The Adsorption of Cetyltrimethylammonium Bromide by Bacteria, its Action in Releasing Cellular Constituents and its Bactericidal Effects

By M. R. J. SALTON

Department of Colloid Science, University of Cambridge

SUMMARY: The form of the uptake curve of cetyltrimethylammonium bromide (CTAB) by *Staphylococcus aureus* and *Escherichia coli* is that of an adsorption isotherm. Tested on six different bacteria, the maximum amounts of CTAB adsorbed showed variations from one organism to another.

When cells of *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli* or *Salmonella pullorum* are suspended in water, the ultra-violet spectrum of the supernatant liquid has a maximum absorption at a wave-length of 260 m μ . The height of this maximum is greatly increased when the cells are suspended in CTAB solutions instead of water. Free purines and pyrimidines contribute to this maximum.

There is a parallel relationship between the leakage of 260 m μ -absorbing material, glutamic acid and inorganic phosphorus from two CTAB-treated Gram-positive bacteria, when their release is followed during the initial rapid phase of leakage. There is a similar relationship for *Esch. coli*, although no glutamic acid is released. Release of cell constituents from CTAB-treated *Staph. aureus* continues slowly for some time after the initial process and is accompanied by a decrease in dry weight of the cells and a gradual change from positive to negative in their Gram-staining reaction. The rate of release of cellular constituents was increased by raising the temperature or by treatment with high concentrations of CTAB.

Treatment of suspensions with sufficient CTAB to sterilize them released amounts of cell constituents comparable to those released by placing the cells in boiling water. When smaller amounts of CTAB were used, a quantitative relationship was found between the amount of CTAB present, the proportion of cells killed and the amount of 260 m μ -absorbing material released.

Many surface-active agents are bactericidal and various explanations have been proposed to account for this action. Baker, Harrison & Miller (1941) suggested that detergents disorganize the cell membrane and denature certain proteins essential to metabolism and growth. Kuhn & Bielig (1940) observed that germicidal concentrations of some detergents correspond to concentrations effecting denaturation of proteins. Hotchkiss (1944, 1946) found that treatment of bacterial cells with certain surface-active compounds in bactericidal concentrations released nitrogenous and phosphorus-containing substances. Gale & Taylor (1947) demonstrated that the nitrogenous substances released included the free amino-acids concentrated within the cells of *Strep. faecalis*; Mitchell & Crowe (1947) showed that this action was accompanied by an alteration in the surface structure of the cells, as shown by the electron microscope. By growing *Staph. aureus* and *Pseudomonas fluorescens* on media containing radioactive phosphorus (P^{32}), Salton (1950) showed that there was an increased leakage of P^{32} into the suspending fluid when CTAB was present. The release of purines and pyrimidines from *Staph. aureus* treated with CTAB was reported by Salton & Alexander (1950).

The inhibition of bacterial metabolism by surface-active compounds was interpreted by Hotchkiss (1946) as evidence against the idea that an essential, detergent-sensitive enzyme exists. On the other hand, Knox, Auerbach, Zarudnaya & Spirtes (1949) showed that cell-free preparations of certain enzymes from *Esch. coli* are inhibited at detergent/protein ratios which are bactericidal for the intact cells. They concluded that the specific inhibition of detergent-sensitive enzymes, such as the lactic acid oxidase, could account for metabolic inhibition, cell death and increased permeability observed in bacteria treated with bactericidal amounts of the cationic detergents.

The present communication deals with the adsorption of CTAB by bacteria, the disorganization of their cell membranes, and the subsequent release of certain cellular constituents. Cytological studies of the cellular disorganization were carried out in parallel and are reported by Salton, Horne & Cosslett (1951).

METHODS

Organisms. *Staph. aureus* and *Proteus vulgaris* investigated by Salton (1950); *Strep. faecalis* ST (N.C.T.C. no. 6782); a strain of *Bacillus pumilus* and *Esch. coli* H (kindly given by Dr E. F. Gale); *Salm. pullorum* (kindly given by Dr R. Coombs) were used in these investigations.

