0.50 |
| | 40 mg. per cent | Fresh | 0.50 | 0.20 | 0.60 | 0.60 |
| Cetab | $5 \times 10^{-5}$ M | Fresh | 1.3 | 1.2 | 0.5 | 4.0 |
| | $1.0 \times 10^{-4}$ M | Fresh | 1.2 | 1.0 | 0.7 | 3.8 |
| | $2.0 \times 10^{-4}$ M | Fresh | 0.2 | 0.20 | 0.4 | 1.2 |
| Igepal | 10 mg. per cent | Fresh | 0.30 | 0.20 | 0.40 | 2.1 |
| | 20 mg. per cent | Fresh | 0.70 | 0.40 | 1.1 | 0.8 |
| | 40 mg. per cent | Fresh | 0.40 | 0.10 | 2.8 | 0.4 |
| None | — | Aged 2 hrs. at 37° | 1.0 | 0.10 | 2.1 | 1.1 |
| Dodecyl sulfate | $5 \times 10^{-4}$ | Aged 2 hrs. at 37° | 1.0 | — | 4.0 | — |
| Tween 80 | 24 mg. per cent | Aged 2 hrs. at 37° | 0.90 | — | 2.0 | 0.9 |
| | 40 mg. per cent | Aged 2 hrs. at 37° | 1.0 | | 2.1 | 1.1 |
| Cetab | $1 \times 10^{-4}$ M | Aged 2 hrs. | 1.2 | — | 1.6 | 1.2 |
| | $2 \times 10^{-4}$ M | at 37° | 1.4 | — | 1.5 | 0.9 |
| Igepal | 20 mg. per cent | Aged at 37° 2 hrs. | 1.1 | — | 2.6 | — |
| | 40 mg. per cent | Aged at 37° 2 hrs. | 1.3 | — | 2.9 | — |
| None | — | Frozen | 1.4 | 0.20 | 1.9 | 1.3 |
| Dodecyl sulfate | $5 \times 10^{-4}$ M | Frozen | 1.5 | — | 3.0 | — |
| Igepal | 40 mg. per cent | Frozen | 0.9 | — | 2.9 | — |
| Tween 80 | 24 mg. per cent | Frozen | 1.3 | — | 1.5 | 1.2 |

## RESULTS

In the first experiments (Fig. 1) a comparison was made of the effects of the detergents on the

