THE BACTERICIDAL PROPERTIES OF CERTAIN CATIONIC DETERGENTS

By M. R. J. SALTON*

[*Manuscript received August* 5, 1949]

Summary

Using the F.D.A. method of testing germicides, the bactericidal properties of two cationic detergents, "Cetavlon" and "Fixanol C," were tested against ten organisms at seven pH levels between 5.2 and 8.2. The results for the two detergents were similar. Gram-positive organisms were more susceptible than Gram-negative. There was considerable variation in the relationship between susceptibility and pH. *Staph. aureus, Staph. albus, Strep. faecalis,* and *Proteus vulgaris* were all most suceptible under slightly alkaline conditions; whereas *Ps. fluorescens, Ps. pyocyanea, Achromobacter liquefaciens,* and *Bacterium coli* were most susceptible under slightly acid conditions. The susceptibility of *Corynebacterium equi* was unaffected by pH within the range studied.

One strain each of *Staph. aureus* and of *Ps. fluorescens* were studied in more detail. Plate count disinfection studies confirmed the greater rate of destruction of *Staph. aureus* at pH 8.1, and of *Ps. fluorescens* at pH 5.3. Neither of two anionic detergents reversed the bactericidal action of "Cetavlon." For both types of cells the adsorption of detergent was greatest at pH 8.2. Likewise the inhibition of oxygen uptake showed no marked relationship with mortality. Treatment with detergent caused an increased loss of phosphorus-bearing compounds from both types of cells, but the data are insufficient to show whether susceptibility to the detergent was related to this leakage from the cells.

I. INTRODUCTION

The bactericidal properties of synthetic detergents have been the subject of numerous investigations since Domagk (1935) reported that the cationic detergent "Zephiran" possessed germicidal activity. Factors of molecular structure, such as the length of the alkyl chain and the nature of the anion central atom, have been found to influence the bactericidal activity, and this work has been reviewed by Glassman (1948). The influence of pH on the germicidal efficiency of both cationic and anionic detergents has been reported in many investigations (Gershenfeld and Perlstein 1941*a*, 1941*b*; Gershenfeld and Milanick 1941; Baker, Harrison, and Miller 1941*a*, 1941*b*; Hoogerheide 1945; Quisno and Foter 1946; Eisman and Mayer 1947).

It has been generally accepted from these investigations that, as one departs from a neutral pH, the cationic detergents become more effective with increasing alkalinity, whereas the reverse is true for the anionic detergents.

* Division of Food Preservation and Transport, C.S.I.R.O., Homebush, N.S.W.

However, Quisno and Foter (1946) reported that cetylpyridinium chloride is germicidal in high dilutions under acid as well as alkaline conditions and maintains an even level of bactericidal activity over the pH range 2 to 10. Apart from this finding with cetylpyridinium chloride, there would appear to be a similarity between the cationic and anionic detergents on the one hand, and the basic and acid dyes on the other in their pH-bactericidal relationships. Stearn and Stearn (1924) showed that an increase of pH favoured the disinfecting power of basic dyes, whilst a converse relationship was found with acid dyes.

Baker, Harrison, and Miller (1941*a*) examined the effect of cationic and anionic detergents on the metabolism of Gram-positive and Gram-negative bacteria and found that respiratory and glycolytic activity were markedly reduced. These authors found that inhibition of oxygen uptake was greatest at pH 8.0. In a later work, Baker, Harrison, and Miller (1941*b*) concluded that inhibition of metabolism and destruction of the bacteria were roughly parallel. As Hotchkiss (1946) pointed out, the regularity of this relationship was impaired by frequent observations of the lack of bactericidal activity under the same conditions which markedly inhibited respiration.

The observations that the Gram-negative organism *Pseudomonas fluorescens* was more susceptible to the bactericidal activity of a cationic detergent under acid than under alkaline conditions suggested the possibility that other organisms may also differ in their pH-bactericidal relationships. This led to the present study in which adsorption of detergent, inhibition of respiration, and loss of cellular phosphorus have each been studied in relation to the antibacterial activity at various levels of pH.

II. Experimental Methods

The two cationic detergents used in these investigations were "Cetavlon" (cetyltrimethylammonium bromide) and "Fixanol C" (cetylpyridinium bromide) manufactured by Imperial Chemical Industries. "Cetavlon" was in the form of a white crystalline powder, and "Fixanol C" was a brown paste. Determination of the content of cation-active agent in both compounds was done by titration based on the assay described by Auerbach (1943). Both "Cetavlon" and "Fixanol C" contained approx. 75 per cent. cation-active agent.

The following organisms were used in the initial studies in the effect of pH on the bactericidal properties of "Cetavlon" and "Fixanol C" determined by the United States Food and Drug Administration method.

Gram-positive organisms: *Staphylococcus aureus, Staphylococcus albus, Streptococcus faecalis, Corynebacterium equi;* Gram-negative organisms: *Pseudomonas fluorescens* H 137, *Pseudomonas fluorescens* 542, *Pseudomonas pyocyanea, Bacterium coli, Proteus vulgaris.*

Throughout this study of the bactericidal properties of cationic detergents M/15 disodium hydrogen phosphate-potassium dihydrogen phosphate buffers have been used, and will be referred to subsequently as 'phosphate buffer.'

(*a*) *Food and Drug Administration Method*

The Food and Drug Administration method, described by Ruehle and Brewer (1931), was used in determining the minimum concentration of detergent which produced sterilization of 22-26 hr. cultures in 10 min., but not in 5 min.

The following technique was used: 5.0 ml. of each concentration of detergent was sterilized in phosphate buffer adjusted to seven levels of pH ranging from pH 5.2 to 8.2. To each tube was added 0.5 ml. of a 22-26 hr. culture of the test organisms in F.D.A. broth (containing 0.3 per cent. "Difco" beef extract and 0.5 per cent. "Bacto-Peptone"). At intervals of 5, 10, and 15 min. one loopful (from a standard loop 4 mm. internal diameter) of approx. 0.01 ml. volume was transferred to fresh broth. After incubation for a period of 48-72 hr. the tubes were examined for turbidity and the minimum concentrations showing sterilization in 10 min. and not in 5 min. were recorded. All experiments were performed at 30°C.