Medium, conditions of culture and harvesting. The organisms were grown in a medium consisting of c. 3 % tryptic digest of casein, 0.1 % Marmite and 1 % glucose, at an initial pH 7.4–7.6. Roux bottles of medium inoculated with c. 10 ml. of overnight culture were incubated for 16 hr. at 37°. The cells were harvested from the liquid medium by centrifugation, washed three times with distilled water on the centrifuge and finally suspended in distilled water to give a suspension containing c. 15 mg. dry-weight bacteria/ml.

Dry-weight determinations. The dry weights of the washed bacterial suspensions were determined directly, by drying 1 ml. samples of the dense suspensions to constant weight in an air-oven at 105°.

Adsorption of CTAB. The amount of CTAB adsorbed was determined by adding bacterial suspensions to solutions containing known amounts of CTAB, allowing them to stand at 20° for 15 min., removing the cells by centrifugation and estimating the residual CTAB in the supernatant liquid by the method of Salton & Alexander (1949).

Release of cellular constituents and their estimation in the supernatant fluid. The release of cellular constituents was examined after three types of treatment: (1) cells suspended in distilled water; (2) cells suspended in CTAB solutions; (3) cells pipetted into distilled water at c. 100° and then held at that temperature for 10 min. (referred to subsequently as 'boiled' cells). In each case the final suspension density was c. 1.5 mg. dry-weight cells/ml. Samples from suspensions were centrifuged for 5 min. to remove most of the cells, and the supernatant finally cleared by further centrifugation. The cell-free supernatants were then analysed for released constituents. For the estimation of some constituents (glutamic acid, purines, pyrimidines) it was necessary to concentrate the supernatant liquids by evaporation to dryness *in vacuo* and to re-dissolve the residues in small volumes of distilled water.

The following methods were used in estimating quantities of released cellular constituents:

(a) Ultra-violet spectra were studied by use of the Beckman photoelectric spectrophotometer.

(b) Inorganic phosphorus content was estimated by the method of Fiske & Subbarow (1925). Total phosphorus was determined by the procedure of Umbreit, Burris & Stauffer (1945).

(c) Pentose was determined by the method of Mejbaum (1939). D-Ribose was used as the standard for a calibration curve.

(d) Glutamic acid was estimated by the amino-acid decarboxylase method of Gale (1945).

(e) Purines, pyrimidines and derivatives. Paper chromatography was used for the identification and estimation of the purines, pyrimidines and derivatives in the supernatants from bacterial suspensions. These were concentrated and volumes of not more than 0.1 ml. then placed on Whatman filter-paper No. 1, according to the method of Hotchkiss (1948). Of the solvent systems tried, *n*-butanol saturated with water and 5 % (v/v) formic acid gave the best separations. Chromatograms were run with guanine, adenine, uracil and cytosine as markers. The locations of substances on the chromatograms were found by the method of Markham & Smith (1949). For quantitative estimations, the areas of filter-paper corresponding with spots on the developed chromatogram were cut out, extracted with 4 ml. distilled water and estimated at neutral pH in the Beckman spectrophotometer. Filter-paper blanks were obtained by cutting out adjacent areas of equal size as recommended by Hotchkiss (1948). A check on recovery was obtained by running known amounts of purines and pyrimidines and extracting from the chromatograms.

Plate-count estimation of surviving cells. Samples of bacterial suspensions (1.0 ml.) were withdrawn at intervals and transferred to 99 ml. sterile tap water. Suitable dilutions were then plated out, in duplicate, in the growth medium containing 2 % agar and the colonies counted after 48 hr. incubation at 37°.

