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ROLE OF KREBS CYCLE ACIDS IN PROMOTING ERGOT ALKALOID PRODUCTION BY CLAVICEPS SPECIES

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In further efforts to account for the marked stimulation of alkaloid production by *Claviceps* when Krebs cycle acids are included in the nutrient medium, the ability of *Claviceps* strains to take up and metabolize these compounds was examined. Marked differences were found in the capacity of different strains to grow on carboxylic acids as sole sources of carbon. *Claviceps* strain PRL 1980, which could grow on succinate and fumarate but not on citrate, malate, acetate, or α-ketoglutarate, nevertheless responded with similar increases in aklaloid yield when any of the Krebs cycle acids was added to a production medium. All of these acids were taken up by the fungus mycelium, but only succinate and fumarate achieved intracellular concentrations exceeding those in the uptake solution. Examination of uptake characteristics indicated that the acids entered the mycelium by passive diffusion with differences in rate and duration being markedly influenced by subsequent metabolism. A study of succinate uptake showed that entry of the monoanion was accompanied by influx of 3 potassium ions and efflux of 2 hydrogen ions, suggesting that cation transport, supported by metabolism, may be important in establishing the conditions for prolonged uptake of Krebs cycle acids.

Afin d'expliquer la stimulation prononcée de la production d'alcaloides par *Claviceps* quand on introduit dans son milieu de culture les acides du Cycle de Krebs, on a examiné la capacité de diverses souches de *Claviceps* d'absorber et de métaboliser ces acides. Il y a des différences prononcées entre les taux de croissance des diverses souches lorsque les acides carboxyliques sont leur unique source de carbone. *Claviceps,* souche PRL 1980, peut croitre avec du succinate ou du fumarate comme unique source de carbone mais pas avec du citrate, du malate, de l'acétate ou de l'alpha-cétoglutarate; néanmoins sa production d'alcaloides est accrue de façon égale lorsque n'importe quel acide du Cycle de Krebs est ajouté à son milieu de production. Tous ces acides sont absorbés par le mycélium de champignon, mais seuls le succinate et le fumarate se retrouvent intracellulairement à des concentrations supérieures à celle de la solution d'absorption. L'examen des caractéristiques de l'absorption a indiqué que l'entrée des acides dans le mycélium se fait par diffusion passive et que les différences de taux et de curée d'entrée sont causées par le métabolisme subséquent. Une étude de l'absorption du succinate a montré que l'entrée de ce mono-anion est accompagnée par l'influx de 3 ions potassium et par la sortie de 2 ions hydrogène, ce qui indique que le transport des cations, lui-même supporté par le métabolisme, peut être important pour l'instauration des conditions nécessaires à une absorption prolongée des acides du Cycle de Krebs.

Introduction

To obtain an optimum yield of ergot alkaloids in cultures of *Claviceps,* large amounts of ammonium succinate, citrate, fumarate, or malate are usually included in the medium (Taber 1967). The beneficial effect of these Krebs cycle acids is not solely one of pH control (Brar et al. 1968) and studies on their utilization have been directed toward understanding the physiological responses that favor alkaloid biosynthesis (Taber 1968; 1971; 1973; 1976). *Claviceps* strain

PRL 1980 can metabolize ammonium [^{14}C]succinate to a wide range of cell constitutents and in acidic media grows well with succinate as the only source of carbon. A glucose supplement is needed for growth at pH 7, implicating succinate uptake as a limiting factor under alkaline conditions. The effect of pH on uptake has been confirmed by permeability studies with [^{14}C]succinate. The response to pH is bimodal; it is low at pH 7 and reaches a peak near pH 5 where the concentration of succinate monoanion is at a maximum. The second peak occurs at very low pH values and matches the increasing concentration of undissociated acid. Most studies have centered on uptake of succinate near the pH 5 optimum.

Claviceps strain PRL 1980 differs from several bacteria where transport of Krebs cycle acids has been studied in that uptake occurs without induction and is not catabolite-repressed by glucose (Villarreal-Moguel & Ruiz-Herrera 1967; Kay & Kornberg 1971; Postma & van Dam 1971; Willecke & Pardee 1971; Fournier et al. 1972; Wilkerson & Eagon 1972). Uptake of succinate from solutions similar in concentration to those used in alkaloid production media exhibits an initial rapid component which is insensitive to metabolic inhibitors (Taber 1971). Superimposed on this is a slower, concentration-dependent and inhibitor-sensitive permeation. The combined process equalizes the intracellular and extracellular concentrations within approximately 12 minutes. Up to 85% of the radioactivity can be recovered from the mycelium as succinic acid at this time (Taber 1973). The rate of uptake during the inhibitor-sensitive phase is appreciably higher if the mycelium has been grown in a medium containing ammonium succinate.

