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THE EFFECT OF MERCURIC CHLORIDE AND CERTAIN SULFHYDRYL COMPOUNDS ON THE CHANGES IN OPTICAL DENSITY OF SUSPENSIONS OF *PSEUDOMONAS AERUGINOSA* IN SODIUM, POTASSIUM, AND SODIUM-POTASSIUM BUFFERS

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Abstract—The decrease with time in the optical density of suspensions of *Pseudomonas aeruginosa* was measured in sodium, potassium, and sodium–potassium phosphate buffers. Mercuric chloride after a short interval accelerates the decrease in sodium and sodium–potassium buffers but inhibits the decrease in potassium buffer. Mercaptoethylamine inhibits the decrease in sodium and to a greater extent in sodium–potassium buffers but has no effect in potassium buffer. It is active only in the oxidized form. Cysteine slightly accelerates the decrease in all three buffers and partially antagonizes the effect of mercaptoethylamine. It is active only in the reduced form. Mercaptoethylamine has no effect on the oxidation of succinate. Cysteine inhibits it. Mercaptoethylamine inhibits the induction of the enzyme for the oxidation of benzoate, and cysteine does this less effectively.

SUSPENSIONS of *Pseudomonas aeruginosa* incubated in sodium–potassium phosphate decrease in optical density (O.D.), and this has been correlated with an increase in the size of the organism.[1] Mercuric chloride accelerates the rate of decrease in O.D.[2] The acceleration at first is the result of the influx of sodium ions and their water of hydration (see below) and lysis may occur. Lysis of *Escherichia coli* by sulfhydryl binding agents has been reported by Schaechter and Santomassino.[3] In some animal cells mercuric chloride may also increase sodium influx. Small amounts ,0·2 μmole/200 mg dry weight, increase sodium permeability of the isolated rat ileum.[4] When 0·3 mg/100 g was injected s.c. into rats for several days, the sodium content of the cells was increased at the expense of the potassium.[5] This also occurred when 10^{-3} m-mole mercuric chloride/liter was added to human red cells *in vitro*.[6] These facts suggest that sulfhydryl groups are of importance, directly or indirectly, for the transport of sodium ions. It was therefore of interest to test the effects of sulfhydryl compounds which might interact with such groups. For this purpose mercaptoethylamine HCl (MEA) and cysteine HCl were added to the washed organisms under various conditions, and changes in O.D. were determined. Apparently there has been only one study on the effect of MEA on bacteria. Weinberg *et al.*[1] found that it caused lysis in certain strain of bacillus but not in any of the other organisms studied.

METHODS

A strain of *Ps. aeruginosa* maintained in this laboratory for 15 years was grown at 34° for 24 hr on Difco nutrient broth. The organisms were spun down and washed twice with water by centrifugation. They were then suspended in water and adjusted to a suitable optical density. When an aliquot was added to buffers of various strengths, an immediate increase in O.D. occurred which was complete within 40 sec. At this time the O.D. of the suspension, which had a final volume of 2·0 ml, was determined in the Coleman Jr. spectrophotometer at 500 mμ and recorded at $2 - \log T \times 1000$. All determinations were made in duplicate or triplicate. The suspension was then placed in a test tube and incubated at 37° without shaking. Readings were taken at 10-min intervals for 30 min. Compounds to be tested were added to the suspension after the organisms had been put into the buffer. There was a decrease in O.D. with time of incubation, but if sucrose was used as the osmotic agent no such decrease occurred unless an inorganic cation was added.[1] The increase in cell size (decrease in O.D.) must therefore be the result of ion entry. Direct measurement of cation concentration within the cells after their exposure to salt solutions is not possible, since the cells rapidly lose cations when washed with water.[1]