RESULTS

Adsorption of CTAB by bacteria

The amount of CTAB taken up by *Staph. aureus* and *Esch. coli* is expressed in Fig. 1 as a function of the CTAB concentration in the supernatant. The results suggest typical adsorption isotherms. Similar curves were obtained with *Strep. faecalis*, *B. pumilus*, *Salm. pullorum* and *Pr. vulgaris*. Marked agglutination of the suspensions was observed in the region of maximum CTAB uptake. The level of maximum CTAB adsorption varies somewhat from one organism to another. Table 1 shows maximum amounts of CTAB adsorbed and the weight ratios of bacteria:adsorbed CTAB, for a number of bacterial species.

Release of ultra-violet absorbing material and identification of some of its components

An examination of the ultra-violet spectra of supernatants from suspensions of *Staph. aureus* in distilled water and solutions containing 90 µg. CTAB/ml.,

Table 1. *Comparison of maximum amounts of cetyltrimethylammonium bromide adsorbed by various bacteria*

Organism	CTAB adsorbed (μ g. CTAB/mg. dry-wt. bacteria)	Weight ratio Bacteria:CTAB
<i>Staph. aureus</i>	320	3:1
<i>Strep. faecalis</i>	430	2.3:1
<i>B. pumilus</i>	600	1.7:1
<i>Esch. coli</i>	420	2.4:1
<i>Salm. pullorum</i>	340	3:1
<i>Pr. vulgaris</i>	355	2.8:1

revealed a maximum absorption at a wave-length of 260 $m\mu$. A marked increase in the 260 $m\mu$. absorption of the supernatant from CTAB-treated cells was observed. Fig. 2 shows the ultra-violet spectra of these supernatant fluids. Spectra of supernatants from untreated and CTAB-treated *Strep. faecalis*, *Esch. coli* and *Salm. pullorum* also showed maxima at 260 $m\mu$., with marked increases in the 260 $m\mu$. absorption when cells had been treated with 90 μ g. CTAB/ml. The absorption of the supernatants at 260 $m\mu$. obeyed Beer-Lambert's Law up to *c.* 1.2 optical density units.

Paper chromatography of concentrated supernatants from untreated and CTAB-treated suspensions of *Staph. aureus* showed the presence of a number of components which contribute to the ultra-violet maximum at 260 $m\mu$. Guanine, adenine and uracil were found in the supernatants from untreated suspensions, while guanine, adenine, uracil and adenine riboside were the main components in the supernatant from the CTAB-treated cells. The identification of the free bases guanine, adenine and uracil was confirmed by comparing the spectra of eluted supernatant spots with the spectra of recovered markers and standard solutions of the appropriate bases. The presence of adenine riboside was established by elution from the chromatogram, hydrolysis in N-HCl for 1 hr. and running the hydrolysate on a chromatogram; only one spot, corresponding to adenine, then appeared on the chromatogram. Quantitative data on the release of adenine and uracil which appeared to be present in greater amounts than the other components are given below.

Release of 260 m μ .-absorbing material relative to other cellular constituents

The release of glutamic acid (known to be concentrated as the free amino-acid in the internal environment of Gram-positive bacteria; Taylor, 1947) was followed concurrently with the appearance of 260 $m\mu$.-absorbing material and inorganic P. The parallel relationship between the release at 20° of all three cellular constituents from *Staph. aureus* treated with 90 μ g. CTAB/ml. is shown in Fig. 3. Similar leakage curves were also found with CTAB-treated *Strep. faecalis* and *Esch. coli*, although no glutamic acid was released from the latter organism. Untreated suspensions of the three bacteria showed a steady release of 260 $m\mu$.-absorbing material, but there appeared to be no significant leakage of inorganic P and glutamic acid. The data for the leakage from untreated and

Fig. 1. Adsorption of CTAB by *Staph. aureus* and *Esch. coli* plotted as a function of the CTAB concentration in the supernatant fluid.