These experiments suggest that succinate diffuses into the cell as a result of the concentration gradient. Whether or not diffusion is facilitated by a carrier has not been unequivocally determined but there is no strong evidence of active transport. Uptake of succinate in excess of that equalizing the intracellular and extracellular concentrations can be attributed to metabolic drag. We report here additional experiments bearing on this question. Earlier studies on the inhibition of succinate uptake by other Krebs cycle acids suggested that these compounds might compete for a common permeation process (Taber 1971). Because of the value of this information in establishing the uptake mechanism, as well as in clarifying the role of Krebs cycle acids in the physiology of alkaloid production, we have reexamined both the uptake of citric, fumaric, malic, and α-ketoglutaric acids by *Claviceps* strain PRL 1980 and the ability of these substances to support growth and alkaloid production. To assess whether the ability to use such compounds as sole carbon sources for growth is widely distributed in *Claviceps,* we have also surveyed additional strains.

Materials and Methods

Cultures

Claviceps strain PRL 1980 was isolated from *Pennisetum typhoides* and is probably *Claviceps fusiformis* (Loveless 1967). Strains PRL 1565, 1578, and 1598 are isolates of *Claviceps purpurea* (Taber & Vining 1960) and strain Ta 1 is an isolate of *Claviceps paspali* (Brar et al. 1968).

Media

Medium T-4M, a modification of medium T-4 (Taber 1973), was used to grow mycelium for measuring uptake of organic acids. It contained (g l^{-1}): D-glucose (autoclaved separately), 10; NH_4NO_3, 0.8; KH_2PO_4, 1.6; K_2HPO_4, 2.4; $MgSO_4 \cdot 7H_2O$, 0.3; and the following micronutrients (mg l^{-1}): $FeSO_4 \cdot 7H_2O$, 10; $ZnSO_4 \cdot 7H_2O$, 4.4; $CaCl_2 \cdot 2H_2O$, 6.0; $CuCl_2 \cdot 2H_2O$, 0.03; $CoCl_2 \cdot 2H_2O$, 0.02; $MnSO_4 \cdot H_2O$, 3.0; NaCl, 2.5; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.8, and biotin, 0.05.

Medium 10-B-2 (Taber 1967), and modifications with succinic acid replaced by another organic acid at equimolar concentrations, was used to produce ergot alkaloids. It contained (gl^{-1}): mannitol, 50; succinic acid, 7.2; KH_2PO_4, 0.25; $MgSO_4 \cdot 7H_2O$, 0.3 and the same micronutrients as medium T-4M except that cobalt chloride was omitted, and ferrous sulphate was used at half concentration. The pH was adjusted to 5.2 with ammonia. Media containing α-ketoglutaric acid and acetic acid were sterilized by filtration.

Medium T-6, used to measure growth on organic-acid carbon sources, contained (gl^{-1}): carbon from organic acid, 4.07; NH_4NO_3, 0.8; KH_2PO_4, 3; $MgSO_4 \cdot 7H_2O$, 0.3 and the same micronutrients as medium T-4M. Half of each batch of medium was adjusted to pH 6.8-7.0 with KOH; the other half was adjusted to pH 4.0-4.1 and the amount of potassium equalized by adding KCl.

Uptake Experiments

Uptake solutions contained a ^{14}C-labeled organic acid at the specified concentration in 90 ml of 30 mM potassium phosphate buffer, pH 4.0-4.1 or 6.8-7.1. In testing the effect of phosphate, the buffer salts were omitted; the pH was adjusted with KOH and the potassium content was brought to equivalency with KCl. Each uptake solution contained 12.5 μCi of radioactivity. Of the compounds used, [2,3-^{14}C]succinic acid was obtained from New England Nuclear Corporation, Boston, Massachusetts; α-keto-(5-^{14}C)glutaric acid, (1,5-^{14}C)citric acid, L-(U-^{14}C)malic acid and (2,3-^{14}C)fumaric acid were obtained from Amersham/Searle, Des Plaines, Illinois. The radiochemical purity of each compound was verified by paper chromatography followed by scanning with a Packard model 7201 radiochromatogram scanner.