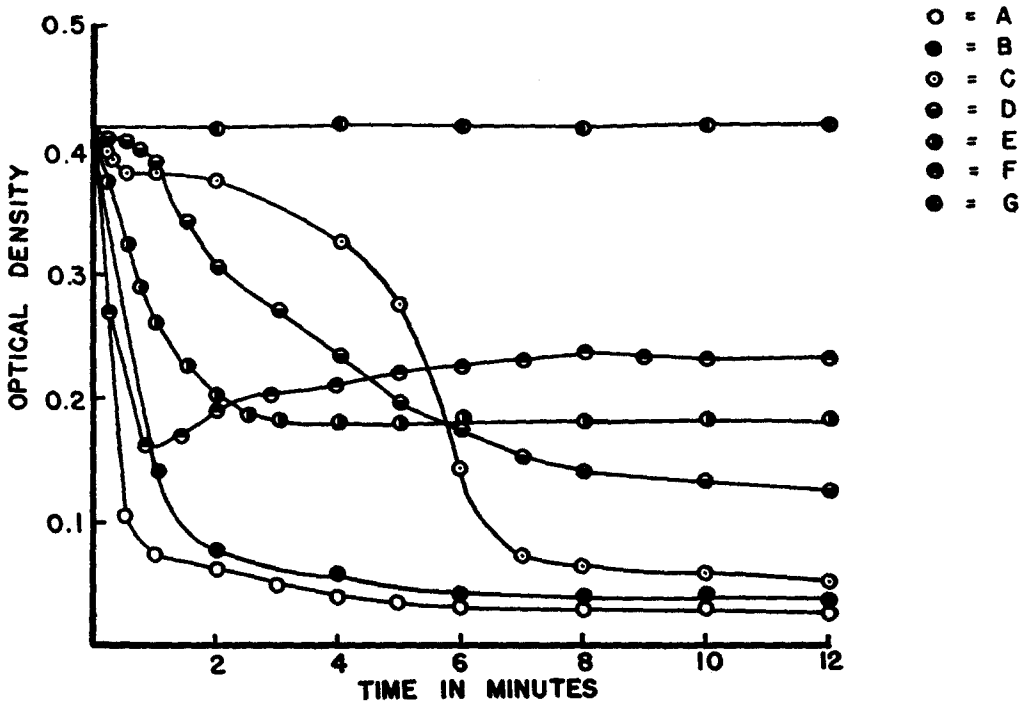


FIG. 1. Effect of detergents on the swelling of mitochondria in 0.44 M sucrose containing $6.7 \times 10^{-3}$ M versene, pH 6.2.

A, $9 \times 10^{-5}$ M dodecyl sulfate.

B, $9 \times 10^{-5}$ M Na dodecyl—or Na decyl—benzene sulfonate.

C, $9 \times 10^{-5}$ M Na cetyl sulfate.

D, 0.25 mg. igepal.

E, $9 \times 10^{-5}$ M cetyl trimethyl or octyl trimethyl ammonium chloride.

F, $9 \times 10^{-5}$ M cetyl pyridine chloride.

G, 0.5 mg. anatarox G100, antaron F-C-34, tween 80 or 20, pluronic L-62, or control.

turbidity of dilute suspensions of mitochondria in 0.44 M sucrose plus $6.7 \times 10^{-3}$ M versene, pH 6.2, at which pH the organelles exhibit maximal stability (18, 19). The non-ionic detergents, with the exception of a type containing a nonyl phenoxy group (igepal), did not influence the turbidity of the mitochondrial suspension, even when the period of observation was extended to 30 minutes. The detergent (igepal) containing aromatic groups caused a rapid drop in the optical density of the suspension. A similar drop was noted with anionic detergents. It seems unlikely that the sample of igepal used contained anionic detergent since no titratable acidic groups were present, and besides the non-ionic detergent was purified by a method which would remove traces of these contaminants (see Experimental Section).

In agreement with results of studies (23) on the precipitation of protein by detergents, it was found (Fig. 1) that the ionic detergent dodecyl sulfate was more effective than cetyl sulfate in promoting the swelling of isolated mitochondria. However, little difference was noted between the benzene sulfonates containing alkyl chains of 10 carbons and those containing 12 carbons.

The cationic detergents also caused a decrease in the optical density of the suspension; the salts of pyridine appeared to be slightly more effective in this regard than did the derivatives of ammonia. Study (Fig. 2) of the effects of the ratio of detergent to mitochondria on the optical density of the suspension revealed that the changes in turbidity of the mitochondrial suspension were much more complex with the cationic than with the anionic detergents. As the ratio of anionic detergent to mitochondria increased, the rate of decrease in optical density became progressively greater. On the other hand, as shown in Fig. 2, at low concen-