Fig. 2. Ultra-violet spectra of supernatants from *Staph. aureus* suspensions (1.5 mg. dry-wt. cells/ml.) held at 20°. ×——×, distilled water: curve 1, initial sample; curve 2, 180 min. ●——●, treated with 90 μg. CTAB/ml.; curve 3, 5 min. exposure; curve 4, 180 min. exposure.

CTAB-treated suspensions of the three organisms are shown in Table 2. Both untreated and treated cells of *Staph. aureus* and *Strep. faecalis* stained Gram-positive throughout the course of the experiment.

Table 2. *Release of cellular constituents from untreated and CTAB-treated bacteria at 20°*

A, 260 m μ -absorbing material log [I_0/I]. B, inorganic phosphorus (μ g./mg. dry wt. cells). C, glutamic acid (μ g./mg. dry wt. cells)

Organism		Time (min.)						
		0	5	10	30	60	120	180
		Quantities of cellular constituents released						
<i>Staph. aureus</i> *								
Untreated	A	0.05	—	—	0.07	0.10	0.14	0.19
CTAB-treated,	A	—	0.30	0.36	0.52	0.71	0.86	0.90
90µg./ml.	B	—	0.9	1.3	2.3	3.0	3.4	3.6
	C	—	6.1	7.7	12.6	15.6	—	19.2
<i>Strep. faecalis</i> *								
Untreated	A	0.05	—	—	0.06	0.07	—	0.10
CTAB-treated,	A	—	.50	—	0.59	0.65	—	0.73
45µg./ml.	B	—	3.1	—	3.3	3.4	—	3.7
	C	—	9.6	—	11.2	11.9	—	14.0
<i>Esch. coli</i> †								
Untreated	A	0.07	—	—	0.09	0.10	0.12	0.14
CTAB-treated,	A	—	0.30	0.34	0.42	0.49	0.57	0.62
90µg./ml.	B	—	0.5	0.6	0.8	1.0	1.2	1.3

* Dry wt., 1.5 mg./ml. suspension.

† Dry wt., 1.4 mg./ml. suspension.

Effect of temperature on the release of cellular constituents from Staphylococcus aureus

When *Staph. aureus* cells were treated with 90 μ g. CTAB/ml. at 0, 20 and 30°, appreciable differences in the rates of release of cellular constituents were observed. Plate-count estimations of survivors revealed 99.99 % killing in 5 min. at both 20 and 30°. The initial rates of release of 260 m μ -absorbing material and inorganic P from CTAB-treated cells were determined over periods of 200, 40 and 20 min. at 1, 20 and 30° respectively. Control rates of release of 260 m μ -absorbing material from untreated cells were determined over 180 min. at the three temperatures. The data are summarized in Table 3.

Comparison of the amounts of cellular constituents released from CTAB-treated cells with those from 'boiled' cells

On exposure of cells of the three organisms *Staph. aureus*, *Strep. faecalis* and *Esch. coli* to 90 μ g. CTAB/ml. at 20° for 180 min., virtually all of the cells were killed (99.99 % mortality). Under these conditions, the amounts of constituents released into the suspending fluid were observed to approach maxima (e.g. Fig. 3 for *Staph. aureus*). Analyses of supernatants from CTAB-treated bacteria revealed quantities of cellular constituents comparable to the amounts released when suspensions were pipetted into water held in a boiling water-bath. The

Table 3. *Influence of temperature on the rate of release of cellular constituents from Staph. aureus*

Temp. (° C.)	Rate of release of constituents		
	Control, 260 m μ - absorbing material*	Treated with 90 μ g. CTAB/ml.	
		260 m μ - absorbing material*	Inorganic phosphorus†
0	0.085	0.880	0.005
20	0.551	7.615	0.056
30	0.744	18.950	0.122

* Rate expressed as $10^3 \times \log I_0/I$ /mg. dry wt. bacteria/min.† Rate expressed as μ g. phosphorus/mg. dry wt. bacteria/min.Table 4. *A comparison of the quantities of cellular constituents released from CTAB-treated bacteria with those from 'boiled' cells*A, untreated suspensions held for 180 min. at 20°. B, suspensions treated with 90 μ g. CTAB/ml. for 180 min. at 20°. C, 'boiled' cells.