The mycelium used in uptake studies was collected by centrifugation from 4 125-ml Erlenmeyer flask cultures incubated with shaking for 24 hours in medium T-4M after receiving 10 ml of homogenized inoculum (Taber & Vining 1959). It was washed 4 times with either phosphate buffer or water (the latter only when determining the effect of phosphate on uptake) of the same temperature as that of the uptake mixture. About 3 ml of the fourth wash was left behind to ensure that cell water was not drawn out of the mycelium during decantation. Approximately 36 ml of washed mycelium, formed as a paste with the 3 ml of water or buffer, was added to each uptake solution. The amount added was approximately 6 mg per ml and was measured in each experiment.

The uptake mixture was aerated at a rate of 1 volume of air per volume of mixture per minute. At intervals, samples of approximately 3 ml were withdrawn into a large-bore pipet and discharged on to a course sintered glass filter under suction. The mycelium was quickly washed with 7 ml of buffer (or water when appropriate), freeze-dried, heated to 100° C for 15 minutes and stored in a desiccator for combustion as described by Taber (1976). In experiments where CO_2 was collected, the air stream from the uptake mixture after final addition of sulfuric acid was vented into 0.25 N NaOH. One gram of dried mycelium was considered to represent 4 ml of cell water (Benko et al. 1967).

Uptake and efflux of sodium and potassium ions were measured in an unbuffered reaction mixture containing 41 mM succinic acid (adjusted to pH 4 with KOH) after 1 and 15 minutes contact with mycelium that had been previously washed 4 times with distilled water. Amounts of mycelium equivalent to 747 mg dry weight were added to the succinic acid solution and to an equal volume of distilled water which served as the control. The temperature of the incubation mixtures was 24°C. Sodium and potassium were measured with a Perkin Elmer model 2903 atomic absorption spectrophotometer.

Other Procedures

Cultures testing ergot alkaloid production were grown with shaking in 33 ml of medium contained in 125-ml Erlenmeyer flasks and were harvested by freeze-drying. The residual solids, basified with aqueous ammonia, were extracted with diethyl ether. Alkaloids were transferred from the ether into 0.2 N H_2SO_4 and reacted with van Urk reagent (Vining & Taber 1959). Alkaloid content was expressed as ergometrine equivalents.

Growth was estimated as the weight of mycelium collected by filtration, washed with water and dried at 95°C for 24 hours. To examine uptake mixtures for sugars and amino acids, filtered samples were acidified and extracted with ether to remove succinic acid, freeze-dried and subjected to paper chromatography (Taber 1973). The amount of unchanged [2,3-^{14}C]succinic acid accumulated in the mycelium was estimated from the radioactivity extracted by ether (Taber 1976). Radioactivity in samples of the initial uptake solution and of mycelium was measured, after combustion to carbon dioxide and collection as barium carbonate, with a Nuclear Chicago model 1152 low-background gas-flow counter.

Results

Growth on Krebs Cycle Acids

Claviceps strains differed in their ability to grow in media containing a carboxylic acid as the sole source of carbon (Table I). The variation was not due solely to species differences since the three strains (PRL 1565, PRL 1578, & PRL 1598) of *Claviceps purpurea* showed a wide range of tolerance. In general, growth

Table I. Growth ($g\,l^{-1}$) of *Claviceps* strains on organic acids as sole carbon sources.*

Strain	Initial pH	Acetate	Citrate	α-Keto glutarate	Succinate	Fumarate	Malate
PRL 1980	4	0	0	0	1.6	0.8	0
	7	0	0	0	0	0.4	0
PRL 1598	4	0	2.3	2.3	2.3	2.3	2.3
	7	2.8	2.9	1.0	2.7	2.5	2.3
PRL 1565	4	0	0	0	2.7	1.4	0.28
	7	0	0	0.4	0	0.4	0.1
PRL 1578	4	0	0	0	1.0	0.7	0
	7	0	0	0	0	0.5	0.5
Ta1	4	0	0	0.3	3.6	1.1	0.35
	7	0	0	0	0	0.4	0.16

*Cultures were incubated stationary in medium T-6 at 24°C. Strain PRL 1578 was harvested at 34 days, all others at 28 days. The initial pH was adjusted with KOH. KCl was used to equalize the potassium content of acid and neutral media.

occurred more readily at pH 4 than at pH 7 but the ability of strain PRL 1598 to grow on acetate only at the higher pH value was a notable exception. *Claviceps* strain PRL 1980 was relatively fastidious and grew only with succinate or fumarate. Moreover, with succinate, no growth occurred at pH 7. When, after 22 days incubation, glucose was added to this medium and to other media on which strain PRL 1980 had failed to grow, a mycelium formed rapidly on all cultures except those containing acetate. Preparing the vegetative inoculum in a medium containing the carboxylic acid to be tested improved the growth rate but did not increase the number of permissible carbon sources.