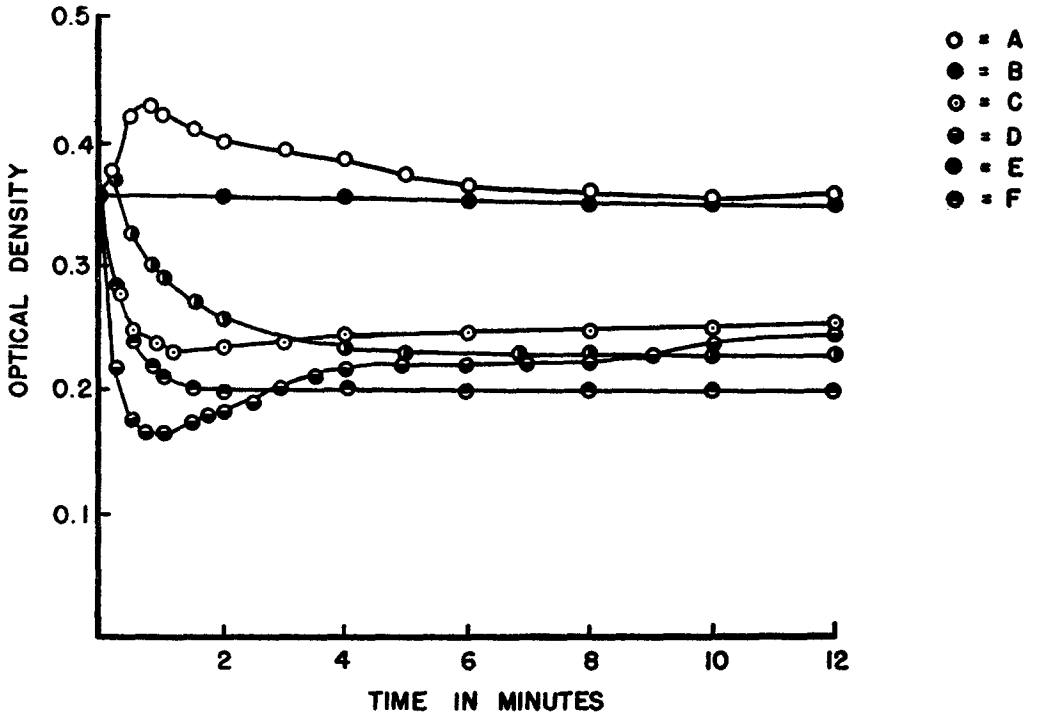


FIG. 2. Effect of concentration of cetyl trimethyl ammonium chloride on the swelling of mitochondria at pH 6.2 in 0.44 M sucrose containing $6.7 \times 10^{-3}$ M versene.

A, $5 \times 10^{-5}$ M cetyl trimethyl ammonium chloride.

B, control.

C, $1.2 \times 10^{-4}$ M cetyl trimethyl ammonium chloride.

D, $9 \times 10^{-5}$ M cetyl trimethyl ammonium chloride.

E, $6 \times 10^{-5}$ M cetyl trimethyl ammonium chloride.

F, $7.5 \times 10^{-5}$ M cetyl trimethyl ammonium chloride.

trations of cetyl trimethyl ammonium chloride the optical density at first rose above, and then declined back to the level of the control. At intermediate concentrations the turbidity immediately fell below the control values. At high concentrations of cetyl trimethyl ammonium chloride the fall in optical density was followed by a rise. These results indicated that the cationic detergent influenced the suspensions of mitochondria in a manner different from that of the anionic detergent.

Examination of the preparation under the phase microscope showed that the mitochondria were agglutinated by the cationic detergents, but swollen and lysed in the presence of the anionic detergents and igepal. Many "ghost" mitochondria could be seen in the presence of the latter two detergents. On the other hand, the complex changes in the optical density observed with the mitochondria treated with cationic detergents were undoubtedly the results of a change in particle size which occurred during agglutination of the mitochondria. At higher concentrations of cetyl trimethyl ammonium chloride and mitochondria (0.1 ml. of mitochondria plus $1 \times 10^{-3}$ M cationic detergent in 0.5 ml. 0.44 M sucrose), the organelles were actually precipitated out of solution and settled to the bottom of the tube.

