CHOLINE SULFATE PERMEASE: TRANSFER OF INFORMATION FROM BACTERIA TO HIGHER PLANTS?

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This communication[2] describes induction of a permease for choline sulfate in plant tissues in the presence of certain bacteria. Induced plant uptake requires induction of the corresponding bacterial permease, contact between induced bacteria and plant tissue, and plant protein synthesis. This phenomenon represents a new type of interaction between bacteria and higher plants and may involve a transfer of information.

Choline sulfate has been found in higher fungi (for references see Itahashi, 1961), lichens (Lindberg, 1955a), algae (Lindberg, 1955b), and higher plants (Nissen and Benson, 1961). It is formed by the transfer of sulfate from 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to choline by choline sulfotransferase (Kaji and McElroy, 1958; Orsi and Spencer, 1964). Bacteria and most lower fungi lack this enzyme (Harada and Spencer, 1960) and cannot synthesize choline sulfate. Certain bacteria can utilize choline sulfate as their sole sulfur source (Harada, 1964), presumably by induction of a permease and a sulfatase for this ester. Takebe (1960) has partially purified and characterized an inducible choline sulfatase from a strain of Pseudomonas. A repressible sulfatase (Spencer et al., 1968; Scott and Spencer, 1968) and permease (Bellenger, Nissen, Wood and Segel, unpublished) for choline sulfate have been studied in filamentous fungi. Higher plants possess two or more constitutive transport mechanisms for choline sulfate (Nissen and Benson, 1964a; Nissen, unpublished).

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2 Preliminary accounts of this work have been given (Nissen, 1966; Nissen, 1967).

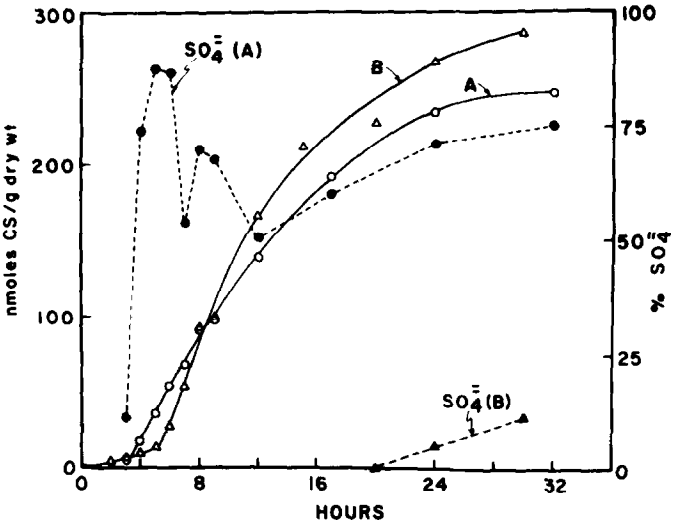


Fig. 1. Time-course of uptake (——) and hydrolysis (----) of 10^{-6}M choline sulfate by roots of 'Herta' barley. Nonsterile conditions, no bacteria added. Results of two experiments (A and B).

Materials and Methods. Seedlings of barley, Hordeum vulgare, cvs. Herta and Arivat, were grown essentially as described by Epstein (1961). Roots were excised from 6 or 7-day-old plants; leaf slices, 0.5 mm thick, were prepared from 6-day-old etiolated 'Arivat' plants according to Smith and Epstein (1964). Fresh weights of the root samples were 0.5-1.0 g; the leaf samples consisted of 20 slices in a cheesecloth bag. Uptake experiments, using S^{35}-labeled choline sulfate, were done at 21°C in 50-1000 ml of aerated solutions ('Herta' roots) or at 28°C in 100 ml of solution on a shaker ('Arivat' roots and leaf slices). Experimental solutions contained $2x10^{-4}$M $CaCl_2$ (roots) or $5x10^{-4}$M $CaCl_2$ (leaf slices). The pH of these unbuffered solutions was approximately 5.6. Experiments were terminated by rinsing the plant tissues with water for 30 minutes. Desorption experiments indicated that this removed all choline sulfate outside the plasma membrane.