Production of Alkaloids

Claviceps strain PRL 1980 produced ergot alkaloids on media containing citrate, α-ketoglutarate, succinate, fumarate, and malate but not acetate (Table II). The type of Krebs cycle acid present in the medium affected the yield, but the differences were modest. In cultures containing fumarate and malate, production may have been limited by high pH. Those with citrate remained acidic and attainment of maximum yield appears to have been delayed.

Table II. Effect of organic acids on alkaloid production (mg l^{-1}) by *Claviceps* strain PRL 1980.*

Days	Succinate		Fumarate		Malate		α-Keto glutarate		Citrate	
	Alkaloid	pH	Alkaloid	pH	Alkaloid	pH	Alkaloid	pH	Alkaloid	pH
6	88 ± 5	7.4	102 ± 8	6.1	73±9	5.1	62 ± 7	6.2	34± 2	4.2
9	121±16	7.4	154 ± 4	7.6	179±6	7.4	178±13	6.8	112±13	4.6
12	226 ± 4	7.3	186±24	7.8	183±7	7.7	264±24	6.3	151±11	5.2

*Values are the mean and standard deviation for 3 flasks. Cultures, in 50 ml of medium 10-B-2 containing the appropriate organic acid, were shaken in 250-ml Erlenmeyer flasks at 24°C.

Uptake of Succinate

When mycelium from *Claviceps* strain PRL 1980 was incubated at 24°C in potassium phosphate buffer, pH 4.0, containing a low (0.83 mM) concentration of [2,3-^{14}C]succinic acid, radioactivity was taken up rapidly during the 20-minute observation period (Fig 1A). Measurements, after 15 minutes, of the distribution of radioactivity in the mycelium and effluent showed that only 0.1% of the total radioactivity entering the cells was oxidized to carbon dioxide. However, only 6% was recovered as succinate. If the [2,3-^{14}C]succinate had remained intact and in solution, the intra- and extracellular concentrations estimated from radioactivity would have been equalized during the first 3 minutes of incubation. Such a conclusion assumes that the factor of 4 ml g^{-1} dry weight used by Benko and coworkers to estimate the cell-water content of filamentous fungi applies to *Claviceps*. A similar though not identical value was adopted by Slayman and