Constituents estimated	<i>Staph. aureus</i>			<i>Strep. faecalis</i>			<i>Esch. coli</i>		
	A	B	C	A	B	C	A	B	C
	μ g. released constituent/mg. dry wt. bacteria								
Inorganic phosphorus	0	3.6	3.5	0	3.6	4.1	0	1.1	1.0
Total phosphorus	0	5.4	5.5	0	5.1	5.2	0	1.9	3.3
Pentose	0	4.9	6.6	0	7.3	7.7	0	4.8	13.0
Glutamic acid	0	26.6	27.8	0	10.7	11.2	0	0	0
Adenine									
(a) Unhydrolysed supernatant	0.4	1.8	—	—	—	—	—	—	—
(b) Hydrolysed supernatant, N-HCl, 1 hr. at 100°	—	2.4	2.8	—	—	—	—	—	—
Uracil	0.6	1.4	0.9	—	—	—	—	—	—

amounts of adenine and uracil contributing to the leakage of 260 m μ -absorbing material from untreated suspensions of *Staph. aureus* were estimated. The data are shown in Table 4.

Effect of CTAB concentration on the release of 260 m μ -absorbing material

The amounts of 260 m μ -absorbing material appearing in the suspending fluids were estimated for a range of CTAB concentrations from 22.5 to 900 μ g./ml. Samples were taken after 5, 30 and 180 min. exposure at 20° to the CTAB solutions. At least four estimations were made for each CTAB concentration and sampling-time. Fig. 4 shows the results obtained with *Staph. aureus* and illustrates those obtained also with *Strep. faecalis* and *Esch. coli*; the complete data are recorded in Table 5.

The data show that leakage of 260 m μ -absorbing material was a slower process for *Staph. aureus* and *Esch. coli* than for *Strep. faecalis* when the CTAB

Table 5. *Effect of CTAB concentration on the release of 260 m μ -absorbing material from various organisms*

CTAB concentration (μ g./ml.)	<i>Staph. aureus</i>			<i>Strep. faecalis</i>			<i>Esch. coli</i>		
	5 min.	30 min.	180 min.	5 min.	30 min.	180 min.	5 min.	30 min.	180 min.
	260 m μ -absorbing material released (log (I_0/I)/mg. dry wt. cells)								
22.5	0.017	0.035	0.182	0.182	0.220	0.300	0.036	—	—
45	0.036	0.090	0.420	0.344	0.378	0.435	0.069	0.096	0.124
90	0.120	0.325	0.450	0.440	0.445	0.465	0.178	0.228	0.325
135	0.250	0.448	0.450	0.454	0.456	0.457	0.231	—	—
180	0.355	0.453	—	0.460	0.456	0.453	0.272	0.321	0.323
270	0.430	0.450	0.450	0.455	0.456	0.453	0.281	0.325	0.325
450	0.375	0.400	0.424	0.403	0.410	0.414	0.257	0.325	> 0.500
630	0.345	0.370	0.447	0.356	0.358	0.377	0.326	0.384	> 0.500
900	0.438	0.480	0.504	0.356	0.375	0.400	0.336	0.440	> 0.500