The roots were assayed for radioactivity by liquid scintillation counting (Nissen and Benson, 1964b). The leaf slices were assayed in 5-7 ml of scintillation liquid. For determination of the hydrolysis of choline sulfate, the roots were extracted with hot aqueous ethanol, the extract was subjected to paper electrophoresis, and the spots containing choline sulfate and sulfate were cut out and assayed by liquid scintillation counting.

In initial experiments the ambient bacterial flora of the roots was used as the sole source of bacteria. Later the bacteria used were Pseudomonas tolaasii PT 109 (International Collection of Phytopathogenic Bacteria, Department of Bacteriology, University of California, Davis) and an isolate of the same species from the roots of 'Arivat' barley (P. tolaasii 'Arivat'). The bacteria were grown on a rotatory shaker at room temperature in synthetic citrate No. 3 medium (Benko et al., 1967) containing 1 g Na_2SO_4 per liter. Exponentially growing bacteria were harvested by centrifugation, resuspended in water, and added to experimental solutions to give optical densities of 0.02-0.05 at 650 mμ (0.4-$1x10^7$ cells/ml). Bacterial uptake of choline sulfate was determined by filtering 5-ml samples of bacteria onto Metricel GA 6 membranes, washing the membranes with 25 ml water, and measuring their radioactivity by liquid scintillation counting.

Choline sulfate was synthesized according to Schmidt and Wagner (1904). "Carrier-free" choline sulfate-S^{35} was prepared as described by Segel and Johnson (1963). The L-threo isomer of chloramphenicol was donated by Parke, Davis & Co. Abscisic acid was obtained from Dr. J. van Overbeek.

Results. The uptake of choline sulfate by excised barley roots was linear for short periods, but increased sharply after several hours (Fig. 1). This increase either was (experiment A), or was not (experiment B), accompanied by hydrolysis of the choline sulfate taken up. The reason for this difference is not known. Available data indicate that choline sulfate is taken up intact and independent of any subsequent hydrolysis. Using choline sulfate labeled with tritium and S^{35} it was shown that both moieties are taken up at identical rates. Furthermore, neither choline nor sulfate did competitively inhibit the increase in uptake.

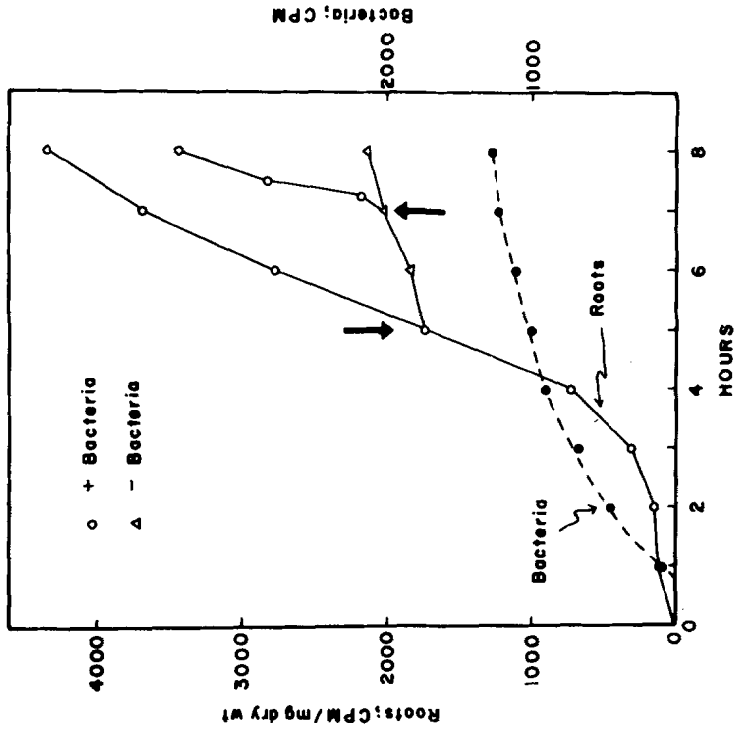
The increase in uptake was not due to aging, but required the presence of choline sulfate as an inducer. No induction occurred when roots were allowed to take up choline sulfate under sterile conditions. Several species of bacteria were isolated from the roots of 'Herta' and 'Arivat' barley and tested for ability to induce plant uptake. One species from each cultivar of barley was found to be effective and was, in both instances, identified as Pseudomonas tolaasii by phage typing and physiological tests. This species was previously isolated from cultivated mushrooms, where it causes, or is associated with, a rot disease (Tolaas, 1915; Paine, 1919; Lelliott et al., 1966).