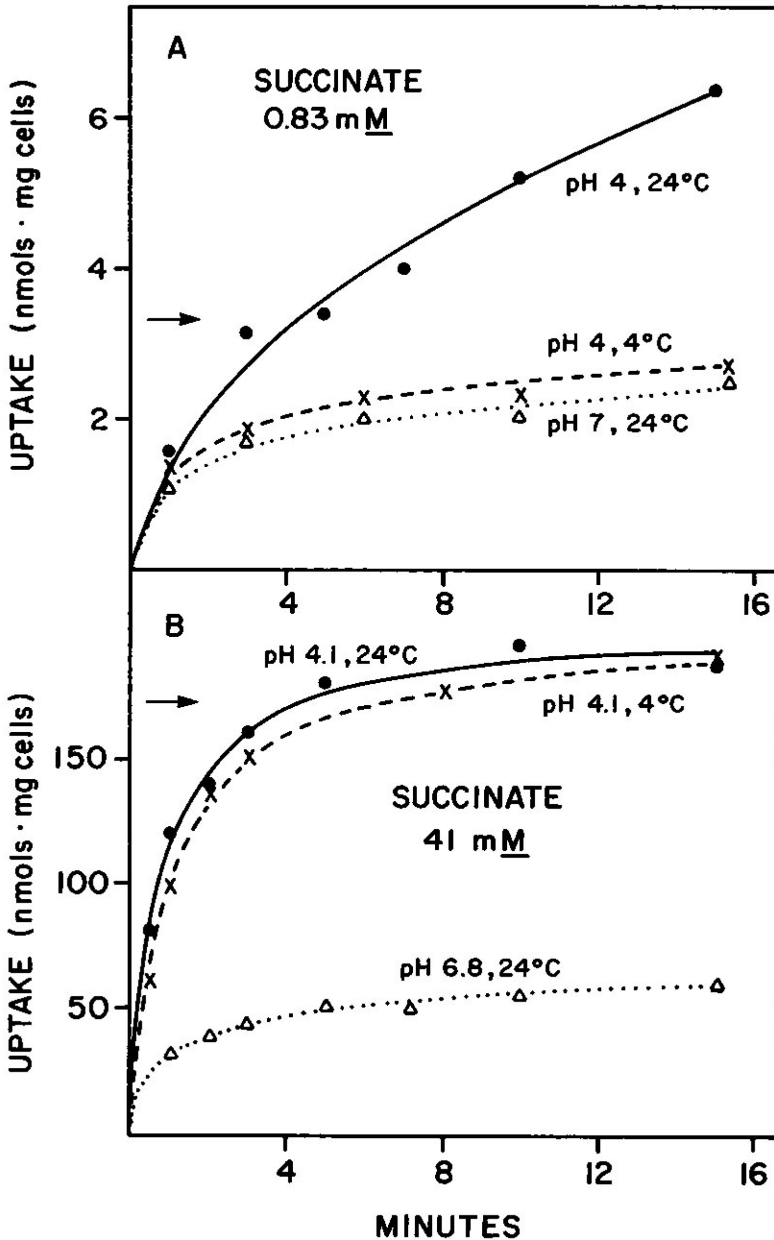

Fig 1. Effect of temperature and pH on the uptake of [2,3-^{14}C]succinic acid by *Claviceps* strain PRL 1980. Solutions were prepared as described in the text. Uptake was measured as the amount of radioactivity accumulated in the mycelium. The arrow indicates the amount needed to equalize the intra- and extracellular succinate concentrations. The uptake solution contained either 0.83 mM (panel A) or 42 mM succinate (panel B).

Slayman (1970) for *Neurospora crassa.* In contrast to the sustained uptake of labeled succinate observed at 24°C, incorporation of radioactivity at 4°C became very slow after the first minute. At the reduced rate of entry, it reached a value corresponding to balanced intracellular and extracellular succinate concentrations only after 30 minutes. A similar pattern was obtained at 24°C when the pH of the uptake solution was raised to 7.0.

When the concentration of [2,3-^{14}C]succinic acid in an uptake solution at pH 4.1 was increased to 41 mM, *Claviceps* strain PRL 1980 accumulated radioactivity rapidly at first but the rate declined as the intracellular and extracellular concentrations equalized (Fig 1B). This pattern was not changed substantially by lowering the temperature of the incubation mixture to 4°C. The value of Q_{10} at pH 4.1 was 0.5, substantially lower than the Q_{10} of 2 expected for a chemical reaction. Although not shown in Figure 1B, the rates of uptake at 24°C and 4°C were also very similar when the incubation mixture was at pH 7.0. As noted previously (Taber 1973), substantially less substrate entered at pH 7.0 than at pH 4.1. Ether extraction of cells incubated for 15 minutes at pH 4.1 and 24°C recovered 75% of the radioactivity; previous studies have shown this fraction to consist mainly of unchanged succinic acid (Taber 1973; 1976).

Results obtained in the earlier studies showed that, whereas phosphate could be omitted from the incubation mixture without altering the rate of succinate uptake, sodium and potassium ions affected the process. To detect a possible influx or efflux of these cations, a 41 mM succinic acid solution in water adjusted to pH 4.1 with KOH was incubated for 15 minutes with mycelium at 24°C. The cells took up 0.143 mmol of succinate and the content of potassium ions in the reaction solution decreased by 0.441 mmol. This represented an influx of approximately 3 mol K^+ accompanying each mole of succinate. The sodium content of the reaction mixture increased by 1.68×10^{-3} mol, an efflux too small to be significant. The pH of the solution increased from 4.09 to 4.18. Allowing for the buffering capacity of the succinate present, the equivalent loss of protons was 0.34 mmol, which corresponds to 2.3 mol of H^+ per mole of succinate taken up. In a control experiment lacking succinic acid there was a small loss of both potassium (3.8×10^{-2} mmol) and sodium (2.9×10^{-3} mmol), presumably due to leaching. Paper chromatographic examination of the uptake mixtures after incubation detected small amounts of mannitol in both the succinic acid and control solutions. The succinic acid solution, but not the control, also contained two amino acids. Their R_f values and color reactions matched those of glutamic acid and glutamine but their concentrations were too low to be significant.

Uptake of Other Krebs Cycle Acids by Claviceps Strain PRL 1980

Uptake of α-keto [5-^{14}C]glutaric acid at 24°C from a 41 mM solution at pH 4.0 was similar to that of succinate except that the amount of radioactivity in the mycelium declined rapidly after 4 to 6 minutes (Fig 2). At its maximum, the accumulated radioactivity was close to the value estimated to equalize the intra- and extracellular substrate concentrations. When the temperature of the uptake solution was reduced to 4°C, radioactivity accumulated in the mycelium at a similar rate but the subsequent decline was much slower. Shifting the pH of the uptake solution from 4.0 to 7.4 also changed the pattern of ^{14}C accumulation in the mycelium.