concentrations were below *c.* 200 μ g./ml. With *Strep. faecalis* the release was virtually complete in 5 min. for CTAB concentrations between 90 and 270 μ g./ml. As the CTAB concentration was increased above 270 μ g./ml., the 260 m μ -absorbing material appearing in the supernatants from the three bacteria fell to a minimum and rose again. These discontinuities appeared in the region of maximum CTAB uptake by the cells. To test several possibilities which may account for the discontinuities, *Staph. aureus* was treated with 270 μ g. CTAB/ml. for 30 min. Under these conditions most of the CTAB was adsorbed, concentrations of residual CTAB were found to be less than 10 μ g./ml. and the release of 260 m μ -absorbing material had ceased (see Fig. 4). When CTAB was added to give final concentrations in the supernatant of 100–500 μ g. CTAB/ml., no change in the optical density or absorption maximum could be detected. Further, the addition of CTAB to supernatants showed no traces of turbidity or precipitate formation. The addition of more CTAB to suspensions previously treated with 270 μ g. CTAB/ml. resulted in a 5 % decrease in the amount of 260 m μ -absorbing material found in the supernatant. Adsorption of some of the 260 m μ -absorbing material on the CTAB-saturated cells, would therefore partly account for the fall to a minimum shown in Fig. 4.

Relationship between bactericidal effects, amount of CTAB and release of 260 m μ -absorbing material

The bactericidal activity of CTAB was followed concomitantly with the release of 260 m μ -absorbing material from suspensions of *Staph. aureus*, *Strep. faecalis*, *Esch. coli* and *Salm. pullorum*. Samples for estimating surviving bacteria and 260 m μ -absorbing material released were taken after 5 and 30 min. exposure to CTAB at 20°. Estimations of viable cells from untreated suspensions held for 30 min. at 20° showed no differences from the initial populations. The bactericidal effects of 22.5 and 45 μ g. CTAB/ml. on known weights of the four organisms, together with the amounts of 260 m μ -absorbing material released, are shown in Table 6.

Fig. 3. The course of release of cellular constituents from *Staph. aureus* (1.5 mg. dry-wt. cells/ml.) suspensions at 20°, the amount of constituents appearing in the supernatant being plotted against time. $\times$ — $\times$, 260 $m\mu$ -absorbing material; $\odot$ — $\odot$, glutamic acid; $\triangle$ — $\triangle$, inorganic P; A, cells suspended in water; B, cells suspended in a solution containing 90 μ g. CTAB/ml.

Fig. 4. Effect of CTAB concentration on the release of 260 $m\mu$ -absorbing material from *Staph. aureus* at 20°. The amount of 260 $m\mu$ -absorbing material released into the suspending fluid was estimated for: $\times$ — $\times$, 5 min. exposure; $\odot$ — $\odot$, 30 min. exposure; $\triangle$ — $\triangle$, 180 min. exposure.

Table 6. *Relationship between percentage cells killed, amount of CTAB and release of 260 m μ .-absorbing material at 20°*

Organism	Dry-wt. bacteria (mg./ml.)		22.5 μ g. CTAB/ml.		45 μ g. CTAB/ml.	
			5 min. exposure	30 min. exposure	5 min. exposure	30 min. exposure
<i>Staph. aureus</i>	1.8	Killed (%)	24	35	50	62
		260 m μ . material released (log I_0/I)	0.036	0.061	0.071	0.116
<i>Strep. faecalis</i>	1.5	Killed (%)	43	55	85	97
		260 m μ . material released (log I_0/I)	0.255	0.315	0.508	0.571
<i>Esch. coli</i>	1.4	Killed (%)	16	28	31	57
		260 m μ . material released (log I_0/I)	0.049	0.069	0.094	0.137
<i>Salm. pullorum</i>	1.5	Killed (%)	26	31	54	62
		260 m μ . material released (log I_0/I)	0.156	0.175	0.314	0.347

Autolysis initiated by CTAB treatment

When the appearance of cellular constituents in the suspending fluid was followed over an extended period, autolytic breakdown of the cells did contribute to the amount of materials found in the supernatants, as suggested by the data of Hotchkiss (1944, 1946). The appearance of 260 m μ .-absorbing material and pentose in the suspending fluid from *Staph. aureus* treated with 90 μ g. CTAB/ml. at 30° was followed together with the Gram-stain reaction of the cells and changes in the dry weight of centrifuge-deposited untreated and CTAB-treated bacteria (i.e. cells freed from the supernatant fluid). These measurements are recorded in Table 7.