The effects of the detergents were next investigated at pH 7.4 and at pH 8.0, under which conditions the mitochondria are less stable than at pH 6.2 and hence might be more sensitive to the presence of the surface active compounds (18, 19). Again (Fig. 3), none of the non-ionic detergents, with the exception of igepal which contains the nonylphenoxy group, had an effect on the optical density of the suspensions. Igepal appeared to be somewhat more effective at pH 8.0 than at 6.2, and substitution of versene buffer by tris buffer did not alter these results. Apparently the presence of divalent metals ions is not necessary for the

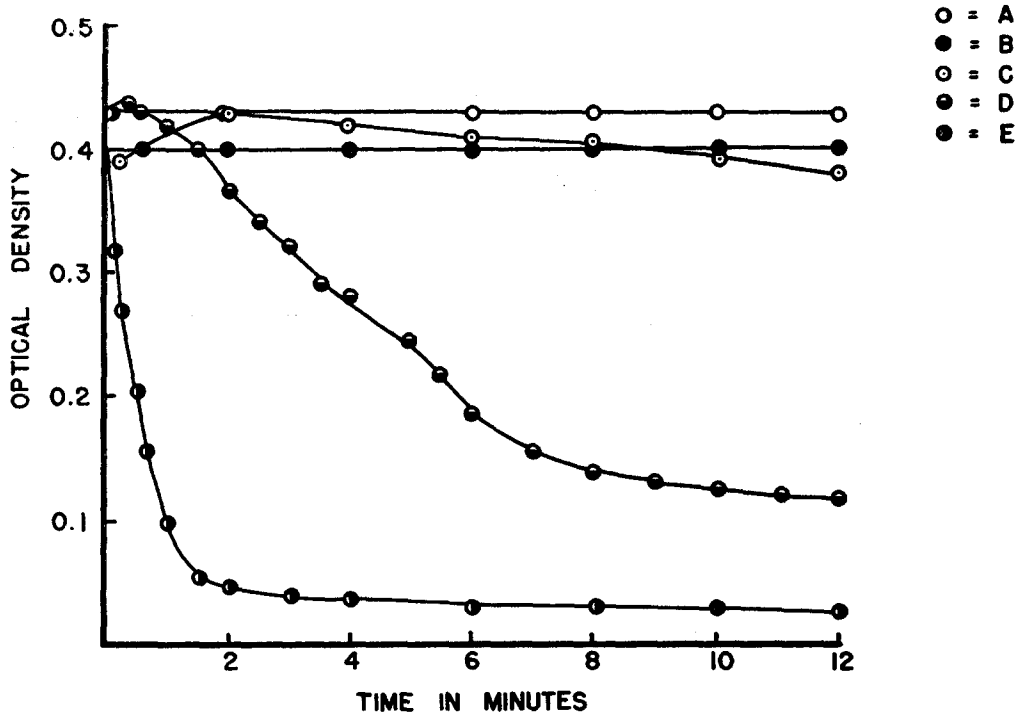


FIG. 3. Effect of pH and versene on the swelling of mitochondria in 0.44 M sucrose containing non-ionic detergents. Tris or versene buffer were $6.7 \times 10^{-3}$ M in concentration.

A, control, 0.5 mg. or 1.0 mg. of anatarox G100, tween 20 or 80, or pluronic L-62 in tris, pH 7.4 or versene, pH 6.2, 7.4, or 8.0.

B, antaron F-C-34, versene, pH 6.2, 7.4, or 8.0; tris pH 7.4.

C, control, 0.5 or 1.0 mg. of antarox G100, anataron F-34, tween 80 or 20, pluronic L-62, tris pH 8.0.

D, 0.2 mg. igepal, versene, pH 6.2.

E, 0.2 mg. igepal, versene or tris, pH 7.4 and pH 8.0.

action of igepal. The cationic detergent cetyl trimethyl ammonium chloride appeared to be more effective at pH 7.4 than at pH 8.0 or pH 6.2 (Fig. 4). In contrast to previous results with anionic detergents (2), Mg did not prevent the action of this cationic detergent. The anionic detergent (Fig. 5) dodecyl sulfate was more effective at pH 6.2 than at pH 8.0, when versene was the only other ionizable compound added. Apparently versene exerted a protective effect at pH 7.4 or 8.0, since the activity of dodecyl sulfate in tris buffer at pH 7.4 or 8.0 was the same as that of the detergent in versene or barbiturate buffer at pH 6.2. In contrast, versene appeared to enhance the destructive power of cetyl trimethyl ammonium chloride (Fig. 4). The protective effect of versene against dodecyl sulfate cannot be due to the complexing of a trace metal, since versene had no effect at lower concentrations or when some of the complexing agent was replaced by another salt such as potassium chloride (Fig. 5). It is suggested as a working hypothesis that versene may compete with the detergent for the same binding site on the mitochondrial surface. These results explain the apparent discrepancy in previous experiments from this laboratory (2) in which versene was found not to have a protective effect against dodecyl sulfate, since in these studies a small amount of versene was used in the presence of another buffer.