With 41 mM [2,3-^{14}C]fumaric acid at pH 4.1 and 24°C, the intracellular and extracellular concentrations of substrate were equalized over a similar time interval to that observed with succinic acid (Fig 3). Uptake continued beyond the equilibration point; the pattern resembled more closely that obtained with

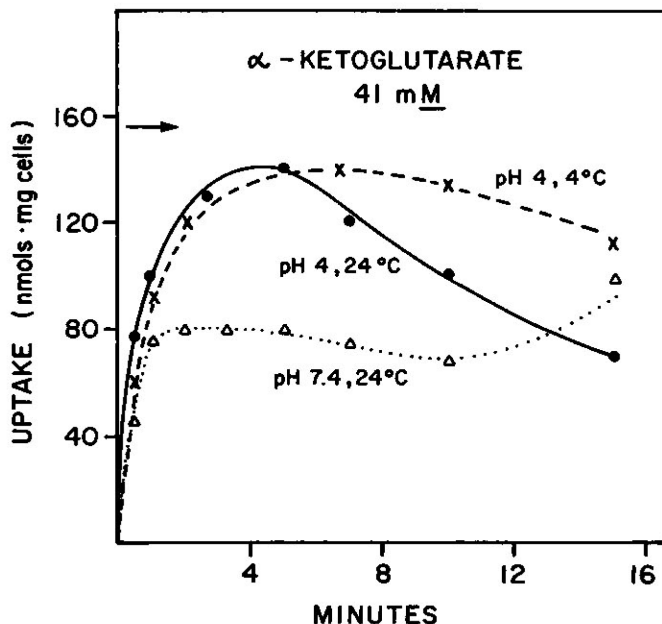

Fig 2. Effect of temperature and pH on the uptake of α-keto-[5-^{14}C]glutaric acid from a 41 mM solution. See Figure 1 and text for details.

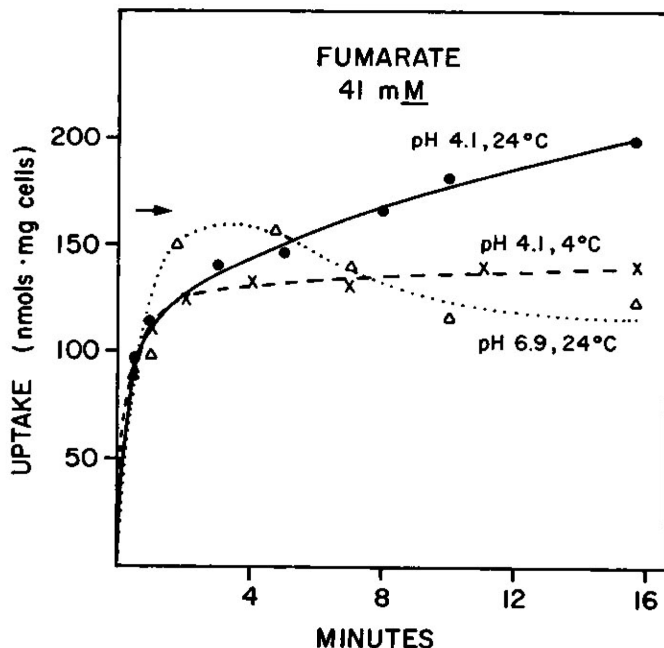

Fig 3. Effect of temperature and pH on the uptake of [2,3-^{14}C]fumaric acid from a 41 mM solution. See Figure 1 and text for details.