The results show an initial leakage process which is completed in 3–4 hr. This is then followed by a slower release of constituents, accompanied by a gradual change in staining from Gram-positive to Gram-negative and a progressive decrease in dry-cell mass. The results with this organism therefore appear to differ from those of Hotchkiss (1946) in that autolytic breakdown of the cells does not appear to contribute significantly to the amounts of cellular constituents released within the first few hours of exposure of the cells to CTAB.

DISCUSSION

Bacterial cells have a high affinity for CTAB molecules. For the six organisms studied, the amount of CTAB adsorbed per cell, at saturation levels, was found to be more than the amount corresponding to a closely packed monolayer of the detergent. CTAB molecules occupy an area of *c.* 45 Å.² (Friend & Schulman, private communication). The amount of CTAB taken up at saturation of *Staph. aureus* would, therefore, occupy an area *c.* 15 times the cell surface area (assuming a sphere of 1 μ . in diameter). The corresponding ratio for *B. pumilus* is

Table 7. Release of cell constituents, changes in dry weight and Gram-stain of untreated and CTAB-treated suspensions of *Staph. aureus* held at 30°

A, untreated cells. B, cells treated with 90 µg. CTAB/ml.

Time	260 mµ.-material log [I ₀ /I]		Pentose (µg./ml.)		Dry wt. (mg./10 ml. sample)		Gram stain (approx. % Gram- positive)	
	A	B	A	B	A	B	A	B
0 min.	0.12	0.12	0	0	19.7	19.7	100	100
5 min.	—	0.38	—	—	—	19.4	100	100
10 min.	—	0.45	—	3.2	—	19.5	100	100
20 min.	—	0.64	—	4.2	—	18.5	100	100
40 min.	—	0.96	—	6.3	—	18.3	100	100
60 min.	0.26	1.12	0.9	8.1	19.6	17.5	100	100
120 min.	0.40	1.31	1.0	9.0	19.2	16.6	100	100
180 min.	0.56	1.42	—	9.8	—	16.8	100	100
4 hr.	0.70	1.44	—	9.7	18.9	16.3	100	100
6 hr.	0.94	1.54	—	—	—	15.9	100	90
8 hr.	—	1.64	—	12.3	—	15.6	100	75
10 hr.	1.30	1.76	2.0	—	18.4	15.3	100	50
12 hr.	—	1.84	—	—	18.6	15.1	100	25
18 hr.	1.57	1.92	2.2	15.0	—	15.1	100	10
22 hr.	—	—	—	21.0	18.4	14.8	100	0
28 hr.	—	2.40	—	24.3	—	18.3	100	0

c. 18 times the cell-surface area, assuming cell dimensions of 0.8 µ. width, 3 µ. length. This suggests that CTAB is adsorbed at sites additional to those available at the cell surface, if it be all adsorbed as a monolayer. Alexander & McMullen (1949) and McQuillen (1950) also suggested the possibility of CTAB adsorption at sites within the bacterial cytoplasm. Valko & Diblee (1944) reported that adsorption of dimethyldodecylbenzylammonium chloride by *Esch. coli* corresponded to an equivalent weight of the bacterium of c. 600, an equivalent weight about the same as that of many animal proteins. It would seem, therefore, that both surface and internal components of the bacterial cell are capable of titrating with cationic detergents. McQuillen (1950) suggested the possibility of some detergent being held as concentric shells around the bacterial cell, but his electrophoresis data afforded no confirmation of this. However, the agglutination of bacterial suspensions at CTAB-saturation levels indicates that the surface of the cells has become hydrophobic, a phenomenon analogous to the agglutination of red blood cells with tannic acid, observed by Schulman & Rideal (1937).