For some organisms plate counts of the 22-26 hr. F.D.A. broth cultures were made.

(*b*) *Rate of Disinfection*

A study of the rate of disinfection of the two organisms, *Staph. aureus* and *Ps. fluorescens* 542, by "Cetavlon" was made at four levels of pH. For these studies the organisms were grown on fresh meat agar (medium prepared from minced ox heart infusion 500 g., peptone 15 g., sodium chloride 5 g., agar 10 g. (from Davis Gelatine (N.Z.) Ltd., Christchurch, N.Z.), made up to 1 litre, the pH adjusted to 7.6-7.8), cultures being incubated at 30°C. for 18 hr. Cells were harvested by washing from the surface of the agar with saline and centrifuging. Two washings in saline followed and the cells were finally resuspended in saline.

Phosphate buffer (M/15) adjusted to levels of pH 5.3, 6.2, 7.2, and 8.2, containing "Cetavlon" to give a final concentration of 100 μg./ml., was sterilized prior to inoculation with the bacterial suspension of which 1.0 ml. was added to 9.0 ml. of the "Cetavlon" solutions. Samples of 1.0 ml. were withdrawn at various intervals and transferred to 99 ml. of saline. Dilutions for the determination of the numbers of survivors were plated out in brain heart infusion agar and counted after 48-72 hr. at 30°C. All experiments on the rates of disinfection were performed at 30°C.

(*c*) *Effect of Anionic Detergents on Treated Cells*

Several experiments with *Ps. fluorescens* 542 were carried out to test the effect of the two anionic compounds, "Aerosol OT" and sodium cetyl sulphate, on the numbers of organisms surviving 15 min. exposure to "Cetavlon" at 30°C. Suspensions of *Ps. fluorescens* 542 were prepared as for the disinfection rate studies. In one experiment, samples of bacteria were pipetted into 99 ml. saline containing 2 μg./ml. "Aerosol OT," after having been exposed to solutions containing 100 and 200 μg. "Cetavlon"/ml. Dilutions were plated on brain heart infusion agar. In another experiment, dilutions of bacteria exposed to "Cetavlon" (100 μg./ml.) for 15 min. at 30°C. were plated out in brain heart infusion agar containing 10 and 100 μg./ml. of "Aerosol OT" and sodium cetyl sulphate. For both experiments "Cetavlon" was in phosphate buffer at pH 7.

(*d*) *Adsorption*

Determinations of the amount of "Cetavlon" adsorbed by the two organisms, *Staph. aureus* and *Ps. fluorescens* 542, were made using the colorimetric assay of quaternary ammonium salts described by Auerbach (1944).

Organisms were grown on fresh meat agar and harvested after 18 hr. incubation at 30°C., washed twice, and resuspended in saline, as for the disinfection rate studies. 1.0 ml. of bacterial suspension was added to 9.0 ml. of "Cetavlon" in phosphate buffer, to give final concentrations of 100, 200, 400, and 800 μg. "Cetavlon"/ml. After 15 min. exposure to "Cetavlon" at 30°C., samples were taken for estimations of the numbers of survivors. The bacteria-"Cetavlon" mixture was then transferred to centrifuge tubes, the cells removed, and estimations of the residual quaternary ammonium salt in the supernatant were made. These experiments were performed at pH levels of 5.2, 6.2, 7.2, and 8.2.

This method of measuring the adsorption of quaternary ammonium compounds by suspensions of bacteria is apparently similar to the method used by Valko and Dibblee (unpublished data) referred to by Valko (1946).

(*e*) *Inhibition of Respiration*

Inhibition of oxygen uptake by suspensions of *Staph. aureus* and *Ps. fluorescens* 542 was measured manometrically. Organisms were grown on fresh meat agar, harvested after 18 hr. incubation at 30°C., washed twice, and resuspended in saline.

The manometric technique was essentially the same as that used by Baker, Harrison, and Miller (1941*a*) in their studies on the action of synthetic detergents on bacterial metabolism. Phosphate buffer adjusted to pH levels of 5.2, 6.2, 7.2, and 8.2 was used; 1.7 ml. of buffer being added to each Warburg flask. 0.1 ml. of 0.6M glucose solution (making final concentration of glucose 0.02M), 1.0 ml. bacterial suspension, 0.2 ml. "Cetavlon" in phosphate buffer (in side-arm), and 0.3 ml. of 20 per cent. (w/v) KOH solution (centre well) were added to each flask. Two flasks for each treatment were prepared. Oxygen uptake at 30°C. was followed over a period of one hour, readings being recorded at 10 min. intervals.

At the conclusion of the manometric observations, suspensions from the two flasks were pooled and samples were taken for the estimation of the number of surviving bacteria.

(*f*) *Loss of Phosphorus-containing Compounds from Treated Cells*

The bacteria were grown on fresh meat agar to which was added radioactive phosphorus (P^{32}) in the form of H_3PO_4. Cells were harvested after 18 hr. incubation at 30°C., washed twice, and resuspended in saline as for previous studies.

The amount of P^{32} taken up by the cells was determined by drying (at 100°C.) suitable aliquots of the suspension on discs and recording Geiger-Mueller counts. The loss of P^{32} from bacterial cells exposed to a "Cetavlon" concentration of 100 μg./ml. at 30°C. for 15 min. was determined by recording Geiger-Mueller counts on aliquots of filtrate obtained by removing the cells with sintered glass filters. These observations were made with phosphate buffer at pH levels of 5.2 and 8.2 in the presence and absence of "Cetavlon." Five counts per aliquot were made with the Geiger-Mueller counter, the aliquots being adjusted, where possible, to give counts of the order of 300/min.

