with histological studies, seems good evidence that most of the Walker tumor becomes an infarct without blood flow following this combined treatment. Long-term survival studies to be reported elsewhere show that a closely related type of combined treatment, with an X-ray dose that by itself has little effect on tumor growth, can be made consistently effective in producing permanent regression of the Walker tumor(9).

Summary. A dose of 1500 R X-ray to rat Walker carcinosarcoma 256, followed by a sublethal dose of endotoxin, produced routinely a condition that gross and histological study suggested was an infarct involving all or nearly all tumor tissue. Tracer studies with intravenously administered I^{131} antibody to rat fibrin showed a pronounced localization of radioactivity about the periphery of treated tumors with no radioactivity in the central portions of the tumor, a picture consistent with a lack of blood circulation there. X-ray or endotoxin, administered alone, at the same doses, produced no obvious effect on tumor histology or antibody localization.

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Received October 28, 1966. P.S.E.B.M., 1967, v124.

Influence of Na^+ on Synthesis of a Substrate Entry Mechanism in A Marine Bacterium.* (31894)

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Marine bacteria require Na^+ for growth (1,2) and for transport of oxidizable substrates(3) as well as a non-metabolizable amino acid analogue, α-amino isobutyric acid (AIB)(4). We noted(5) that an elevated concentration of K^+ (0.26 M) increased the rate of penetration into marine bacteria of two non-ionizing substrates, mannitol and L-arabinose, but not an ionizing compound, glucuronate. The only other set of ions or ionic substitutes in suspending media used in these studies that permitted entry of substrates was a combination of 0.25 M Na^+ and 0.01 M K^+. Working with animal cells, Kipnis and Parrish(6) observed that the presence of an elevated concentration of K^+ in suspending media for uptake experiments permitted entry of mannitol and other substrates into rat diaphragm cells, but the presence of Na^+ was required for the transport of AIB. They concluded that the elevated extracellular K^+ functioned to increase diffusion of the substrates into the cells, whereas those substances that were not able to enter in this way were transported by mechanisms influenced by Na^+. The present work was undertaken to investigate the possibility suggested by earlier work(5) that there is a function for Na^+ exerted during the *synthesis* of an induced entry mechanism, separate from the involvement of Na^+ in *operation* of the entry mechanism.

Experimental. Suspensions of resting cells of the marine bacterium *Pseudomonas natriegens,* harvested from sea water nutrient broth cultures and washed with 0.052 M $MgCl_2$,

*This study was supported by National Science Foundation grant GB-3108.

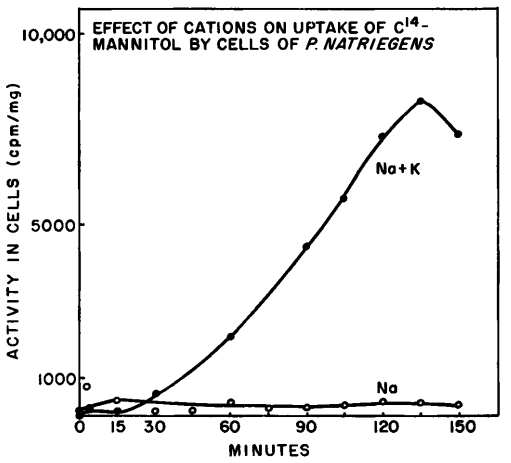

Fig. 1. Effect of cations on uptake of C^{14}-mannitol by cells of *P. natriegens*. Reaction mixtures contained mannitol-1, 6-C^{14} (20 μmoles/3 ml), 0.04 M di- and mono-sodium phosphate buffer and the following ions: Na + K = 0.25M Na Cl and 0.01 M KCl. Na = 0.25 M NaCl. At various intervals as indicated, 1 ml of each suspension was removed, filtered immediately through Millipore filters (HA), washed twice with 1 ml lots of 0.052 ffl $MgCl_2$, dried in planchets and assayed for radioactivity. Inclusion of 10 μg/ml of choramphenicol at time zero resulted in no uptake of the labeled substrate.

were incubated with C^{14}-labeled mannitol in media containing Na^+ or a combination of Na^+ and K^+. There was no uptake of the labeled substrate in the media containing 0.25 M Na^+ as the only cation, and uptake of the C^{14}-labeled mannitol in the media containing 0.25 M Na^+ and 0.01 M K^+ began only after incubation for 45 minutes (Fig. 1). There was then no entry mechanism initially present, and chloramphenicol added to the suspending media prevented the induction of one.

Despite the failure of the cells in the medium containing Na^+ and no inhibitor to take up the substrate, an entry mechanism was induced. This was demonstrated by harvesting cells, incubated concurrently with Na^+ and cold substrate, and washing and resuspending them with C^{14}-labeled mannitol in media containing 0.25 M Na^+, 0.01 M K^+ and chloramphenicol or 0.26 M K^+ and chloramphenicol. The cells took up C^{14}-labeled mannitol linearly from "zero" time in these media, but not in those containing other combinations of cations (0.25 M Na^+ and 0.01 M K^+ singly or combined with 0.052 M Mg^{++}, or the latter singly) or in unsupplemented buffer.

Induction of the capacity of resting cells of *P. natriegens* to permit entry of labeled mannitol in media containing 0.25 M Na^+ and 0.01 M K^+ was partially inhibited by ouabain (Table I). The inhibitory effect was increased in the presence of Mg^{++}. Induction in media containing 0.26 M K^+, on the other hand, was insensitive to ouabain supplied at the same concentration. An additional difference in the sensitivity of the induction processes was discerned when the cells were incubated with 0.01 μmole of 2,4-dinitrophenol (2,4-DNP), which inhibited the uptake of labeled mannitol by only 25% in the medium containing both Na^+ and K^+ but completely suppressed uptake by cells incubated in the medium containing 0.26 M K^+.

