TABLE IV. Effects of Iodinated Casein on Coenzyme Q Content of Liver Homogenates from Chickens Kept at 70° and 85°F.

Ambient temp	Iodinated casein (% of diet)	Coenzyme Q* Male	Female
70°	.00	77 (35)	76 (30)
"	.05	102 (10)	84 (13)
"	.10	120 (20)	103 (18)
"	.20	114 (18)	137 (28)
85°	.00	86 (12)	89 (13)
"	.05	97 (20)	102 (11)
"	.10	97 (37)	99 (24)
"	.20	88 (8)	60 (15)

( ) Standard error of the mean.
* μg of Coenzyme Q/g liver (wet wt).

homogenates from thyroxine treated rats(1). The differences in response obtained from chickens kept at 85°F are of interest, and at present cannot be explained. Further work on the status of the electron transport and protein synthetic mechanisms of both the rat and chicken under various thyroid states and at several environmental temperatures should be examined in the hope of gaining a better understanding of the role of thyroxine in thermoregulation and of changes in responsiveness of the tissue to a given thyroxine titer.

1. Moury, D. N., Crane, F. L., Biochemistry, 1964, v3, 1068.
2. Weiss, W. P., Sokoloff, L., Science, 1963, v140, 1324.
3. Michels, R., Cason, J., Sokoloff, L., ibid., 1963, v140, 1417.
4. Tata, J. R., Ernster, L., Lindberg, O., Arrhenius, E., Pederson, S., Hedman, R., Biochem. J., 1963, v86, 408.
5. Tata, J. R., Nature, 1963, v197, 1167.
6. Wharton, D. C., Griffith, D. E., Arch. Biochem. Biophys., 1962, v96, 103.
7. Crane, F. L., Dilley, R. A., Methods of Biochemical Analysis, 1963, v11, 279.
8. Gornall, A. G., Bardawill, C. J., Methods of Biochemical Analysis, 1949, v177, 751.
9. Beyer, R. E., Noble, W. M., Hirschfeld, T. J., Canad. J. Biochem. Physiol., 1962, v40, 511.

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Effect of Dodecyl Succinic Acid on *Pseudomonas aeruginosa* and Its Reversal by Formaldehyde and Iron. (29968)

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Anionic detergents react with positively charged groups on proteins(1,2). The positive charge is primarily contributed by amino and amide groups and anionic compounds are more effective in acid solutions(3). Their effect on bacteria is probably the result of a similar interaction with proteins of the cell membrane since work with protoplasts(4,5) shows membrane disruption in the presence of the compounds. If reaction with amino groups is important for the action of these compounds on bacteria, then pretreatment with small amounts of formaldehyde should counteract some of their effects. Cations such as calcium and iron which may be present on cell membranes could also react with anionic detergents and thus addition of small amounts of these ions to the cells may also counteract the effects. To test these possibilities a strain of *Pseudomonas aeruginosa* was used. This organism as well as many other Gram negative ones rapidly decreases in size when placed in salt solutions(6). Upon incubation a slow increase in size occurs as a result of the influx of ions with their water of hydration(7). The change in size can be measured by the change in optical density and the validity of the method has been checked with electron micrographs(7). The effect of detergents on the rate of cell size increase was studied in the presence and absence of formaldehyde and ferrous salts. Some experiments on the effect of mercury and EDTA are also included.

Materials and methods. A strain of *Pseudomonas aeruginosa* maintained in this laboratory for 15 years was grown at 34° for 24 hours on Difco nutrient broth. The cells were spun down and then washed twice with water by centrifugation. They were then sus-

TABLE I. Effect of Formaldehyde and Ferrous Sulfate on the O.D. Change Caused by Dodecyl Succinic Acid (DDS) and $HgCl_2$. Ratios obtained by dividing the O.D. change of control into the O.D. change of experimental.