While the presence of certain bacteria is required for induction to occur, the observed phenomenon is not due simply to bacterial contamination: (i) The time-courses of choline sulfate uptake in bacteria and in roots do not coincide. Induced uptake occurred about 2 hours earlier in the bacteria (Figs. 2 and 3) and usually ceased before that in the plant (Fig. 3). (ii) In the absence of an energy source, bacterial uptake of choline sulfate was low compared to that of the plant. Certain bacterial strains which caused a weak but significant induced uptake by the plant, had no measurable uptake of choline sulfate. (In these strains the bacterial permease may have been at least partially induced but not expressed due to the unfavorable conditions for bacterial metabolism). Furthermore, there was no correlation between the amount of choline sulfate taken up by effective strains and that taken up by leaf slices induced by these strains. (iii) Examination, by light and electron microscopy, of suspension cultured tobacco cells induced for uptake of choline sulfate, revealed no bacteria inside the cells. Removal of newly induced roots from bacterial suspensions abolished induced root uptake within 5 minutes (see also Fig. 3), indicating that most of the bacteria readily are washed off the roots. The addition of bacteria to experimental solutions containing roots grown under nonsterile conditions did not increase the number of bacteria remaining on the roots after desorption. The contribution of these bacteria (about 5×10^5 per mg dry weight of roots) to the radioactivity of the roots was probably less than 5 per cent. Similarly, leaf slices exposed to

bacteria heavily labeled with choline sulfate-S^{35} contained very little radioactivity after a desorption period.

The following findings indicate that the induced uptake observed in plant tissues is indeed carried out by the plant: (i) Induced leaf uptake was inhibited by a mild heat treatment of the leaf slices prior to addition of the bacteria; the integrity of the metabolic apparatus of the plant is therefore essential. (ii) Cycloheximide inhibited induced plant uptake, but had no effect on the bacterial uptake of choline sulfate (Fig. 2, Table I), indicating the requirement for plant protein synthesis. (iii) The inducible and constitutive plant uptake mechanisms were equally dependent on Ca^{++}. This indicates the involvement of plant membranes, since Ca^{++} is necessary for their integrity. Bacterial uptake of choline sulfate did not require Ca^{++}. (iv) While the bacteria were required during the first few hours of induced plant uptake, the plant tissue could later be transferred to solutions containing no bacteria with little or no loss in uptake rates. Prolonged periods in bacteria-free solutions did, however, result in a loss of induced uptake, possibly due to a breakdown of the induced plant permease.

The effects of inhibitors of nucleic acid and of protein synthesis on constitutive and induced uptake of choline sulfate are shown in Table I. In summary: (i) Actinomycin D inhibited induced root uptake, probably indicating the requirement for RNA synthesis in the bacterial component of the system. (ii) Constitutive leaf uptake was inhibited by cycloheximide and other inhibitors of plant protein synthesis, but not by chloramphenicol. As previously shown, induced plant uptake was also inhibited by cycloheximide. This indicates that protein synthesis by 80s ribosomes is involved in induced plant uptake as well as in constitutive leaf uptake (Vazquez and Monro, 1967). Constitutive root uptake was not inhibited by high concentrations of inhibitors of protein synthesis. (iii) The L-threo isomer of chloramphenicol did not inhibit induced root uptake. This indicates that the effect of the D-threo isomer is not on oxidative phosphorylation, but on bacterial protein synthesis (Hanson and Krueger, 1966). The main effect of streptomycin and chlortetracycline was probably also on bacterial protein synthesis. (iv) Puromycin and the amino acid analogues L-ethionine and p-fluorophenylalanine inhibited protein synthesis both by the bacteria and by the plant tissues. The previously reported enhancement by puromycin (Nissen, 1966) was probably due to an inactive batch of the inhibitor (Studzinski and Baserga, 1966), which may have served as substrate for the bacteria. (v) Synthesis of plant nucleic acids may not be required for induced plant uptake, since the induction was not inhibited by abscisic acid (dormin, abscisin II), which is believed to inhibit DNA and RNA synthesis in other plant systems (van Overbeek et al., 1967; Chrispeels and Varner, 1967).