The numbers of survivors were again estimated at the end of the 15 min. exposure period, the samples being withdrawn just prior to filtration.

III. Results

(a) *Food and Drug Administration Method*

The results for ten organisms are shown in Figures 1 and 2 for "Cetavlon" and "Fixanol C" respectively. Each curve is drawn through seven points, each of which represents the arithmetic mean of not less than four determinations of the concentration of detergent which resulted in sterile sub-cultures after ten, but not after five, minutes exposure at the pH specified. Although the points often fall on a reasonably smooth curve, irregularities characteristic of such "end-point" determinations were recorded frequently. In this respect the experience is similar to that reported by Quisno and Foter (1946) and Klarmann and Wright (1946).

Table 1

PLATE COUNTS OF ORGANISMS GROWN IN F.D.A. BROTH

Organism	No. of Estimates	Mean Population/ml. of Culture
Gram-positive:		
Staph. aureus	11	4.3×10^8
Strep. faecalis	9	2.4×10^8
Corynebacterium equi	10	2.7×10^6
Gram-negative:		
Ps. fluorescens 542	11	5.4×10^8
Ps. pyocyanea	10	1.0×10^9
Proteus vulgaris	9	6.8×10^8
Achromobacter liquefaciens	13	6.9×10^8

Approximate estimates of the numbers of each organism added to each tube of disinfectant were obtained by plate counts of the cultures used as inoculum. The average values for the plate counts are shown in Table 1. As

0.5 ml. of broth was added to 5 ml. of detergent solution, and the sample for the test of sterilization was approximately 0.01 ml., the initial number of viable cells per loop sample is approximately 1/1000 of the plate counts listed. It is apparent, therefore, that under F.D.A. test conditions the initial number of viable cells will vary from one organism to another, and that differences as great as 100-fold may be recorded.

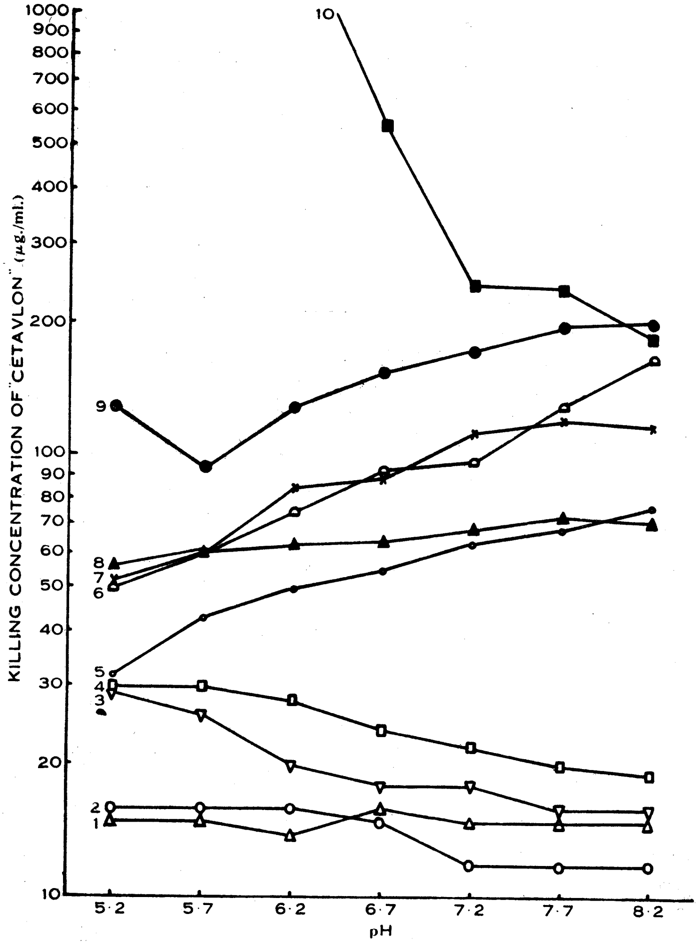

Fig. 1.—F.D.A. killing concentrations of "Cetavlon" for ten bacteria. 1. *Corynebacterium equi;* 2. *Streptococcus faecalis;* 3. *Straphylococcus albus;* 4. *Staphylococcus aureus;* 5. *Pseudomonas fluorescens* H137; 6. *Pseudomonas fluorescens* 542; 7. *Achromobacter liquefaciens;* 8. *Bacterium coli;* 9. *Pseudomonas pyocyanea;* 10. *Proteus vulgaris.*

Figures 1 and 2 indicate a marked similarity between the two compounds tested, and some substantial differences between the organisms. For both compounds the Gram-positive organisms are generally more susceptible than Gram-negative organisms, a finding which confirms earlier reports. The susceptibilities of the various organisms also show considerable differences in relation to pH. *Staph. aureus, Staph. albus, Strep. faecalis,* and *Proteus vulgaris* are more susceptible under slightly alkaline than under slightly acid conditions, such results being in accord with the findings of Gershenfeld and Milanick (1941), and Gershenfeld and Perlstein (1941*a*, 1941*b*) for *Staph. aureus.* For *Ps. fluorescens, Ps. pyocyanea, A. liquefaciens,* and *Bact. coli* a converse relationship applies, and the organisms are more susceptible in a