Since cationic and anionic detergents form ionic complexes, it was of interest to determine the effect of mixtures of cationic and anionic detergents on the stability of mitochondria. In Fig. 6 it is shown that the mixture of equivalent amounts of dodecyl sulfate and cetyl trimethyl ammonium chloride had virtually none of the ability of the individual detergents to destroy the mitochondria. If dodecyl sulfate was first added to the mito-

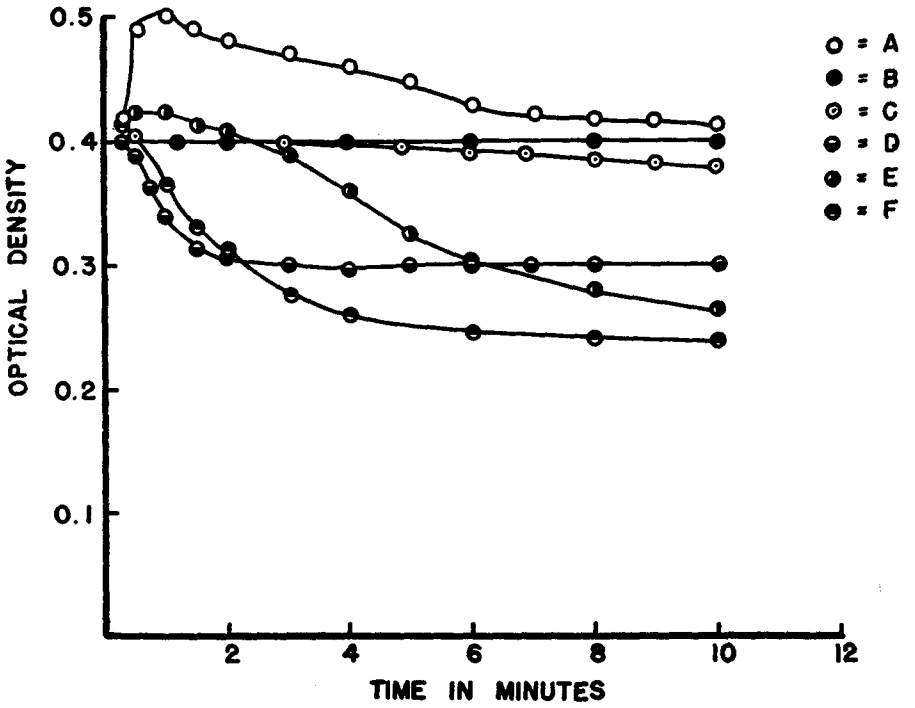


FIG. 4. Effect of pH and versene on the swelling of mitochondria in 0.44 M sucrose containing the cationic detergent cetyl trimethyl ammonium chloride. The concentration of cetyl trimethyl ammonium chloride was $5 \times 10^{-5}$ M and that of tris or versene was $6.7 \times 10^{-3}$ M.

A, cetyl trimethyl ammonium chloride, versene, pH 6.2.

B, control versene, pH 6.2, 7.4, or 8.0.

C, control tris, pH 7.4.

D, cetyl trimethyl ammonium chloride and tris, pH 7.4.

E, cetyl trimethyl ammonium chloride and versene, pH 8.0.

F, cetyl trimethyl ammonium chloride and versene, pH 7.4.

chondria, the subsequent addition of cetyl trimethyl ammonium chloride did not prevent the change in optical density of the mitochondrial suspension. These results indicate that the charged groups on the ionic detergents are important for their activity.