RESULTS

The initial shrinking (increase in O.D.) depends within limits on the concentration, i.e. osmotic pressure, of the buffer. The rate of swelling is greater the higher the buffer concentration (Table 1) which indicates that the diffusion gradient aids in the entry of the ions. In the presence of potassium as the only added cation, the rate of swelling is greater than in the presence of sodium or an equimolar mixture of sodium and potassium. This latter fact indicates that sodium interferes in some way with potassium influx and becomes the rate-limiting factor when both cations are present. It should also be noted that the rate of swelling in sodium–potassium buffer increases linearly with the increase in salt concentration at the 20- and 30-min values. Thus in 0·05, 0·1, and 0·15 M buffer, the figures are 21, 42, 63 at 20 min and 26, 50, 75 at 30 min. This relationship does not hold in sodium or potassium buffer at the 0·15 M concentration.

TABLE 1. THE EFFECT OF MOLARITY OF SODIUM–POTASSIUM, POTASSIUM AND SODIUM PHOSPHATE BUFFERS, pH 7·7 ON THE DECREASE IN O.D., 37°

	0·05 M			0·1 M			0·15 M		
Minutes:	10	20	30	10	20	30	10	20	30
Na–K	13	21	26	33	42	50	42	63	75
K	24	33	37	52	64	76	51	77	91
Na	18	25	31	33	42	49	28	53	66

The figures in this and the other Tables are the mean of 8–15 experiments

When a readily oxidizable substrate is added to the suspension, the rate of swelling is accelerated.[8] This fact and a decrease in rate in the presence of cyanide indicate that the entry of the ions into the organism is linked in part to energy-producing mechanisms which may affect the semipermeability of the membrane. Since mercuric chloride inhibits many SH enzymes involved in oxidative reactions, it should have an effect

similar to that of cyanide. Table 2 shows that the agents behave differently. Cyanide inhibits the rate of swelling in both sodium and potassium buffers. Mercuric chloride inhibits the rate in potassium buffer throughout the 30-min period but inhibits in sodium buffer only for 10 min, after which it causes a rapid acceleration. Thus potassium entry is inhibited at a time when sodium entry is increased. If one assumes that

TABLE 2. THE EFFECT OF $7\cdot4 \times 10^{-6}$ M MERCURIC CHLORIDE AND 1×10^{-3} M POTASSIUM OR SODIUM CYANIDE ON THE DECREASE ON O.D. IN 0·1 M POTASSIUM OR SODIUM PHOSPHATE BUFFER pH 7·7, 37°

Minutes:	10	20	30		10	20	30
Control (K)	52	68	78	Control (K)	52	56	76
+ $HgCl_2$	32	52	62	+ KCN	32	46	62
Control (Na)	33	42	51	Control (Na)	27	38	45
+ $HgCl_2$	26	48	94	+ NaCN	20	26	29

the inhibition in potassium by mercuric chloride is the result of the inhibition of metabolic processes, as in the case of cyanide (the percentage inhibitions by the two compounds are almost the same), then the acceleration in sodium is probably the result of inactivation of specific SH groups which in some way regulate the entry of this cation. On this assumption, a sulfhydryl compound which could react with such groups might also affect sodium entry, although not necessarily in the same direction.

In phosphate buffer, pH 7·7, MEA is rapidly oxidized in the presence or absence of the organisms. A quantitative nitroprusside test[9] showed that 94% disappeared in 30 min at 37°, but at pH 6·7 only 20% disappeared in the same time. The corresponding figures for cysteine were 17 and 0·5%. Table 3 shows that reduced MEA inhibits the

TABLE 3. THE EFFECT OF $0\cdot7 \times 10^{-3}$ M REDUCED AND OXIDIZED MEA·HCl OR CYSTEINE·HCl ON THE DECREASE IN O.D. IN 0·1 M SODIUM–POTASSIUM BUFFER, pH 7·7, 37°

Minutes:	10	20	30		10	20	30
Control	47	58	67	Control	38	51	59
Reduced MEA	27	32	37	Reduced cysteine	44	62	72
Oxidized MEA	17	27	35	Oxidized cysteine	36	52	58

rate of swelling and cysteine increases it. If MEA is oxidized to the disulfide form before it is added to the suspension, the inhibition during the first 10 min is greater than when the reduced form is used. After 30 min the inhibition by both forms is the same. This indicates that the active form of MEA is the disulfide. On the other hand, previously oxidized cysteine is inactive.

