The metabolism of succinic semialdehyde by a psychrophilic basidiomycete
by Parker Nelson Davies

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Microbiology
Montana State University
© Copyright by Parker Nelson Davies (1968)

Abstract:

It has been established that succinic semialdehyde was oxidized to succinic acid by a coenzyme dependent dehydrogenase found in brain and bacterial extracts, Strobel (196?) recently reported preliminary evidence that the enzyme was present in a psychrophilic basidiomycete (strain W-2, J. B. Lebeau, Lethbridge, Alberta, Canada). This preliminary evidence had not been further investigated. When a mycelial-mat was incubated at 23°C with uniformly labeled succinic semialdehyde (14C), 12.7% of the total 14C administered was recovered as 14CO₂ providing evidence that succinic semialdehyde was metabolized by the fungus. Four mycelial mats equal in size were incubated with labeled succinic semialdehyde (14C). Each was extracted at a specific time and these extracts were separated into fractions using Dowex columns. Labeling in the cation, anion, neutral, and cell wall fractions was found to increase with incubation time. Investigation of the most heavily labeled fraction, the 4 hr anion fraction, showed that there were two predominantly labeled organic acid peaks. Paper chromatography and two dimensional autoradiochromatography established that the two compounds were succinic semialdehyde and succinic acid. The labeling in both of these compounds increased as time increased. When labeled succinic semialdehyde (14C) was mixed with an acetone enzyme preparation of the fungus, it was found that after the control had been subtracted 22.1% of the initial or labeled succinic semialdehyde had been converted to succinic acid.

Boiled and frozen extracts of the fungus demonstrated a 100% loss of the activity that a control demonstrated. Using the acetone preparation, a soluble succinic semialdehyde dehydrogenase was characterized as to pH optimum, coenzyme specificity, substrate specificity, and K_m. Electrophoresis of an acetone preparation showed that there were three active electrophoretic forms of this enzyme. In summary it was found that succinic semialdehyde was metabolized and directed to all fractions of the fungus. Furthermore, the fungus was found to have a DPN dependent succinic semialdehyde dehydrogenase.

184

THE METABOLISM OF SUCCINIC SEMIALDEHYDE BY A
PSYCHROPHILIC BASIDIOMYCETE

by

PARKER NELSON DAVIES, JR.

A thesis submitted to the Graduate Faculty in partial
fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

Microbiology

Approved:

William G. Walter

Head, Major Department

Gary Strobel

Chairman, Examining Committee

A. Goering

Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

August, 1968.

ACKNOWLEDGEMENT

I want to express my gratitude and appreciation to Dr. Gary A. Strobels for his advice and guidance in my graduate studies.

I would also like to thank Dr. Strobels, Dr. Walter, Dr. Nelson, and Dr. Rodgers for their help in preparing this manuscript.

I am grateful to Darlene Harpster who typed this manuscript.

TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGEMENT	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT	viii
INTRODUCTION	1
MATERIALS AND METHODS	4
Culturing	4
Materials	4
General Methods	4
Radioactivity Determinations	5
Chromatography	5
Administration of Succinic Semialdehyde ^{14}C to Cultures	5
Conversion of Succinic Semialdehyde ^{14}C to $^{14}\text{CO}_2$	6
Succinic Semialdehyde Dehydrogenase Studies	7
Assay of Succinic Semialdehyde Dehydrogenase	7
Preparation of Succinic Semialdehyde Dehydrogenase	7
Electrophoresis of Succinic Semialdehyde Dehydrogenase	8
EXPERIMENTAL RESULTS	
Demonstration of Succinic Semialdehyde Metabolism	9

	Page
Collection of $^{14}\text{CO}_2$	9
Distribution of Labeling in Four Fractions	9
Examination of the Organic Acid Fraction	9
Conversion of Succinic Semialdehyde to Succinic Acid by Fungal Mats	15
Enzyme Studies	15
Purification of Succinic Semialdehyde Dehydrogenase . .	21
Inactivation and Stability of the Enzymes	21
Effect of pH on Activity	21
Coenzyme Specificity	24
Substrate Specificity	24
Effect of Substrate Concentration on Enzyme Activity	24
Induction	24
Electrophoretic Forms of Succinic Semialdehyde Dehydrogenase	24
DISCUSSION	29
SUMMARY	32
LITERATURE CITED	33