*ATPase Activity:*

The results of the studies of the effects of detergents on Mg- or DNP-ATPase are presented in Table II. The ratio of detergent to mitochondria in these experiments was similar to that in the experiments in which mitochondrial stability was investigated. All of the detergents tested inhibited DNP-ATPase activity, although the cationic detergent cetyl trimethyl ammonium chloride had the least inhibitory action. At low concentrations this cationic detergent by itself activated the ATPase of fresh mitochondria even in the presence of versene, indicating that divalent metal ions were not needed for activation. Cetyl trimethyl ammonium chloride formed a precipitate with ATP; however, this decrease in the concentration of ATP could not explain the inhibition of Mg-ATPase by higher concentrations of the cationic detergent, since only 13 per cent of the ATP was precipitated[2] by the highest concentration of cetyl trimethyl ammonium chloride tested.

The presence of Mg prevented activation by the cationic detergent. On the other hand Mg was required for maximal activation with the anionic detergent dodecyl sulfate and the non-ionic detergent igepal. The enzyme activity cata-

[2] The concentration of ATP was estimated in suitable extracts at 260 mμ in the Beckman spectrophotometer.

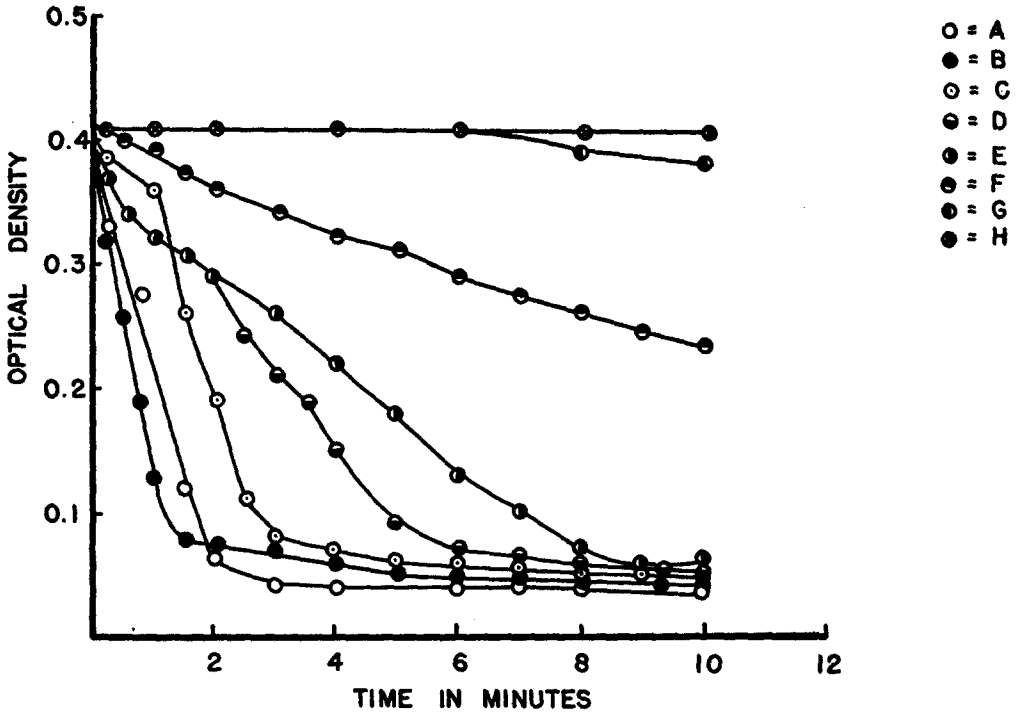


FIG. 5. Effect of pH and buffer on the swelling of mitochondria in 0.44 M sucrose in the presence of the anionic detergent dodecyl sulfate ($9 \times 10^{-5}$ M).

A, dodecyl sulfate and $6.0 \times 10^{-3}$ M versene, pH 6.2.