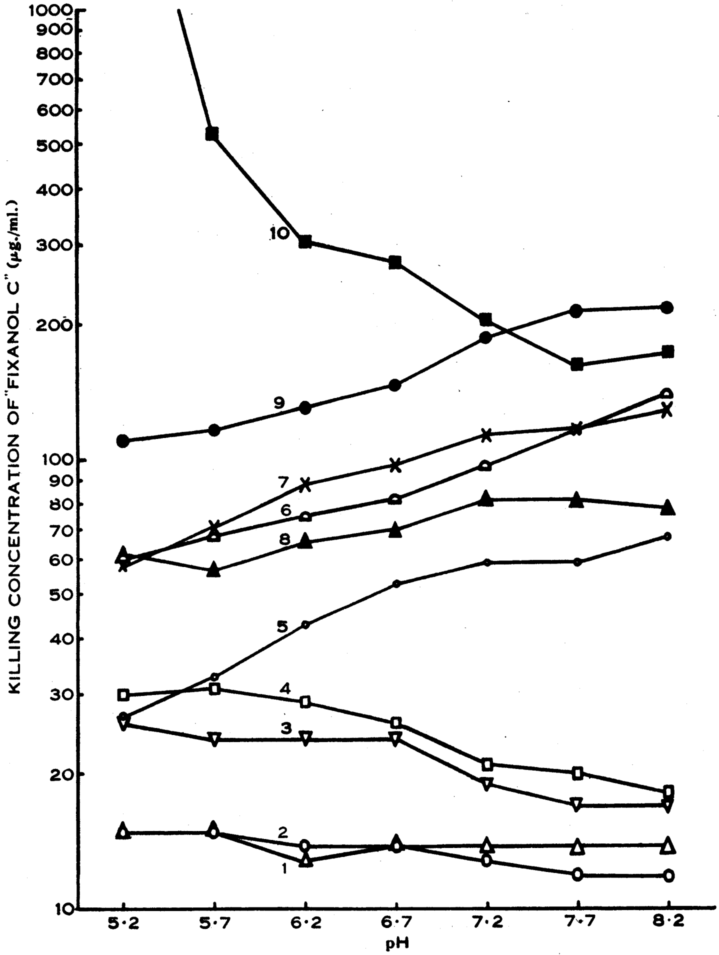

Fig. 2.—F.D.A. killing concentrations of "Fixanol C" for ten bacteria. Symbols as for Figure 1.

slightly acid environment. Although Quisno and Foter (1946) report that the bactericidal activity of cetylpyridinium chloride against *Staph. aureus* and *E. typhosa* was virtually unaffected by pH in the range 2 to 10, there appear to be no reports of increased bactericidal effects under slightly acid conditions. No significant change in the susceptibility of *Corynebacterium equi* is apparent over the pH range studied.

(*b*) *Rate of Disinfection*

The principal aim of studying the rate of disinfection was to determine whether the pH-susceptibility relationships indicated by the F.D.A. method were revealed also by plate-count estimates of survivors after various times of exposure. The experiments were carried out, therefore, with two organisms (*Staph. aureus* and *Ps. fluorescens* 542) showing, by the F.D.A. technique, different susceptibility-pH relationships. Estimates of survivors were made when the initial populations were between 10^9 and 10^{10} cells/ml., and also with smaller initial populations between 10^7 and 10^8/ml. Figures 3 and 4 show the results for *Staph. aureus* and *Ps. fluorescens* 542 respectively, each point representing the average of four determinations for the higher initial population and two for the lower.

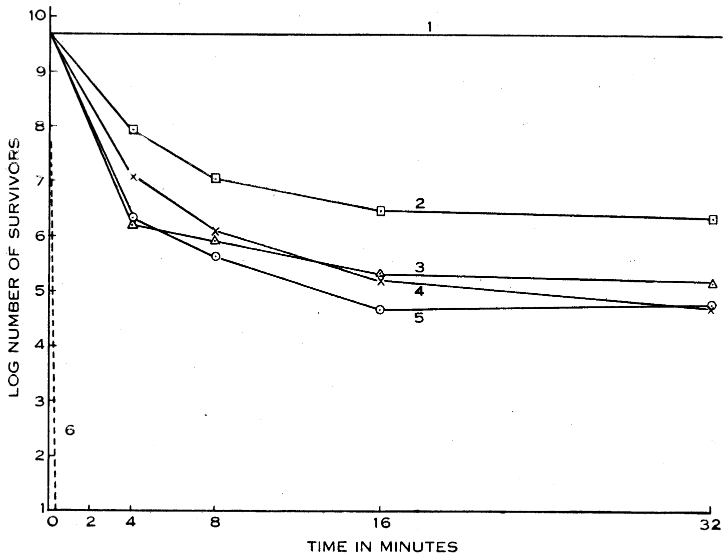

Fig. 3.—Disinfection of *Staph. aureus* by 100 μg./ml. of "Cetavlon." 1. Controls (no "Cetavlon") at four pH levels; 2. "Cetavlon" at pH 5.3; 3. "Cetavlon" at pH 7.2; 4. "Cetavlon" at pH 6.2; 5. "Cetavlon" at pH 8.1; 6. Initial number of cells $10^{7.7}$/ml. "Cetavlon" at all four pH levels.

Figure 3 shows that for an initial population of $10^{9.7}$/ml. the rate of decrease of the survivors is least at pH 5.3, with little, if any, difference between the rates at the three higher pH levels. When the logarithms of the numbers of survivors were plotted against the cube root of the exposure time, significant linear regressions were obtained, the slope of the curve being significantly less at pH 5.3 than at pH 6.2, 7.2, and 8.1. It will be noted that the rate of death gradually falls, and the population of survivors becomes virtually constant after 16 minutes. This decline in the rate of death is no doubt due to the fact that this concentration of cells is sufficient to adsorb almost all the detergent from solution during the first few minutes (see Sub-section III(*d*) below). When the initial number of cells was reduced to approximately $10^{7.7}$/ml. the number of survivors was less than 100/ml. after 30 seconds exposure at all four pH levels. This greater rate of death is undoubtedly due to the fact that the actual concentration of detergent would be almost unaffected by adsorption.