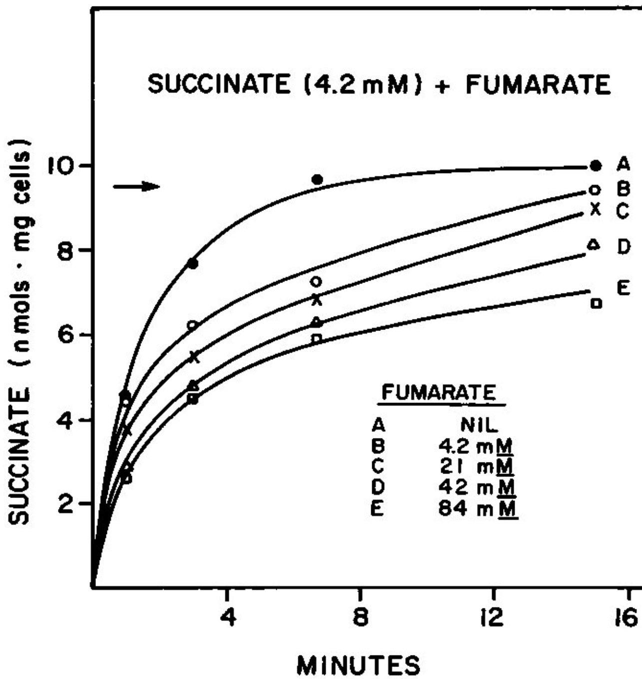

Fig 4. Uptake of succinic acid in the presence of various concentrations of unlabeled fumaric acid. The uptake solution contained 4.2 mM [2,3-^{14}C] succinic acid at pH 4.0. See Figure 1 and text for additional details.

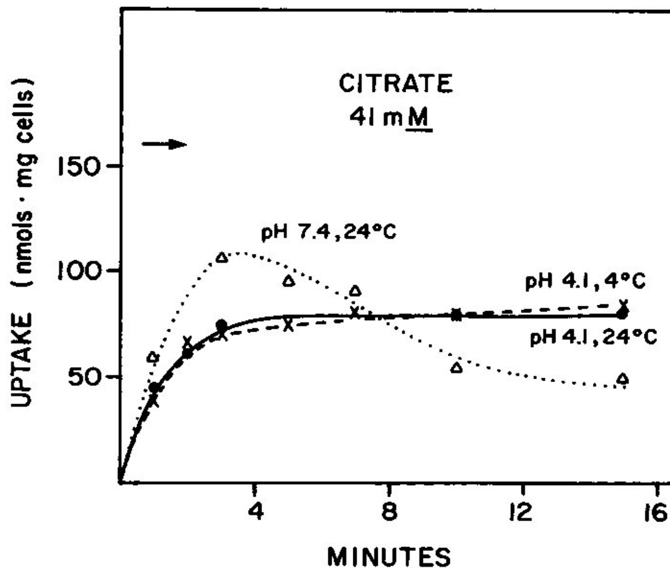

Fig 5. Effect of temperature and pH on the uptake of [1,5-^{14}C]citric acid from a 42 mM solution. See Figure 1 and text for details.

mycelium adapted to succinate (Taber 1971) than the one shown in Figure 2 where the cells had not previously been exposured to this substrate. Adding fumaric acid to an uptake mixture containing [2,3-^{14}C]succinic acid at 4.2 mM reduced the rate at which radioactivity was incorporated into the mycelium (Fig 4). However, the total accumulated radioactivity at each concentration of fumarate eventually approached a value corresponding to equal intracellular and extracellular concentrations. When the temperature of the uptake mixture containing [2,3-^{14}C]fumarate was reduced to 4°C, uptake beyond the initial phase was extremely slow and did not equalize the internal and external concentrations during the course of the experiment. A similar pattern was observed for the uptake of L-malic acid under all conditions of pH and temperature tested (data not shown) and for citric acid at both 4°C or 24°C when the uptake solution was at pH 4.1 (Fig 5). With fumarate and citrate, when the pH of the uptake solution was changed to 7.4, there was an unexpected increase in the rate of entry; the amount of radioactivity accumulated in the mycelium passed through a maximum after about 3 minutes, then declined. Apparently the substrate initially taken into the cells was being lost by efflux or metabolism.

Discussion

It is evident that *Claviceps* strains vary considerably in their ability to use carboxylic acids as the sole source of carbon for growth. Since *Claviceps* strains PRL 1980 and Ta 1 produce good yields of alkaloid whereas strains PRL 1565, 1578 and 1598 are poor producers, ability to assimilate the acids and ability to produce alkaloids are not closely linked. In media with mannitol as the main carbon source, *Claviceps* strain PRL 1980 produces alkaloids more abundantly if nitrogen is supplied as the ammonium salf of an organic acid than if an inorganic ammonium salt is used (Vining & Nair 1966; Brar et al. 1968). The results presented here indicate that the various Krebs cycle acids support comparable yields of alkaloid. Again, the response is not correlated with ability to serve as a sole carbon source for growth and uptake characteristics are not a determining factor.

