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Influence of Salt Upon Diffusion from Bacterial Cells.

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Numerous experiments in this laboratory and elsewhere[1] have shown that all cations in low concentration stimulate, and in higher concentration inhibit, bacterial viability. It has been suggested that the stimulating effect was associated with increased diffusion through the cell wall—the inhibitive effect with decreased diffusion. Shaughnessy and Winslow[2] demonstrated that salt concentrations known to be favorable did show an increase in ammonia in the menstruum surrounding the cells while toxic concentrations showed a reverse effect. No bacterial counts were made in these experiments, however; and the purpose of the present study was to check these earlier results by parallel determinations of bacterial numbers and diffusion products.

Heavy cultures of *Esch. coli* were washed off in distilled water, from 12-18 hour agar surface growths in Blake bottles, filtered through paper or cotton to remove clumps or agar, and centrifuged twice to remove soluble foreign materials. The washed cells were then divided into 4 portions which were resuspended respectively in distilled water, in 0.05-0.08 Molar NaCl, in 0.8-1.0 Molar NaCl and in 2.0 Molar NaCl. They were allowed to stand in contact with these menstrua for 30 minutes at 37°. The cells were then removed by centrifugation and the ammonia in the menstruum determined by direct Nesslerization after preliminary clarification by the Standard Methods for Water Analysis of the A.P.H.A. with slight modification. This modification consisted of comparing each specimen with a separate set of color standards containing a corresponding amount of clarified NaCl, since it was found that the different concentrations of NaCl employed caused different degrees of interference with normal Nessler color development.

The basic data for 5 experiments are presented in Table I, with computations of rate of ammonia yield per cell per hour based on the mean of the initial and final counts.

The initial counts of course varied in different experiments; and

[1] Reviewed by Winslow and Dolloff, *J. Bact.*, Balt., **15**, 67; Winslow and Haywood, *J. Bact.*, Balt., **22**, 49.

[2] Shaughnessy and Winslow, *J. Bact.*, Balt., **14**, 69.

TABLE I.

Exp.		1	2	3	4	5
Initial bacteria, billions per 10 cc.		151	257	48	97	187
Final bacteria, billions per 10 cc.	0.0 NaCl	118	284	41	89	176
	0.05-0.08 M NaCl	157	316	37	92	198
	0.8-1.0 M NaCl	114	220	25	74	62
	2.0 M NaCl	67	198	26	76	127
Ammonia nitrogen, mg. per 10 cc.	0.0 NaCl	.013	.016	.008	.016	.027
	0.05-0.08 M NaCl	.020	.022	.011	.017	.038
	0.8-1.0 M NaCl	.011	.013	.005	.008	.021
	2.0 M NaCl	.007	.009	.003	.009	.018
Ammonia nitrogen, mg. x 10^{-11} per cell per hour	0.0 NaCl	.019	.012	.037	.034	.030
	0.05-0.08 M NaCl	.026	.015	.052	.035	.039
	0.8-1.0 M NaCl	.017	.011	.027	.018	.034
	2.0 M NaCl	.013	.008	.017	.021	.023

the amount of diffused ammonia per cell per hour also varied somewhat widely from experiment to experiment in a given menstruum. Such variation might be expected on account of differences in initial vitality of culture, degree of clumping, etc.; but the relative results for different menstrua in a given experiment check closely. In every instance but one the dilute NaCl solution (0.05-0.08 M) showed a higher final count than the distilled water and in every instance it showed greater ammonia diffusion per cell. In every instance the medium strength salt (0.8-1.0 M) showed lower final counts than the distilled water and in every instance but one it also showed a lower rate of ammonia diffusion per cell. The strongest salt (2.0 M) showed sometimes higher and sometimes lower counts than the 0.8-1.0 M concentration (which might be expected from the sharp variations in numbers which occur in such salt concentration[3]) and ammonia diffusion was in all but one instance less. These relative relationships remain essentially the same when, as a check procedure, ammonia production per cell per hour is computed on the basis of either initial or final counts rather than on the mean between these values.

The percentage results presented in Table II make these relations clearer.

It seems evident that the influence of salt content upon bacterial viability runs closely parallel to its effect on diffusion of ammonia

[3] *J. Bact.*, Balt., **24**, 185.

TABLE II.
Influence of Salt upon Bacterial Viability and Diffusion of Ammonia into the Menstruum in Terms of mg. per Cell per Hour Expressed as Percentages of Results in Salt-free Control.

	M NaCl	Exp. 1	2	3	4	5	Average
Final	0.05-0.08	133	111	90	103	112	110
Bacterial	0.8-1.0	97	77	61	83	35	71
Count	2.0	57	70	63	85	72	69
Diffused	0.05-0.08	137	125	141	103	130	127
Ammonia per	0.8-1.0	89	92	73	53	113	84
Cell per Hour	2.0	68	67	46	62	77	64

outward through the cell wall. The short time period employed, in the absence of nutrient materials, makes it reasonably clear that changes in production of ammonia within the cell are not the determining factors. We may conclude then, that a dilute stimulating concentration of NaCl increases and a strong toxic concentration decreases diffusion of metabolic products outward through the cell wall. Whether this is due to an influence on the permeability of the cell membrane or to an effect on physico-chemical conditions within or without the cell is uncertain.

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Cellular Responses to Acetone-Soluble Lipoids from Mycobacteria.

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The cellular responses to phosphatides and the so-called waxes isolated by Anderson[1] from acid-fast bacilli have been studied by Sabin and Doan,[2] Sabin, Doan and Forkner,[3] and by Smithburn and Sabin.[4] Anderson[1] has separated from the bacilli a third lipoidal component which is soluble in acetone and which is recovered as a soft brown, salve-like solid with an odor not unlike that which arises from cultures of tubercle bacilli. This substance was shown[1]

[1] Anderson, R. J., *Physiol. Rev.*, 1932, **12**, 166.

[2] Sabin, F. R., and Doan, C. A., *J. Exp. Med.*, 1927, **46**, 645.

[3] Sabin, F. R., Doan, C. A., and Forkner, C. E., *J. Exp. Med.*, 1930, **52**, Suppl. No. 3, 3.

[4] Smithburn, K. C., and Sabin, F. R., *J. Exp. Med.*, 1932, **56**, 867.