LIST OF TABLES

	Page
Table I Distribution of Labeling in the Basidiomycete After the Administration of Succinic Semi- aldehyde (^{14}C)	12
Table II R_f Values of Organic Acids and Peaks 1 and 2	16

LIST OF FIGURES

		Page
Figure 1	Conversion of succinic semialdehyde ^{14}C to $^{14}\text{CO}_2$. . .	11
Figure 2	Radiochromatoscan of the 4 hr anion fractions from the feeding experiment.	14
Figure 3	Co-chromatography of succinic acid, succinic semi-aldehyde, and peak 1 with x-ray film overlay.	18
Figure 4	Evidence for the conversion of succinic semialdehyde to succinic acid within the organism.	20
Figure 5	Effect of pH on the activity of the crude extract . .	23
Figure 6	The effect of substrate concentration on the activity of the crude extract	26
Figure 7	Corresponding bands from disc-gel electrophoresis of a crude extract.	28

Abstract

It has been established that succinic semialdehyde was oxidized to succinic acid by a coenzyme dependent dehydrogenase found in brain and bacterial extracts. Stobel (1967) recently reported preliminary evidence that the enzyme was present in a psychrophilic basidiomycete (strain W-2, J. B. Lebeau, Lethbridge, Alberta, Canada). This preliminary evidence had not been further investigated. When a mycelial mat was incubated at 23°C with uniformly labeled succinic semialdehyde (^{14}C), 12.7% of the total ^{14}C administered was recovered as $^{14}\text{CO}_2$ providing evidence that succinic semialdehyde was metabolized by the fungus. Four mycelial mats equal in size were incubated with labeled succinic semialdehyde (^{14}C). Each was extracted at a specific time and these extracts were separated into fractions using Dowex columns. Labeling in the cation, anion, neutral, and cell wall fractions was found to increase with incubation time. Investigation of the most heavily labeled fraction, the 4 hr anion fraction, showed that there were two predominantly labeled organic acid peaks. Paper chromatography and two dimensional autoradiochromatography established that the two compounds were succinic semialdehyde and succinic acid. The labeling in both of these compounds increased as time increased. When labeled succinic semialdehyde (^{14}C) was mixed with an acetone enzyme preparation of the fungus, it was found that after the control had been subtracted 22.1% of the initial or labeled succinic semialdehyde had been converted to succinic acid. Boiled and frozen extracts of the fungus demonstrated a 100% loss of the activity that a control demonstrated. Using the acetone preparation, a soluble succinic semialdehyde dehydrogenase was characterized as to pH optimum, coenzyme specificity, substrate specificity, and K_m . Electrophoresis of an acetone preparation showed that there were three active electrophoretic forms of this enzyme. In summary it was found that succinic semialdehyde was metabolized and directed to all fractions of the fungus. Furthermore, the fungus was found to have a DPN dependent succinic semialdehyde dehydrogenase.

INTRODUCTION

Green (1942) first implicated succinic semialdehyde in intermediary metabolism. He reported that heart extracts were able to catalyze the anaerobic decarboxylation of α -ketoglutarate yielding succinic semialdehyde. Ochoa (1944) also worked on succinic semialdehyde metabolism using heart extracts. He found that aerobic decarboxylation produced a succinyl derivative. He was unable to isolate free succinic semialdehyde and concluded that only the anaerobic decarboxylation of α -ketoglutarate produced free succinic semialdehyde. Shemin and Wittenberg (1951) suggested that free succinic semialdehyde might be a precursor in protoporphyrin synthesis, but no evidence for this hypothesis has been forthcoming. Bessman, et al. (1953) using brain tissue extracts, discovered a γ -amino butyric acid transaminase yielding succinic semialdehyde and glutamate from γ -amino butyric acid and α -ketoglutarate. The discovery of this transamination reaction led to further work by Albers and Salvador (1958). They reported that rat brain extracts oxidized succinic semialdehyde to succinate. This reaction was dependent on the coenzyme DPN. Using cell free extracts of Pseudomonas, Scott and Jakoby (1958) found a transaminase functionally similar to that of Bessman. They also found a soluble dehydrogenase functionally similar to that of Albers and Salvador. The dehydrogenase was specific for succinic semialdehyde. The enzyme was both DPN and TPN dependent, although the TPN gave eight times more activity than DPN. Nirenberg and Jakoby (1960) characterized a DPN dependent γ -hydroxybutyric acid dehydrogenase from Pseudomonas. They also characterized two different

succinic semialdehyde dehydrogenases. One required DPN, the other TPN. Consequently succinic semialdehyde was shown to be a common product in two different pathways.