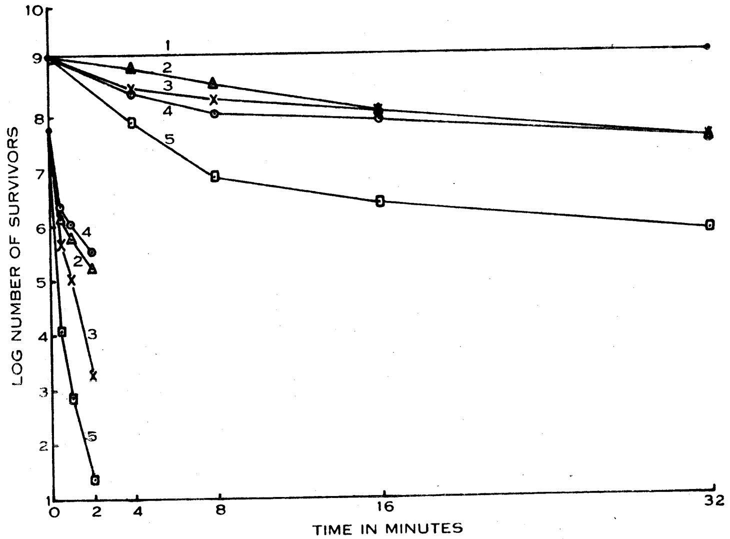

Fig. 4.—Disinfection of *Ps. fluorescens* 542 by 100 µg./ml. of "Cetavlon." 1. Controls (no "Cetavlon") at four pH levels; 2. pH 7.2; 3. pH 6.2; 4. pH 8.0; 5. pH 5.3.

In Figure 4 the data for *Ps. fluorescens* 542 show that the greatest rate of death occurs at pH 5.3 for initial populations of 10^9 and $10^{7.7}$/ml. For the higher initial population adsorption of most of the detergent again resulted in rates of death which decrease substantially with increasing time of exposure. Linear regressions were obtained when the logarithms of the numbers of survivors were plotted against the square root of the exposure time, the slope of the curve being significantly greater at pH 5.3 than at the three higher pH levels. When the initial number of cells was reduced to $10^{7.7}$/ml. the rate of death was substantially increased at all pH levels, that at pH 5.3 being greater than at pH 6.2, and this in turn is greater than the rates at pH 7.2 and 8.0.

These results, therefore, provided independent confirmation of the two types of pH-susceptibility relationships indicated by the F.D.A. method.

(*c*) *Effect of Anionic Detergents on Treated Cells*

Several experiments with *Ps. fluorescens* 542 were performed to investigate the possible reversal of bactericidal action by anionic detergents, as had been found for *Staph. aureus* by Valko and Dubois (1944). For cells exposed at pH 7 to "Cetavlon" (100 μg./ml.), resulting in from 1 to 10 per cent. survivors after 15 min., dilution in a solution containing sufficient "Aerosol OT" to neutralize the "Cetavlon" was without effect on the number of survivors. Similarly, the incorporation of both "Aerosol OT" and sodium cetyl sulphate in brain heart agar (10 and 100 μg./ml.) had no detectable influence on the number of survivors. In a single experiment prior exposure to "Aerosol OT" afforded no protection to cells subsequently treated with "Cetavlon." Under these conditions, therefore, there is no indication that the anionic detergents will reverse the bactericidal action of "Cetavlon."

(*d*) *Adsorption of Detergent by Bacteria*

Measurement of adsorption of "Cetavlon" by *Ps. fluorescens* 542 after 15, 30, and 45 min. showed that adsorption was virtually complete in 15 min. Estimates of the amounts adsorbed within shorter periods were not feasible with the technique used.

The results for the adsorption of "Cetavlon" by *Staph. aureus* and *Ps. fluorescens* 542, using total cell populations of 3-5 x 10^9/ml. at four levels of pH are shown in Figures 5 and 6. From inspection of these adsorption isotherms it is evident that adsorption is proportional to the concentration and independent of pH up to approx. 200 μg./ml. initial concentration. As the

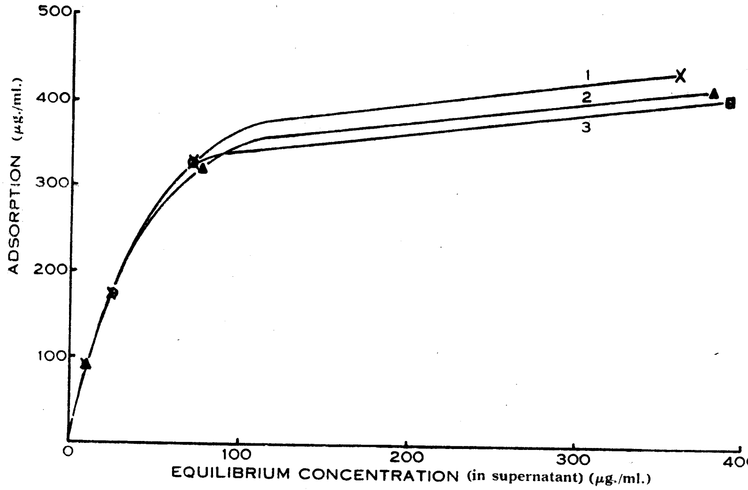

Fig. 5.—Adsorption of "Cetavlon" by approximately 5 x 10^9 cells of *Staph. aureus* at four levels of pH. 1. pH 8.2; 2. pH 7.2; 3. pH 6.2 and 5.2.

concentration is increased, maximum adsorption is achieved, the level of which is dependent on the pH. For both organisms the greatest capacity for detergent is manifest at pH 8.2. At this pH, *Staph. aureus* has adsorbed 1.3 x 10^8 molecules/cell and *Ps. fluorescens* 542, 2.2 x 10^8 molecules/cell. These amounts are rather more than the approximations of detergent per bacterial cell corresponding to a monolayer. It is of interest to note that McMullen and

Alexander (1949) found the amount of "Aerosol OT" adsorbed by *Staph. aureus* at the highest concentration studied corresponded to about one mono-