The effect of oxidized MEA on the rate of swelling in three concentrations of sodium–potassium buffer is shown in Table 4. The percentage inhibition decreases with increase of molarity, as might be expected if MEA were blocking a mechanism necessary for influx. An inhibition greater than 100%, which is invariably seen during the first 10-min incubation in 0·05 M buffer, must be the result of a virtually complete block of ion influx during at least part of this period, which would allow for a further

TABLE 4. THE EFFECT OF $0 \cdot 7 \times 10^{-3}$ M OXIDIZED MEA ON THE DECREASE IN O.D. IN SODIUM–POTASSIUM PHOSPHATE BUFFER OF VARIOUS MOLARITIES, pH 7·7, 37°

	0·05 M			0·1 M			0·15 M		
Minutes:	10	20	30	10	20	30	10	20	30
Control	12	21	28	36	46	51	39	62	68
MEA	−2	2	5	12	13	23	14	25	34
Inhibition (%)	117	91	82	72	72	55	64	60	50

shrinkage of the cells and therefore a further increase in O.D. The effect of MEA on the rate of swelling in various buffers is shown in Fig. 1. The results present an apparent paradox. MEA has no effect in potassium buffer, it inhibits in sodium buffer, but the maximal inhibition is obtained in a mixture of sodium and potassium. In considering

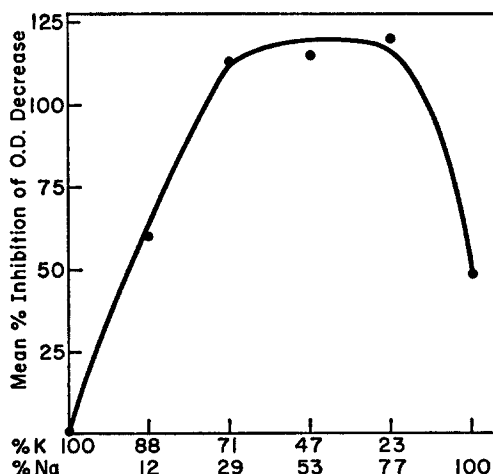

FIG. 1. The effect of different proportions of sodium and potassium in 0·1 M phosphate buffer on the decrease in O.D. by $0 \cdot 7 \times 10^{-3}$ M oxidized MEA, pH 7·7, 37°.

a possible explanation for this, three facts must be taken into account. First, as pointed out above (Table 1), there is an impairment of the entry of sodium and potassium when these cations are added separately, which becomes evident at 0·15 M buffer concentration. Second, the oxidation of certain added substrates by this organism requires both cations (unpublished experiments.) Third, whereas the MEA inhibition decreases as the molarity of the sodium–potassium buffer increases (Table 4), it increases with molarity in sodium buffer. Thus instead of 91 %, 72 %, 60 % inhibitions in 0·05, 0·1, and 0·15 M sodium–potassium buffer at 20 min, the corresponding values in sodium buffer are 50 %, 54 %, 69 %. The presence of both cations is essential for the efficient functioning of the organism, and the MEA inhibits maximally when conditions are optimal. Sodium alone in high concentrations inhibits its own entry, and MEA further impairs it.

The acceleration of cell swelling by cysteine is small and independent of the type of cation. The mean value in the three buffers is 17 %, but the variations are large. In

general the acceleration is greatest in 0·05 M buffer, least in 0·15 M. Both D- and L-cysteine are active. Small amounts of cysteine can partially prevent the effect of MEA This is shown in Table 5. The order of addition to the suspension is immaterial. Nitroprusside determinations show that this concentration of cysteine does not prevent the oxidation of MEA nor does it reduce the disulfide.