The primary results of this early work established that succinic semialdehyde was a free intermediate metabolite in several reactions. The investigators also characterized several enzymes immediately involved in the reactions of succinic semialdehyde. However, their work did not integrate these isolated reactions with functional metabolic pathways. The first attempts to integrate these isolated reactions with a biological scheme were Kretovich et al. (1966) and Strobel (1967). Kretovich, et al. demonstrated that succinic semialdehyde is metabolized in green soybean leaves and roots. This is the first work implicating succinic semialdehyde in plant metabolism. They fed succinic semialdehyde to green soybean leaves and roots and showed a marked increase in glutamine synthesis while the control leaves and roots did not show such an increase. Similar results were observed when the plants were fed γ -amino butyric acid. Marked decreases in free ammonia were noted in plants that had been fed succinic semialdehyde and γ -amino butyric acid. From these data they concluded that succinic semialdehyde and γ -amino butyric acid stimulated glutamine synthesis and are the precursors in this synthesis. Using a psychrophilic basidiomycete Strobel has shown that succinic semialdehyde is a precursor in the biosynthesis of glutamate. With labeled succinic semialdehyde (^{14}C) he found that labeled $\text{H}^{13}\text{C}^{15}\text{N}$ and ammonia react with succinic semialdehyde to form

4-amino-4-cyanobutyric acid. A nitrilase hydrolyzes the nitrile giving glutamate and ammonia. His evidence indicates that glutamate is eventually recycled to succinic semialdehyde by a decarboxylation and a deamination of glutamate. He also pointed out that succinic semialdehyde is oxidized by a DPN dependent dehydrogenase by crude extracts. Certainly one important aspect of these pathways is the oxidation of succinic semialdehyde to succinate. Hence, the purpose of this report is to present evidence for the following: 1) to confirm that succinic semialdehyde is converted to succinate in the psychrophilic basidiomycete, 2) to demonstrate some properties of this succinic semialdehyde dehydrogenase and 3) to implicate succinic semialdehyde in the general metabolism of a lower plant form.

MATERIALS AND METHODS

Culturing

The organism used for the research was a Type B strain of an unidentified psychrophilic basidiomycete supplied by J. B. Lebeau, Research Station, Canada Department of Agriculture, Lethbridge, Alberta. Mycelial mats three weeks old unless otherwise stated were used for both the ^{14}C labeling experiments and the enzyme studies. The stock culture was maintained on Potato Dextrose Agar at 10°C .

Materials

The labeled succinic semialdehyde was prepared from uniformly labeled glutamate (^{14}C) according to the method of Arnoff (1956). The labeled succinic semialdehyde was separated from the other soluble compounds in the mixture by chromatographing on Whatman #1 in n-butanol:acetic acid:water (4:1:5). The compound was then eluted from the paper with distilled water. The eluate was placed on a 1 x 3 cm Dowex 1 Column (formate form), 200-400 mesh, and rinsed with distilled water to remove contaminating cations. The succinic semialdehyde was eluted with 20 ml of 6N formic acid, dried, and stored in a vacuum desiccator. All other chemicals used were reagent grade.

General Methods

Protein was quantitatively determined by the method of Lowry, et al. (1951). All colorimeter measurements were made on a Bausch and Lomb Spectronic 20 Colorimeter.

Radioactivity Determinations

Radioactive samples were counted in a Nuclear Chicago Liquid Scintillation Counter, Model 6804. The solvent used in each counting vial consisted of 1.5 ml methanol and 13.5 ml of toluene containing 4.0 g of 2,5-diphenyloxayole and 100 mg of p-bis-2(5-phenyloxayolyl)-benzene per liter. The radioactive areas on the chromatogram were located by a Packard Radiochromatogram Strip Counter. After location these radioactive areas were cut out, shredded, and placed into a vial and counted. For all cases the counts were converted to dpms by the quench correction method using a standard curve.