Uptake studies with ^{14}C-labeled substrates indicated that the inability of *Claviceps* strain PRL 1980 to grow on citrate, α-ketoglutarate or malate cannot be attributed to substrate impermeability. At appropriate pH values, citrate, fumarate and α-ketoglutarate appeared to be metabolized. Since only acetate was toxic in this organism, the selective ability of succinate and fumarate to support growth might lie either in successful metabolic channeling of the substrate or in facilitated uptake allowing an adequate flow of carbon source into the cells. Earlier studies of succinate uptake demonstrated that it saturates at high substrate concentrations (Taber 1971) and is sensitive to sodium azide which, besides abolishing membrane proton gradients, also inhibits electron transport and thus impairs energy production (Harold 1972). Succinate uptake in *Claviceps* strain PRL 1980 differed from uptake of C_4-dicarboxylic acids in *Neurospora crassa* in being unaffected by the sulfhydryl inhibitor, iodoacetate (Wolfinbarger & Kay 1973). Also unlike *N. crassa* and bacteria, which metabolize Krebs cycle acids as fast as they enter the cell (Kay & Kornberg 1971; Lawford & Williams 1971; Fournier et al. 1972; Wilkerson & Eagon 1972), the *Claviceps* strain accumulated succinate within the mycelium (Taber 1973). However, high succinate concentrations were used in the *Claviceps* study. Under such conditions, if uptake outpaced metabolism of succinate, saturation of the rate of entry at high substrate levels would reflect the limiting rate of endogenous metabolic removal rather than saturation of a mediated transport system.

In the present investigation, *Claviceps* strain PRL 1980 mycelium exposed to a high concentration of [^{14}C]succinate accumulated radioactivity rapidly until the intracellular concentration approached that of the uptake solution. The rate after equalization was appreciably lower than has been observed with cells grown in a medium containing ammonium succinate (Taber 1976). This can be ascribed to reduced metabolic drag in mycelium not previously adapted to the substrate. When a low concentration of [^{14}C]succinate was used in the uptake solution, radioactivity accumulated in the mycelium, without change in rate, after the intracellular and extracellular concentrations had been equalized. Although little of the radioactivity was respired as carbon dioxide, only a small fraction could be recovered as unchanged succinic acid. This is consistent with an unmediated uptake process where the rate of entry associated with the lower external substrate concentration no longer exceeded the rate at which succinate was metabolized. In such circumstances, the decrease in metabolic rate expected at reduced temperature would also explain the different effects of temperature on uptake of succinate from dilute and concentrated solutions.

The evidence that uptake of Krebs cycle acids is insensitive to a large shift in temperature, except where metabolic drag is factor, excludes transport systems mediated by chemical reactions and implicates a diffusion process with a low energy of activation. Uptake from concentrated solutions can be driven at a high rate by the concentration gradient. However, movement of anionic species through the energized plasma membrane neutralizes the proton gradient, and a concurrent energy-dependent charge transfer is required to restore the membrane potential. The unequal exchange of sodium and potassium ions between the intra- and extracellular fluids during succinate uptake suggests that the monionic species is accompanied by a potassium ion. The magnitude of pH change is accounted for by the excess of potassium ions removed from the external solution.

The linear relationship observed between external concentration and uptake of Krebs cycle acids (Taber 1976) is accommodated by a diffusion mechanism with concomitant uptake of potassium contributing to the maintenance of electrical neutrality. Differences in uptake of these acids by *Claviceps* strain PRL 1980 may be due, at least in part, to differences in metabolic capability. Thus, failure to metabolize malate may prevent the generation of energy needed for cation transport and, indirectly, prevent equilibration of intracellular and external malate concentrations. Fumarate resembled succinate in its uptake pattern and it also decreased the rate of succinate uptake in competition experiments. Since the principal effect was to slow the approach towards equalization of cytoplasmic and external concentrations, altered cation transport due to metabolic changes may have been responsible. It is probably significant that succinate and fumarate, alone among the acids tested, were able to support growth of the organism as sole carbon sources.

The most plausible explanation at present for the beneficial effects of Krebs cycle acids on alkaloid production is that rapid diffusion of these substances into the mycelium builds up large intracellular pools which influence regulatory mechanisms in favor of secondary metabolism. Pazoutova and Rehacek (1981) have shown that access to citrate alters the levels of glycolytic and Krebs cycle enzymes in *Claviceps purpurea* strain 129 and influences differentiation of the mycelium. They suggest that this fungus may have included, in its adaptation to parasitic growth on grasses, a biochemical accommodation to the use of phloem sap rich in acids and amino acids related to Krebs cycle activity. Thus the pattern of growth, differentation, and secondary metabolism in media containing large amounts of carboxylic acids may simulate the natural condition for *Claviceps*.

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