As Anson (1939) pointed out, any study of killing and lysis of bacteria by detergents should include a series of concentrations in order to determine the chain of reactions that occur in the organism. From the work reported here, it is possible to appreciate something of the course of events when bacteria are exposed to the cationic detergent CTAB. On exposure of various species to bactericidal amounts of CTAB below that required for 99.99 % killing, the leakage of 260 mµ.-absorbing material in 5 min. is proportional to the percentage of cells killed. These observations are in agreement with the quantitative relationship between the proportion of cells killed by different amounts of

tyrocidin and release of amino-acids reported by Gale & Taylor (1947). When cell suspensions are treated with sufficient CTAB (c. 90 μ g. CTAB/1.5 mg. dry-weight cells) to kill 99.99 % of the cells within 5 min., the rapidity with which cellular constituents appear in the suspending fluid varies from one organism to another. Where the release of cellular constituents was found to be relatively slow (e.g. 3-4 hr. with *Staph. aureus*) temperature affected the rate of leakage of materials into the suspending fluid. Cellular constituents were also more rapidly released when suspensions were treated with amounts of CTAB above those exhibiting maximum killing.

The results reported here support the view that there is a primary process of cell-solute release on treatment of bacteria with CTAB. Autolytic breakdown of the cells does not appear to make a significant contribution to the materials found in the suspending fluid during this initial leakage. This conclusion is supported by the following experimental observations: (1) the parallel relationship between the release of free amino-acid, inorganic P, purines and pyrimidines, etc.; (2) the amounts of cellular constituents released from bacteria exposed to 90 μ g. CTAB/ml. for 180 min. at 20° were comparable to the amounts released from 'boiled' cells; (3) a change in the Gram-staining of *Staph. aureus* was not apparent until several hours after the initial leakage of cell solutes (Table 7). However, a study of cell constituents released when suspensions were treated with 90 μ g. CTAB/ml. and held for periods of more than 3-4 hr. or treated with CTAB concentrations near and above the level required for cell saturation, suggested that processes such as autolytic breakdown, adsorption of released materials on to CTAB-saturated cells and solubilization of cell materials in detergent micelles were involved. Autolysis of bacteria associated with treatment of cells in low and high concentrations of surface-active agents has been well established in previous studies. This phenomenon was reviewed by Dubos (1945), and Hotchkiss (1946) has contributed further information. The possibility of physical solubilization of cellular materials in bacteria+detergent studies has been suggested by the work of Stacey (1949).

That treatment of bacteria and yeasts with detergents results in cellular disorganization is further supported by cytological evidence of cytoplasmic shrinkage, cell rupture, etc. (Dyar, 1947; Mitchell & Crowe, 1947; Meisel & Umanskaya, 1949; Salton *et al.* 1951).

Knox *et al.* (1949) believe that specific inhibition of a detergent-sensitive enzyme, such as the lactic acid oxidase of *Esch. coli*, can account for the metabolic inhibition, cell death and increased permeability observed in bacteria treated with cationic detergents. It does not seem to be necessary to invoke the inhibition of a detergent-sensitive enzyme as being the primary cause of cell death. Indeed, if there were a chain of reactions initiated by a primary metabolic inhibition, a greater time lag before the observation of secondary effects such as the release of cellular constituents might well be expected. However, it appears that leakage of cell solutes, inhibition of sensitive enzymes and cytological damage can all be observed simultaneously with cell death. The interaction between detergent molecules and cell components (e.g.

proteins, enzymes, lipo-proteins etc.) as such, could account for cell death, leakage of solutes, etc. Studies of the penetration of protein and lipo-protein mono-layers by surface-active compounds, discussed by Schulman (1951), appear to have some relevance to the action of these agents on the permeability barrier of bacteria. It would seem that there is little evidence which disagrees with the original hypothesis of cell membrane disorganization proposed by Baker *et al.* (1941). However, it is yet too early to speculate on the primary site or sites of detergent action. A clearer picture will undoubtedly emerge when more is known of the chemical nature of the surface components and outer envelope of the bacterial cell.

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