Chromatography

Sheets of Whatman #1 and #541 were used for paper chromatography. The following solvent systems were used:

- 1) n-butanol-acetic acid- H_2O (4:1:5)
- 2) n-pentanol-5N formic acid (1:1)
- 3) ethanol- NH_4OH - H_2O (80:4:16)

The organic acid spots were located on the chromatograms by an acid-base indicator (Arnoff, 1956). The sugars were located using ammoniacal silver nitrate (Trevelyan et al., 1950). The amino acids were detected by spraying the chromatogram with 0.3% ninhydrin in 95% ethanol.

Administration of Succinic-Semialdehyde ^{14}C to Cultures

Mycelial mats equal in size were rinsed in sterilized distilled water. These mats were aseptically transferred to a 250 ml Erlenmeyer

flask. Uniformly labeled succinic semialdehyde (0.5 μ c) was added to the flask which was sealed with a sterile plug and incubated at room temperature for $\frac{1}{2}$, 1 and 4 hrs. Each mat was ground in a Sorvall Omnimixer at 16,000 rpm for one min. The homogenate was centrifuged at 14,000 x g for ten min to remove the precipitate. An equal volume of ethanol and water (2:1) was added to the supernatant liquid. The precipitate was removed by centrifugation at 20,000 x g for ten min. This supernate was then passed through a column of Dowex 50 (H^+ form, 2 x 3 cm) and then through a column of Dowex 1 (formate form, 2 x 3 cm). Ten ml of 6N HCl and 6N formic acid was added to the Dowex 50 and Dowex 1 columns, respectively, to remove the anion and cation fractions. These two fractions plus the neutral fractions were dried by flash evaporation and stored in an evacuated desiccator.

Conversion of Succinic-Semialdehyde ^{14}C to $^{14}CO_2$

A mycelial mat was rinsed in sterile distilled H_2O and was transferred to an altered 250 ml Erlenmeyer having a center well containing one ml of hyamine hydroxide. Uniformly labeled succinic-semialdehyde ^{14}C , .016 μ c, was added to the flask which was sealed with a sterile plug and incubated at 23°C. At specific time intervals the contents of the center well were removed and counted. An equal volume of fresh hyamine hydroxide was added, and the flask was resealed.

Succinic Semialdehyde Dehydrogenase Studies

Assay of Succinic Semialdehyde Dehydrogenase

Since the assay substrate succinic semialdehyde reduces DPN the activity of the enzyme was followed by measuring the increase in absorbance at 340 m μ in a Beckman D.U. Spectrophotometer with a 1 cm light path. The assay system consisted of 45 μ moles phosphate buffer, pH 8.5; 3 μ moles DPN, 3 μ moles mercaptoethanol, 60 μ moles succinic semialdehyde and .2 ml of enzyme preparation. A unit of activity is defined as a μ mole of substrate converted per min. Specific activity is defined as units per mg protein.

Preparation of Succinic Semialdehyde Dehydrogenase

Five mycelial mats were collected, drained, and washed in 0.05M phosphate buffer, pH 7.0 at 4°C. The mats were ground in a prechilled Servall Omnimixer for 30 seconds. The homogenate was centrifuged at 14,000 x g for ten min. The precipitate was discarded and acetone at -15°C was slowly added to the remaining supernate up to an equal volume. The precipitate was removed by centrifugation at 28,000 x g for ten min and was taken up in 5.0 ml of 0.05M phosphate buffer, pH 7.0, that was reduced with mercaptoethanol to protect possible labile sulphide bonds. The precipitate was removed by centrifugation at 25,000 x g for ten min.

Electrophoresis of Succinic Semialdehyde Dehydrogenase

One and six-tenths mg of protein was subjected to disc gel electrophoresis according to the method of Ornstein and Davis (1964). The protein was submitted to electrophoresis at 2.5 milliamps per gel until the initial boundary was one inch into the small pore gel. One gel was treated with a mixture of nitroblue tetrazolium in order to locate succinic semialdehyde dehydrogenase activity. A second gel was stained with aniline blue black to detect protein. These two gels were scanned in a Joyce Chromoscan Densitometer.