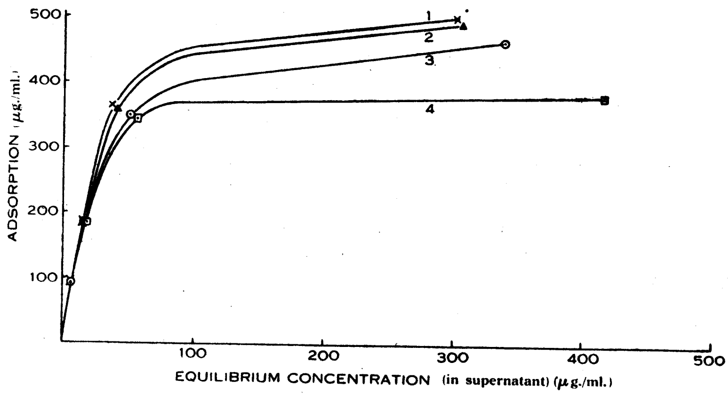

Fig. 6.—Adsorption of "Cetavlon" by approximately 3 x 10^9 cells of *Ps. fluorescens* 542 at four levels of pH. 1. pH 8.2; 2. pH 7.2; 3. pH 6.2; 4. pH 5.2.

layer. Parallel determinations were made on the same suspension of both adsorption and mortality after exposure for 15 min. at each of four concentrations of "Cetavlon" and four levels of pH. The average results for two experiments are shown in Table 2 for *Staph. aureus* and in Table 3 for *Ps. fluorescens* 542.

TABLE 2

INFLUENCE OF pH ON THE NUMBER OF SURVIVORS AND ADSORPTION OF "CETAVLON" BY *STAPH. AUREUS*

	Concentration of Added "Cetavlon" (μg./ml.)								
	0	100		200		400		800	
pH	Log p*	Log p	Adsorption†	Log p	Adsorption	Log p	Adsorption	Log p	Adsorption
5.2	9.7	8.3	90	6.5	176	<2.0	328	<2.0	410
6.2	9.6	6.4	89	5.8	176	"	328	"	410
7.2	9.5	5.2	90	3.7	174	"	323	"	420
8.2	9.6	4.7	92	3.7	175	"	328	"	440

* = log. of plate count/ml., mean of two experiments.
† = amount adsorbed in μg./ml., mean of two experiments.

Although the amount of detergent adsorbed by the cells of both organisms is virtually independent of pH at the lower concentrations of 100 and 200 μg./ml., the numbers of survivors are dependent on pH. Table 2 shows that the disinfection of *Staph. aureus* by both 100 and 200 μg./ml. has been most effective at pH 8.2 and least effective at pH 5.2. At the higher concentrations of detergent less than 100 survivors/ml. were recorded at all pH levels. On the other hand, Table 3 shows that the greatest destruction of *Ps. fluorescens* 542 occurred at pH 5.2 at each of the four concentrations tested.

It is evident, then, that the bactericidal activity of "Cetavlon" at various levels of pH is not correlated with the capacity of the cells to adsorb the detergent from solution.

TABLE 3

INFLUENCE OF pH ON THE NUMBER OF SURVIVORS AND ADSORPTION OF "CETAVLON" BY *PS. FLUORESCENS* 542

	Concentration of Added "Cetavlon" (μg./ml.)								
	0	100		200		400		800	
pH	Log p*	Log p	Adsorption†	Log p	Adsorption	Log p	Adsorption	Log p	Adsorption
5.2	9.3	7.2	94	5.1	182	4.3	345	<2.0	385
6.2	9.4	7.3	94	5.9	183	5.1	350	4.1	463
7.2	9.4	8.3	94	7.8	185	5.5	360	3.0	495
8.2	9.2	8.7	95	7.5	187	5.2	364	2.5	500

* = log. of plate count/ml., mean of two experiments.
† = amount adsorbed in μg./ml., mean of four experiments.

(e) *Effect of Detergent on Respiration*

The relationship between inhibition of oxygen uptake (using glucose as substrate) and disinfection was studied at pH levels of 5.2, 6.2, 7.2, and 8.2. Figures 7 and 8 show the control and inhibited rates of oxygen uptake for *Staph. aureus* and *Ps. fluorescens* 542 respectively.

At the end of the manometric observations, samples were taken for the estimation of the numbers of survivors. The average results for two experiments are shown in Table 4.

TABLE 4

THE NUMBERS OF SURVIVORS AFTER 60 MINUTES EXPOSURE TO "CETAVLON" IN MANOMETRIC EXPERIMENTS

	Concentration of "Cetavlon"	Log Number of Survivors			
Organism	(μg./ml.)	pH 5.2	pH 6.2	pH 7.2	pH 8.2
Staph. aureus	0	9.70	9.73	9.66	9.71
	17	9.64	9.69	9.72	9.70
	25*	9.72	9.64	9.67	9.62
	33	8.97	8.93	8.80	9.05
Ps. fluorescens 542	0	8.71	8.84	8.76	8.80
	17	8.48	8.67	8.72	8.78
	33	7.26	8.40	8.55	8.18

* Result for one experiment only; log no. survivors for control was 9.74.

It is apparent from Figure 7 that pH has had little effect on the control and inhibited rates of oxygen uptake by *Staph. aureus* and there is no marked relationship between inhibition of respiration and destruction of the bacteria. These results with "Cetavlon" differ from those reported by Baker, Harrison, and Miller (1941*a*) for "Zephiran" and "Emulsol 606." These authors found that for these two detergents inhibition of oxygen uptake by *Staph. aureus* was greatest at pH 8.0.