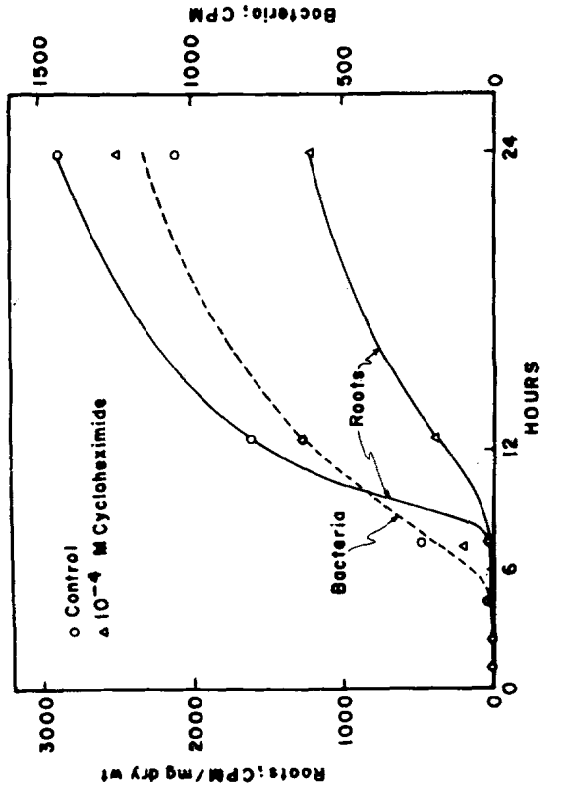


Fig. 2. (left). Time-course of uptake of 10^{-5}M choline sulfate by bacteria and by 'Herta' barley roots in the presence or absence of 10^{-4}M cycloheximide. Nonsterile conditions, no bacteria added.

Fig. 3. (right). Time-course of uptake of 10^{-5}M choline sulfate by P. tolaasii 'Arivat' and by 'Arivat' barley roots. Roots removed from bacterial suspension at 5 hrs and returned at 7 hrs.

Inhibitor	Roots		Leaves		Bacteria
	constitutive	induced	constitutive	induced	induced
Actinomycin D	-	+			
Chloramphenicol, D-threo	-	+	-	+	+
- " - , L-threo	-	-			
Streptomycin	-	+			
Chlortetracycline	-	+			
L-Ethionine	-	+	+	+	+
p-Fluorophenylalanine	-	+	+	+	+
Cycloheximide	-	+	+	+	-
Puromycin	-	+	+	+	+
Abscisic acid	-	-			-

Table I. Effects of inhibitors on uptake of choline sulfate. A plus sign indicates inhibition at low concentrations of the inhibitor, a minus sign indicates little or no inhibition even at high concentrations. Roots ('Herta' barley): Constitutive uptake 2.4 or 4 hrs, induced uptake 24 hrs (constitutive rates extrapolated and subtracted from total rates), 10^{-6}M choline sulfate, nonsterile conditions. Leaf slices ('Arivat' barley): Constitutive and induced uptake 20 or 24 hrs, 10^{-5}M choline sulfate, induced with P. tolaasii 'Arivat'. Bacteria (P. tolaasii 'Arivat'): Conditions as for leaf uptake.