B, dodecyl sulfate, $3 \times 10^{-3}$ M tris, pH 7.4, and 0.003 M KCl.

C, dodecyl sulfate and $6.0 \times 10^{-3}$ M tris, pH 7.4 or 8.0; or dodecyl sulfate, $6.0 \times 10^{-3}$ M tris, pH 7.4 or 8.0, and $6.0 \times 10^{-4}$ M versene.

D, dodecyl sulfate, $3.0 \times 10^{-3}$ M tris, pH 7.4, and $3 \times 10^{-3}$ M versene, pH 7.4.

E, dodecyl sulfate and $3.0 \times 10^{-2}$ M or $6.0 \times 10^{-3}$ M versene, pH 7.4.

F, dodecyl sulfate and $6.0 \times 10^{-3}$ M versene pH 8.0.

G, control, $6.0 \times 10^{-3}$ M tris, pH 8.0.

H, control, $6.0 \times 10^{-3}$ M tris, pH 7.4, or $6.0 \times 10^{-3}$ M versene pH 6.2, 7.4 or 8.0.

lyzed by each of the latter two detergents was much higher than that catalyzed by the cationic detergent. Other experiments not listed in Table II showed that the activation of Mg-ATPase by dodecyl sulfate could be prevented by prior addition of an equivalent amount of cetyl trimethyl ammonium chloride. Evidently the charged group of the dodecyl sulfate is important for the activation of Mg-ATPase. However, the side chain of the non-ionic detergents was also important, since the non-ionic detergents containing aromatic groups (igepal) but not those containing a sorbitan monofatty acid group (tween) could also activate Mg-ATPase. The failure of tween to activate Mg-ATPase was not due to an inhibitory action of this surface active agent, since the activity of this enzyme was not influenced by the presence of the detergent after activation of Mg-ATPase by freezing and thawing or aging of the mitochondria.

## DISCUSSION

The results indicate that a close correlation exists between the ability of a detergent to activate Mg-ATPase and to promote the swelling and lysis of mitochondria. Other methods of activating Mg-ATPase such as freezing and thawing (16, 17), aging (3, 13–15), or the addition of calcium ions (14) also promote the swelling of mitochondria (17–20). All of these observations support the hypothesis that the activation of Mg-ATPase consists in removing a physical barrier to the union of a Mg-APT complex and the enzyme. This