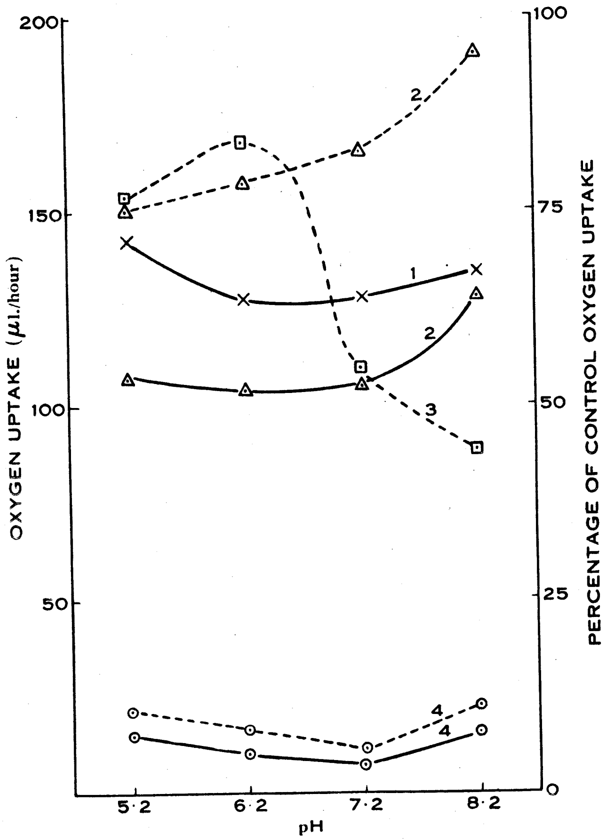

Fig. 7.—Effect of "Cetavlon" on oxygen uptake by *Staph. aureus*. Oxygen consumed in µl./hour: 1. No "Cetavlon"; 2. 17 µg./ml. "Cetavlon"; 3. 25 µg./ml. "Cetavlon"; 4. 33 µg./ml. "Cetavlon." Oxygen consumed as percentage of control is shown by the broken lines.

Figure 8 shows that pH has a marked effect on the control rate of oxygen uptake of *Ps. fluorescens* 542. For this organism the greatest inhibition of respiration by "Cetavlon" occurred at pH 5.2 and this coincided with greatest bactericidal activity. However, at the three higher pH levels there is no obvious correlation between respiration inhibition and mortality.

(*f*) *Loss of Phosphorus-containing Compounds from Cells*

The loss of phosphorus-containing compounds from cells treated with "Cetavlon," and estimates of cells surviving exposure to the detergent are shown in Table 5. For both organisms treatment with "Cetavlon" has increased the loss of phosphorus from the cells, although the amount released was only a small fraction of the original phosphorus content of the cells. The data are, however, insufficient to show whether the pH-susceptibility relationships are correlated with leakage of phosphorus from the treated cells.

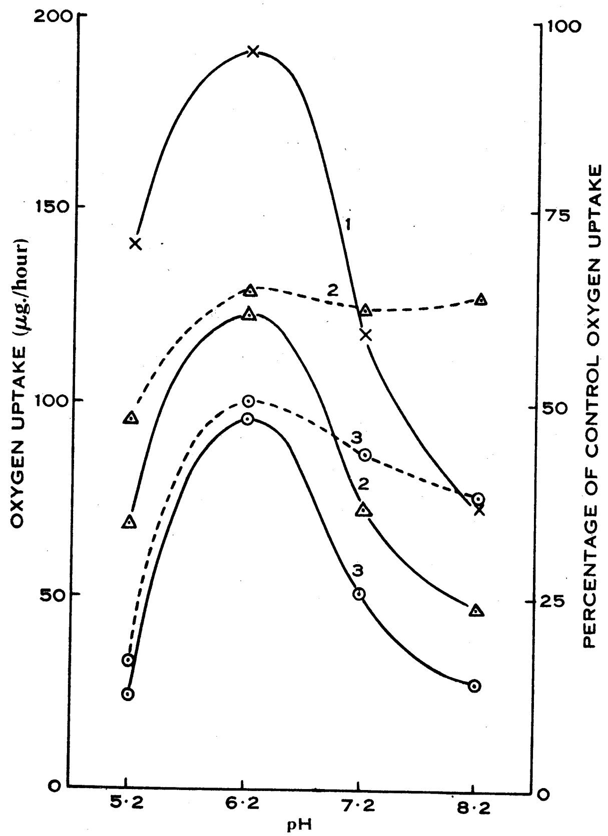

Fig. 8.—Effect of "Cetavlon" on oxygen uptake by *Ps. fluorescens* 542. 1. No "Cetavlon"; 2. 17 µg./ml. "Cetavlon"; 3. 33 µg./ml. "Cetavlon." Oxygen consumed as percentage of control is shown by the broken lines.

IV. Discussion

Consideration of the results of Quisno and Foter (1946), and of those reported here shows that all bacteria do not show an enhanced susceptibility to cationic detergents when exposed under alkaline, rather than acid, conditions. Indeed, it appears that for some organisms the converse relationship exists. In the light of these facts it is difficult to propose a theory which would explain the mode of action of these surface active compounds.

The bacteriostatic activity of basic dyes increases with pH along with an increased adsorption of dye by the bacterial cell (Stearn and Stearn 1924). Valko and Dubois (1944) have suggested that the cationic detergents act in a like manner, and it is true that the capacity of the bacterial cell to combine with "Cetavlon" increases as the pH is raised from 5 to 8. The bactericidal effects of "Cetavlon" are, however, marked at low concentrations where adsorption is apparently independent of pH, and, as shown in this paper, the anti-bacterial effects do change with pH, and in a direction which is dependent on the type of bacteria. It seems then that the anti-bacterial action of these detergents cannot be attributed simply to combination with oppositely charged groups on the bacterial surface.