Phosphate buffer		Tris buffer	
Additions	Ratio	Additions	Ratio
3.5×10^{-4}M DDS	1.88	$.7 \times 10^{-4}$M DDS	3.70
+ $.55 \times 10^{-3}$M formaldehyde	1.48	+ 1.1×10^{-3}M formaldehyde	1.76
+ 1.1×10^{-3}M formaldehyde	1.32	+ 1.8×10^{-5}M $FeSO_4$	1.21
+ 1.8×10^{-5}M $FeSO_4$	1.08		
+ 1.2×10^{-5}M $FeSO_4$	1.53		
+ $.6 \times 10^{-5}$M $FeSO_4$	1.69		
1.0×10^{-6}M $HgCl_2$	1.89	1.0×10^{-6}M $HgCl_2$	1.80
+ 1.1×10^{-3}M formaldehyde	1.96	+ 1.1×10^{-3}M formaldehyde	2.03
+ 1.8×10^{-3}M $FeSO_4$	1.22	+ 1.8×10^{-3}M $FeSO_4$	.60
1.1×10^{-3}M formaldehyde	.98	1.1×10^{-3}M formaldehyde	.95
1.8×10^{-3}M $FeSO_4$	.71	1.8×10^{-3}M $FeSO_4$	.52
$.7 \times 10^{-5}$M EDTA	1.05	$.7 \times 10^{-5}$M EDTA	.98
1.4×10^{-5}M EDTA	.92	1.4×10^{-5}M EDTA	2.22
2.8×10^{-5}M EDTA	1.46	2.8×10^{-5}M EDTA	2.40

pended in 0.05 M Na-K phosphate buffer pH 6.7. At pH 6.0 metabolic activity is greatly depressed; at pH 7.4 the detergent is inactive. Experiments with 0.05 M Tris buffer at pH 6.7 were also done although this pH is at the limit of the Tris buffering capacity. Dodecyl succinic acid kindly donated by the Humphrey Chemical Company, North Haven, Conn., was neutralized to pH 6.7 with NaOH after solution by warming. This alkyl succinic acid as well as others with longer carbon chains when incubated with bacterial suspensions at 37° in concentrations ranging from 1.7×10^{-4} M-3.5×10^{-4} M causes an increase in cell size greater than that which occurs in the control incubated under the same conditions. The change in cell size was measured in the Coleman Junior Spectrophotometer at 500 mμ and expressed as the decrease in optical density (O.D. = $-\log T \times 1000$). O.D. readings were taken after 60, 120, and 180 minutes' incubation at 37°. Depending on conditions, initial readings of 250 units could decrease to 130 units in 180 minutes. The decrease in O.D. at each time was determined and control values were divided into the experimental ones and the average of the 3 readings taken. A ratio greater than 1.0 indicates that the agent increased the rate of swelling; less than 1.0 that it decreased it and 1.0 that it had no effect. Although the cell size increases more slowly in Tris than in phosphate buffer dodecyl succinate (DDS) is much more effective in increasing permeability to the former. The ratio of the control to the experimental containing 3.5×10^{-4} M DDS was 1.8 in phosphate buffer and 3.7 in Tris buffer although only 0.7×10^{-4} M DDS was present.

Table I shows that formaldehyde partially antagonizes the effect of DDS both in phosphate and Tris buffer and that $FeSO_4$ is almost completely effective. The effect of $FeSO_4$ is not the result of salt formation between the iron and DDS for the following reasons. The molar concentration of iron is 5% of the DDS. Removal of this amount of the latter would have only a barely detectable effect on the O.D. change. Second, the ratio remains the same when DDS concentration is doubled. Third, although calcium chloride also antagonizes the DDS effect it is only one-quarter as effective on a molar basis as iron. $FeSO_4$ added to the cells in the absence of DDS slows the rate of swelling and more so in Tris than in phosphate buffer (Table I). Equimolar concentrations of calcium chloride have little or no effect. It has been shown previously(8) that iron plays an important part in the transport mechanism of this organism and the present facts support the previous observations. On the other hand formaldehyde added in the absence of DDS is without effect.