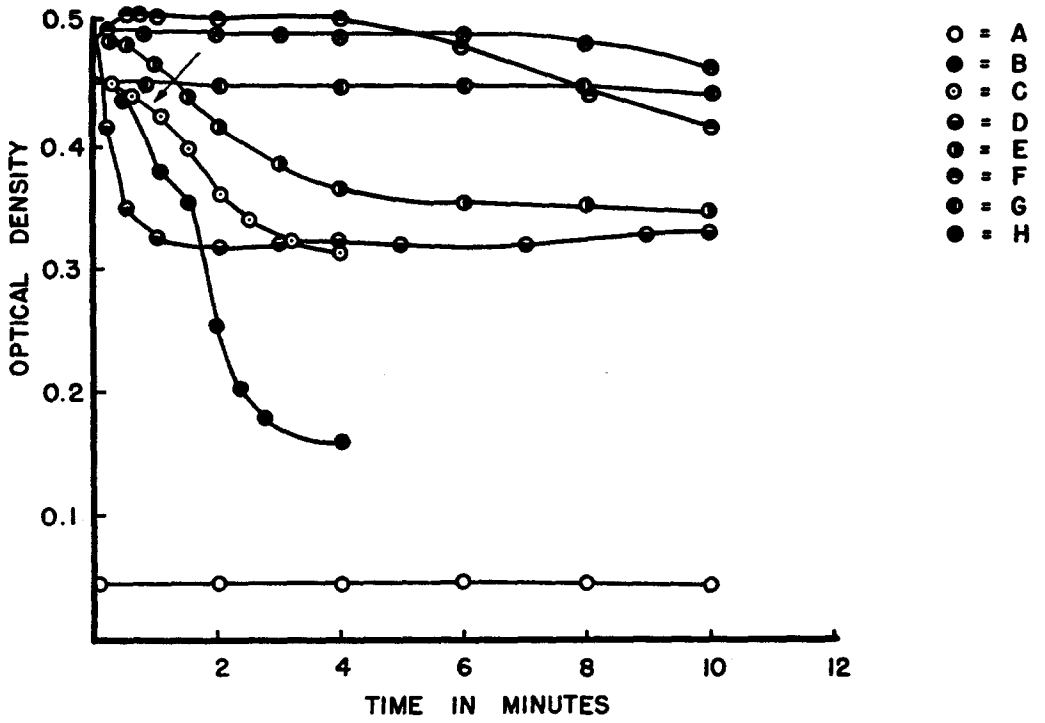


FIG. 6. Effects of the combined addition of dodecyl sulfate and cetyl trimethyl ammonium chloride on the swelling of mitochondria. Each solution contained 0.44 M sucrose and $6.0 \times 10^{-3}$ M tris, pH 7.4.

A, $1.0 \times 10^{-4}$ M cetyl trimethyl ammonium chloride and $1.0 \times 10^{-4}$ M dodecyl sulfate; no mitochondria added.

B, $1 \times 10^{-5}$ M dodecyl sulfate.

C, $1 \times 10^{-4}$ M dodecyl sulfate and then $1 \times 10^{-4}$ M cetyl trimethyl ammonium chloride added at time indicated by arrow.

D, $1 \times 10^{-4}$ M cetyl trimethyl ammonium chloride.

E, $5 \times 10^{-5}$ M cetyl trimethyl ammonium chloride.

F, $5 \times 10^{-5}$ M cetyl trimethyl ammonium chloride, and $1 \times 10^{-4}$ M dodecyl sulfate added before the mitochondria.

G, control.

H, $1 \times 10^{-4}$ M cetyl trimethyl ammonium chloride and $1 \times 10^{-4}$ M dodecyl sulfate added before the mitochondria.

phenomenon may be due either to the removal of an impermeable membrane or to an increase in its permeability.

The results also show that the positive and negative charged centers on the ionic detergents and the type of side chain on the non-ionic detergents are important factors influencing action of these surface active agents on mitochondrial stability and Mg-ATPase. The anionic detergents caused swelling of mitochondria and activated Mg-ATPase; the cationic detergents caused the mitochondria to agglutinate and did not activate Mg-ATPase. Others have also shown that cationic detergents act in a different manner than anionic detergents on biological systems. For example, cationic detergents activate pancreatic lipase, whereas anionic detergents inhibit this enzyme (24).

In accordance with generally accepted theories as to the mode of interaction between protein and detergent (23), it seems likely that in the present experiments the anionic detergent combined with positive centers and the cationic detergent combined with negative centers on the proteins which make up the surface of the mitochondria.

On the other hand, the non-ionic detergents probably did not combine directly with protein (23), but with a lipide which is associated with lipoprotein. Those non-ionic detergents containing the nonyl phenoxy group probably formed inclu-

sion compounds with the lipoproteins of the mitochondrial surface, thereby altering the structure of the lipoprotein and thus the permeability of the mitochondria. It would be of interest to know if the diverse biological effects which have been reported using non-ionic detergents (triton) of the nonyl phenoxy type (7–12) are also dependent on the presence of an aromatic group in the surface active agent.

Apparently, the inhibition of DNP-ATPase by detergents is not related to the type of side chain or charge on the surface active agent, since all detergents tested inactivated this system. These data indicate that a lipoprotein may be necessary for the activity of DNP-ATPase and that the side chains of the various detergents react with the lipide of this protein, thereby altering its properties. The present results also indicate that, in contrast to the data obtained with Mg-ATPase, inhibition of DNP-ATPase is not related to a gross change in morphology of the mitochondria, since inhibition was brought about by all detergents, even those which did not cause swelling of the mitochondria.

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