TABLE 5. THE EFFECT OF $0\cdot14 \times 10^{-3}$ M CYSTEINE·HCl ON THE INHIBITION OF O.D. DECREASE BY $0\cdot7 \times 10^{-3}$ M OXIDIZED MEA·HCl in 0·1 M SODIUM–POTASSIUM BUFFER pH 7·7, 37°

Minutes:	10	20	30
Control	47	58	67
MEA	17	27	35
MEA + cysteine	28	38	48

Finally the effect of MEA and cysteine on the oxidation of succinate and benzoate was determined. These substrates were chosen because the succinoxidase is a sulfhydryl enzyme and because the oxidation of benzoate depends on the induction of its enzyme. The results are shown in Fig. 2. MEA does not significantly inhibit the oxidation of succinate. Cysteine inhibits only when added at the beginning and not when

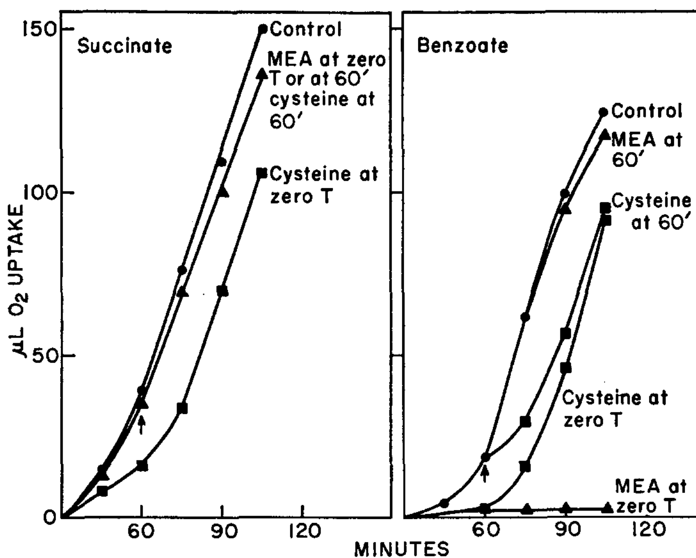

FIG. 2. The effects of $0\cdot14 \times 10^{-3}$ M MEA·HCl or cysteine·HCl (added in the reduced forms) on the oxidation of 1·0 mg sodium succinate $6\cdot H_2O$ and 0·5 mg sodium benzoate at pH 7·7 and 37°.

added after 60 min. It may therefore inhibit the formation of a permease for succinate. MEA completely inhibits benzoate oxidation when added at the beginning and has no effect once the enzyme has been induced. Cysteine inhibits induction for a short time and has a transient effect when added at 60 min. Oxidized cysteine added at this time is without effect. At pH 6·7, where MEA and cysteine are very slowly oxidized,

MEA does not inhibit benzoate induction and again has no effect on succinate oxidation, but cysteine causes a prolonged inhibition of both reactions. Thus MEA is active only in the disulfide form and cysteine only in the reduced state. These effects on oxidation and induction are evident at concentrations as low as $0{\cdot}14 \times 10^{-4}$ M of both compounds, and no antagonism between the two could be demonstrated.

DISCUSSION

It appears unlikely that inhibition of metabolic processes could account for the effect of MEA and cysteine on the rate of cell swelling since both compounds inhibit certain oxidations, but they act in opposite ways on the rate. If it is assumed that SH groups are involved with sodium entry, then one could conceive that the combination of mercury with such groups might allow unimpeded entry, and the oxidation of such groups by oxidized MEA with or without subsequent mixed disulfide formation might inhibit entry and this could be reversed by reduction with cysteine. MEA in the disulfide form has two terminal amine groups. In order to determine whether other diamines were active, putrescine, cadaverine, and ethylene diamine were added in various concentrations. They had no effect. The amino acids corresponding to the first two amines, ornithine and lysine, were also inactive. Penicillamine was also without effect and, since it chelates metals, it is unlikely that chelation accounts for the action of either MEA or cysteine. Mercaptoethanol was inactive.

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