Small amounts of $HgCl_2$ increase the rate of O.D. change but unlike DDS the rate is the same in the two buffers. Formaldehyde

TABLE II. Effect of 7.0×10^{-4}M DDS and 0.9×10^{-3}M Formaldehyde on Oxidation of 1.0 mg Na Succinate 6 H_2O. Control O_2 uptakes have been subtracted. Figures are μl.

Min	Succinate	Succinate + formaldehyde	Succinate + DDS	Succinate + DDS + formaldehyde
30	9	18	11	10
60	27	39	62	30
90	53	63	125	60
120	94	100	203	100

does not antagonize the effect but iron is almost completely effective and calcium partially so. EDTA increases the rate of swelling much more effectively in Tris than in phosphate buffer in this respect acting like DDS.

Table II shows that the rate of succinate oxidation by this organism can be increased by addition of DDS, which allows a more rapid influx of inorganic ions necessary for the oxidation. (No oxidation occurs in Tris buffer.) This effect can be completely inhibited by a concentration of formaldehyde which, if anything, increases the rate of succinate oxidation in the absence of DDS.

Discussion. The carboxyl groups of this anionic detergent apparently react with charged amino groups and divalent cations present in the cell membrane. The importance of this reaction is indicated by the formaldehyde reversal of DDS swelling if it is assumed that formaldehyde is reacting with the amino groups. Although formaldehyde does not inhibit succinate oxidation it inhibits some other oxidations. But in the presence of the detergent, ion influx is primarily a matter of diffusion so that interference with energy-producing mechanisms would have little effect. Moreover, in the absence of DDS cell swelling is energy dependent(7,9) and formaldehyde has little or no effect.

Iron salts, however, slow the rate of swelling in the absence of DDS and this indicates that iron bound to the cell membrane interferes with ion influx and that iron binding sites in the washed cell are not saturated, *i.e.*, additional iron can be bound. Although there is no quantitative relation between the concentrations of DDS and iron to produce any given effect, yet as more iron is bound to the membrane the less effective DDS would be. The binding sites for iron may be SH groups since the effect of $HgCl_2$ in increasing swelling rate can be completely antagonized by sufficient amounts of iron. Undoubtedly other divalent cations, particularly calcium, play a part in regulating membrane permeability and EDTA may remove these as well as iron to increase swelling rate.

Both DDS and EDTA affect the influx of Tris at concentrations which do not have an appreciable effect on inorganic ion influx. On the other hand the effect of mercury on the influx of the two is the same. It is thus not possible to say whether the permeability barrier to organic and inorganic cations involves the same or different groups on the cell membrane.

Summary. The anionic detergent dodecyl succinic acid increases the rate of swelling of cells of *Pseudomonas aeruginosa* incubated in phosphate or Tris buffer pH 6.7. It is more effective in the latter buffer. Formaldehyde and ferrous sulfate partially protect the cell from this action of the detergent. Mercuric chloride increases the rate of swelling equally in both buffers, and ferrous sulfate but not formaldehyde protects the cells. EDTA also increases rate of swelling but more effectively in Tris than in phosphate buffer. These results indicate that chelation of iron or its displacement from the cell membrane by mercury facilitates ion influx. The reversal of the detergent effect by formaldehyde indicates that its carboxyl groups make ionic bonds with amino groups and this also facilitates ion influx.

1. Pankhurst, K. G. A., Surface Chemistry, Interscience Publishers, N. Y., 1949, p109.

2. Putnam, F. W., Advances in Protein Chemistry, 1948, v4, 79.

3. Glassman, H. N., N. Y. Acad. Sci., 1950, v53, 91.

4. Tomcsik, J., Proc. Soc. Exp. Biol. and Med., 1955, v89, 459.

5. Salton, M. R. J., Proc. Intern. Congr. Surface Activity, London, 1957, p245.

6. Mager, J., Kuczinski, M., Schatzberg, G., Avi-Dor, Y., J. Gen Microbiol., 1956, v14, 69.

7. Bernheim, F., ibid., 1963, v30, 53.

8. DeTurk, W. E., Bernheim, F., Arch. Biochem. Biophys., 1960, v90, 218.

9. Packer, L., Perry, M., ibid., 1961, v95, 379.

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