TABLE 5

LOSS OF PHOSPHORUS COMPOUNDS AND NUMBERS OF SURVIVORS OF "CETAVLON"-TREATED CELLS*

		pH 5.2 "Cetavlon" (μg./ml.)				pH 8.2 "Cetavlon" (μg./ml.)			
		0		100		0		100	
Organism	Expt. No.	P^{32}	Log p†	P^{32}	Log p	P^{32}	Log p	P^{32}	Log p
Staph. aureus	2	0.8	9.7	5.6	6.7	6.4	9.6	7.3	4.7
	3	0.8	9.5	9.3	6.1	6.8	9.4	14.5	4.1
Ps. fluorescens 542	1	5.7	9.0	17.2	6.4	2.2	9.1	9.3	8.0
	3	2.6	9.1	8.2	6.6	4.2	9.1	8.5	8.0

* P^{32} released from organisms expressed as a percentage of the P^{32} content of the cells.
† p = plate count/ml. after 15 minutes exposure at 30°C.

The adsorption of detergent as measured chemically, provides no evidence regarding the distribution of the compound between the various types of centres on the bacterial surface. The possibility exists then that combination with one particular type of centre may be of vital importance to the cell. It is unlikely that any such group of vital importance is directly concerned with respiratory activity as the inhibition of oxygen uptake at various pH levels has shown no clear relation with bactericidal effects. Hotchkiss (1946) has also interpreted the evidence of Baker, Harrison, and Miller (1941*a*) as being against the hypothesis that a key enzyme is especially sensitive to the detergent.

It has been shown that, in bactericidal concentrations, the detergents promote the leakage of cellular constituents, including nitrogen and phosphorus compounds (Hotchkiss 1944) and amino acids (Gale and Taylor 1947). Although these authors used Gram-positive organisms only, it is possible that Gram-negative organisms are affected in a similar way. Further investigation of the nature of the cytolytic damage suffered by both Gram-positive and Gram-negative cells would seem to be desirable, and may yet lead to an understanding of the varied pH-bactericidal relationships.

V. ACKNOWLEDGMENTS

The author wishes to thank Mr. G. Ferris for the statistical treatment of the data on rates of disinfection, and Dr. W. P. Rogers for his advice and the loan of facilities used in the studies with radioactive phosphorus.

VI. References

AUERBACH, M. E. (1943).—Germicidal quaternary ammonium salts in dilute solution. *Industr. Engng. Chem.* (Anal. Ed.) **15**: 492-3.

AUERBACH, M. E. (1944).—Colorimetric assay of quaternary ammonium salts. *Industr. Engng. Chem.* (Anal. Ed.) **16**: 739.

BAKER, Z., HARRISON, R. W., and MILLER, B. F. (1941*a*).—Action of synthetic detergents on the metabolism of bacteria. *J. Exp. Med.* **73**: 249-71.

BAKER, Z., HARRISON, R. W., and MILLER, B. F. (1941*b*).—The bactericidal action of synthetic detergents. *J. Exp. Med.* **74**: 611-20.

DOMAGK, G. (1935).—Eine neue Klass von Desinfektionsmitteln. *Dtsch. Med. Wschr.* **61**: 829-32.

EISMAN, P. C., and MAYER, R. L. (1947).—The antibacterial properties of phenoxy-ethyl-dimethyl-dodecyl-ammonium bromide (PDDB). *J. Bact.* **54**: 668-9.

GALE, E. F., and TAYLOR, E. S. (1947).—The assimilation of amino-acids by bacteria. 2. The action of tyrocidin and some detergent substances in releasing amino-acids from the internal environment of *Streptococcus faecalis*. *J. Gen. Microbiol.* **1**: 77-84.

GERSHENFELD, L., and MILANICK, V. E. (1941).—Bactericidal and bacteriostatic properties of surface tension depressants. *Amer. J. Pharm.* **113**: 306-26.

GERSHENFELD, L., and PERLSTEIN, D. (1941*a*).—Significance of H-ion concentration in the evaluation of the bactericidal efficiency of surface tension depressants. *Amer. J. Pharm.* **113**: 89-92.

GERSHENFELD L., and PERLSTEIN, D. (1941*b*).—The effect of Aerosol OT and H-ion concentration on the bactericidal efficiency of antiseptics. *Amer. J. Pharm.* **113**: 237-55.

GLASSMAN, H. N. (1948).—Surface active agents and their application in bacteriology. *Bact. Rev.* **12**: 105-48.

HOOGERHEIDE, J. C. (1945).—The germicidal properties of certain quaternary ammonium salts with special reference to cetyl trimethyl ammonium bromide. *J. Bact.* **49**: 277-89.

HOTCHKISS, R. D. (1944).—Gramicidin, tyrocidine, and tyrothricin. *Adv. Enzymol.* **4**: 153-99.

HOTCHKISS, R. D. (1946).—The nature of the bactericidal action of surface active agents. *Ann. N.Y. Acad. Sci.* **46**: 479-93.

KLARMANN, E. G., and WRIGHT, E. S. (1946).—An inquiry into the germicidal performance of quaternary ammonium disinfectants. *Soap Sanit. Chem.* **22**: (1) 125-37.

MCMULLEN, A. I., and ALEXANDER, A. E. (1949).—Faraday Society Discussion "Recent advances in surface chemistry" (in press).

QUISNO, R., and FOTER, M. J. (1946).—Cetyl pyridinium chloride. I. Germicidal properties. *J. Bact.* **52**: 111-7.

RUEHLE, G. L. A., and BREWER, C. M. (1931).—U.S. Food and Drug Administration methods of testing antiseptics and disinfectants. U.S. Dep. Agric., Circular No. 198.

STEARN, E. W., and STEARN, A. E. (1924).—The chemical mechanism of bacterial behaviour. 1. Behaviour towards dyes; factors controlling the Gram reaction; 2. A new theory of the Gram reaction; 3. The problem of bacteriostasis. *J. Bact.* **9**: 463-509.

VALKO, E. I. (1946).—Surface active agents in biology and medicine. *Ann. N.Y. Acad. Sci.* **46**: 451-78.

VALKO, E. I., and DUBOIS, A. S. (1944).—The antibacterial action of surface active cations. *J. Bact.* **47**: 15-25.