If the basic function of Na^+ in the growth of marine bacteria were restricted to influencing substrate entry, an agent which permitted uptake and did not harm the cells might be expected to replace Na^+ in growth. Incubation of cells in media containing as much as 0.36 M K^+ for 2.5 hours did not diminish viable cell counts. Thus an agent which influences entry but does not harm the cells is available, and experiments to test for replacement in growth were set up. The cells were incubated in minimal culture medium containing various concentrations of K^+

TABLE I. Inhibition of C^{14}-Mannitol Uptake by a Cardiac Glycoside.

	System*	% inhibition
1.	Mannitol + Na^+ + K^+ + ouabain	36.51
2.	" + high K^+ + ouabain	3.5
3.	" + Na^+ + K^+ + ouabain + Mg^{++}	52.15
4.	" + high K^+ + ouabain + Mg^{++}	0.0

* Resting cells were suspended in the 0.04 M phosphate buffer with the indicated ions: Na^+, 0.25 M NaCl; K^+, 0.01 M; Mg^{++}, 0.052 M; and high K^+, 0.26 M KCl. 20 μmoles mannitol-1-6-C^{14} (spec. act. 3.77×10^4 cpm/μmole) was provided as substrate. 10^{-4} M ouabain was added at zero time. Final volume, 3.0 ml. Suspensions were incubated 2.5 hr at 30°C with shaking, harvested, washed five times with 0.052 M $MgCl_2$, and assayed. Uptake in uninhibited systems = 100%. For $Na^+ + K^+ = 5.1 \times 10^3$ cpm/mg dry wt of cells; for high $K^+ = 1.05 \times 10^4$ cpm/mg dry wt of cells.

TABLE II. Effect of Elevated Concentrations of K^+ on Growth of Cells of *P. natriegens*.

	System*	Growth (observed 5 days)
1.	Substrate†	—
2.	" + .1 M KCl	—
3.	" + .15 M KCl	—
4.	" + .2 M KCl	—
5.	" + .25 M KCl	—
6.	" + .30 M KCl	—
7.	" + .25 M NaCl + 0.01 M KCl	+++
8.	" + .1 M KCl + 40 μmoles/ml Na buffer	—
9.	" + .25 M KCl + 40 μmoles/ml Na buffer	—

* Systems 1-7 contained the following minimal medium—5 g/l $(NH_4)_2HPO_4$, 2.5 g/l substrate, H_2SO_4 to neutralize.

Systems 8-9 contained a minimal medium in which .04 M di- and mono-sodium phosphate buffer was the suspending medium for—5 g/l $(NH_4)_2HPO_4$, 0.5 g/l $(NH_4)_2SO_4$, 2.5 g/l substrate. Inoculum for each was .1 ml of a suspension of cells from a 24-hr sea water nutrient broth culture. The cells were washed aseptically with .052 M $MgCl_2$, resuspended to original volume in sterile .052 M $MgCl_2$ and dispensed. Addition of this or increased quantities of sterile $MgCl_2$ to these systems did not change the results.

† Substrate either L-arabinose or mannitol.

but no Na^+, or a very dilute (0.04 M) sodium phosphate buffer was used as diluent; but there was no growth even after 5 days of incubation (Table II). If Na^+ was provided at 0.25 M, however, growth occurred within 24 hours.

These findings are consistent with the hypothesis that a penetration mechanism is synthesized during incubation of cells in Na^+-containing medium, and a combination of Na^+ and K^+ is required(5) to operate the penetration mechanism. But, uptake in the media containing 0.26 M K^+ is due to increase in the rate of diffusion of the non-ionizing substrate into the cells.

These findings lead us to suggest that resting cells of marine bacteria, which can form induced entry mechanisms from the components of the metabolic pool, afford ideal systems for determining Na^+ effects exerted prior to operation of entry mechanisms. In these inducible resting cells, the effect of Na^+ on synthesis of the uptake mechanism can be studied in isolation from growth-related functions of bacterial cultures and quite differently from transport-related phenomena in animal cells. It seems likely that studies of influences of Na^+ at the membrane level on synthesis in animal cells would be fruitful as well.

Summary. Incubation of resting *P. natriegens* cells in a suspending medium containing 0.26 M Na^+ and mannitol yielded cells which were able to take up mannitol in media containing a combination of 0.26 M Na^+ and 0.01 M K^+, or 0.26 M K^+, and chloramphenicol. Cells incubated in media containing a combination of 0.25 M Na^+ and 0.01 M K^+, or 0.26 M K^+ were induced by and took up C^{14}-labeled mannitol. The uptake in the former medium was partially inhibited by ouabain and 2,4-DNP whereas that in the latter was unaffected by ouabain and completely suppressed by 2,4-DNP. Although incubation of the cells in 0.26 M K^+ permitted entry of the substrate mannitol, the need for Na^+ for growth of the bacteria was not replaced by K^+. It is suggested that Na^+ influences *synthesis* as well as *operation* of entry mechanism for substrates, whereas a high K^+ concentration increases rate of diffusion of non-ionizing substrates into marine bacterial cells.

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Received November 2, 1966. P.S.E.B.M., 1967, v124.