Contact between live, induced bacteria and plant tissue seems to be required for induction of the plant permease. As previously mentioned, no induction occurred in plants under sterile conditions. The plant growth hormones kinetin, indoleacetic acid, and gibberellic acid had no inductive effect. No induction occurred with filtrates from induced bacteria or from induced bacteria subjected to sonication or treated with lysozyme. The inductive effect of the bacteria was not transmitted through a membrane filter (pore size 0.45 μ). Heat treated or noninduced bacteria had no inductive effect.

Induced leaf uptake of choline sulfate was completely inhibited by 10^{-4}M periodate, while bacterial uptake and constitutive leaf uptake were less affected. Periodate also has been shown to inhibit bacterial conjugation (Sneath and Lederberg, 1961) and the initial binding of DNA by competent bacteria (Ranhand and Lichstein, 1966; Polsinelli and Barlati, 1967). No selective inhibition of induced plant uptake was obtained with nucleases or proteases.

Figure 3 indicates that there are at least two processes in the development of induced plant uptake. There is, initially, a lag period between the expression of the bacterial and of the root permease. If, after removal of the roots from the bacterial suspension, the roots are returned, induced plant uptake will start immediately, i.e., the lag period need not be repeated.

Similar results were obtained with the induction of alpha-amylase in barley aleurone layers (Chrispeels and Varner, 1967).

Available evidence indicates that induction of the bacterial permease is a prerequisite for induced plant uptake: (i) The ability of bacteria to induce bacterial and plant uptake was correlated. Of 43 strains from different species of the Gram-negative bacteria Pseudomonas, Agrobacterium, and Rhizobium, 28 strains, phytopathogenic as well as nonpathogenic, were able to take up choline sulfate. These were all good inducers of leaf uptake of choline sulfate. The remaining 15 strains did not, or only weakly, induce the plant. Bacteria from 13 other genera have been tested; none induced plant uptake, and only strains of Corynebacterium (Gram-positive) induced their own permease for choline sulfate. (ii) Induced bacterial uptake of choline sulfate preceded induced plant uptake (Figs. 2 and 3), and lag periods for bacterial and plant uptake were equally shortened or lengthened by, respectively, prior sulfur starvation or heat treatment of the bacteria.

Discussion. The findings presented in this communication suggest the existence of a mechanism for the transfer of information from bacteria to higher plants. The requirement for bacterial induction, bacteria-plant contact, and plant protein synthesis for the induced uptake of choline sulfate by plant tissues, warrants the raising of this possibility.

The mechanism of interaction is not understood. The requirement for contact and the differential effectiveness of Gram-negative and Gram-positive bacteria may indicate that bacterial induction produces a change on the bacterial surface. Whether the inductive effect of the bacteria is a contact phenomenon or involves the transfer of molecules (nucleic acids?) from bacteria to plants is not known. While it is difficult to conceive how the mere contact between induced bacteria and plants can cause the plants to produce specific proteins, the ease by which the bacteria are removed from the plant tissues renders the concept of a direct transfer of molecules difficult. (The bacteria could, conceivably, act by the production of an extracellular inducer too large or unstable for filtration). The required presence of induced bacteria for induced plant uptake is also puzzling. Information is needed on the genetic origin of the induced plant permease, and on the requirements for plant nucleic acid synthesis.

These findings may also have other implications: (i) Elucidation of the role of the bacteria in the induction of uptake and hydrolysis of choline sulfate by plants may help clarify the differences between the regulatory capabilities of bacteria and higher plants: Why do the plants require contact with induced bacteria to develop their permease? (ii) This type of inter-

action between bacteria and higher plants may play an important role in Nature. The bacteria in the rhizosphere (such as Pseudomonas, Agrobacterium, and Rhizobium) may confer some of their metabolic versatility and adaptability to the plant roots. This type of interaction may also be involved in certain permeability phenomena in plant disease relationships. (iii) It may be possible to extend the metabolic capabilities of plants in useful ways by employing appropriate bacteria and substrates.

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