EXPERIMENTAL RESULTS

Demonstration of Succinic Semialdehyde Metabolism

Collection of $^{14}\text{CO}_2$

Uniformly labeled ^{14}C (35,000 dpms) of succinic semialdehyde was fed to a fungal mat to determine if it is metabolized. CO_2 was collected at specific time intervals and counted. After incubation for 48 hrs at 23°C at least 12.7% of the original ^{14}C in succinic semialdehyde was collected as $^{14}\text{CO}_2$. $^{14}\text{CO}_2$ production was immediate in that 41.1% of the total $^{14}\text{CO}_2$ collected was given off by the 6th hr of incubation (Fig. 1).

Distribution of Labeling in Four Fractions

Uniformly labeled ^{14}C (1.1×10^6 dpms) succinic semialdehyde was fed to 4 fungal mats of equal size in order to determine the distribution of labeling. At specific times each mat was extracted and fractioned as previously described. The cation, anion, neutral, and cell wall fractions of the mycelia were examined for radioactivity. The results show that as incubation time increased, there was a concurrent increase of labeling in all four fractions (Table I).

Examination of the Organic Acid Fraction

The four hour organic acid fraction was separated by paper chromatography in solvent 3 and scanned to locate the radioactivity. Although several peaks of radioactivity were observed, two predominated (Fig. 2).

Figure 1 Conversion of succinic semialdehyde ^{14}C to $^{14}\text{CO}_2$.

Uniformly labeled ^{14}C (35,000 dpm) was fed a fungal mat and CO_2 collected in a center well containing 1 ml of hyamine hydroxide.

Table I

Distribution of Labeling in the Basidiomycete
After the Administration of Succinic Semialdehyde (^{14}C).

Time	Cation	Anion	Neutral	Cell Wall
½ hr	12,220	43,700	800	38.4 $\frac{\text{dpm}}{\text{mg}}$
1 hr	26,400	52,800	6,400	20.4
2 hr	12,600	59,540	12,600	21.5
4 hr	15,340	80,660	34,600	43.0

Figure 2

Radiochromatoscan of the 4 hr anion fraction from the feeding experiment.

Radioactive material from the 4 hr anion fraction (Experimental Results) was chromatographed on Whatman #1 in solvent system 3. The radioactive areas on the paper were located and recorded by a Packard Radiochromatogram Strip Counter.

Table II shows that the R_f values of these two peaks corresponded to succinic semialdehyde and succinic acid. Further confirmation on peak 1 was done by two dimensional co-chromatography in solvents 2 and 3 using authentic succinic acid as a reference. Autoradiography of the chromatogram using Kodak No-Screen X-ray film revealed that the exposed spot on the x-ray film corresponded to the succinic acid spot on the chromatogram, (Fig. 3).

Conversion of Succinic Semialdehyde to Succinic Acid by Fungal Mats

Figure 4 shows that as the incubation time increased there was a corresponding increase in labeled succinic semialdehyde within the cells. It also suggests that labeled succinic semialdehyde may be directly converted into succinic acid upon entry into the cell.

Enzyme Studies

The previous studies provided evidence that succinic semialdehyde may be converted to succinic acid within the cell. Enzyme studies demonstrated the presence of a succinic semialdehyde dehydrogenase. A 1.2 ml fraction of crude mycelial extract was added to 6.25 μ moles of uniformly labeled ^{14}C succinic semialdehyde (150,000 dpm), 31 μ moles of pyrophosphate buffer, pH 8.5; 68 μ moles of DPN, 25 μ moles of mercaptoethanol and incubated at 23°C. The control contained all of the above ingredients minus the crude extract in a .5 ml volume, and was incubated at 22°C for 4 hrs. Aliquots of .5 ml were removed from the reaction vessel at $\frac{1}{2}$, 1, and 4 hr time intervals. These fractions were

Table II

 R_f Values of Organic Acids and Peaks 1 and 2

	Pentanol-Formic Acid	$\text{NH}_4\text{OH-Ethanol-H}_2\text{O}$
Succinate	.68	.58
Succinic Semialdehyde	.68	.73
Peak 1	.68	.58
Peak 2	.68	.73
γ -Hydroxy Butyric Acid	.67	.87
Malate	.34	.53

Figure 3 Co-chromatography of succinic acid, succinic semialdehyde, and peak 1 with x-ray film overlay.

Succinic semialdehyde, succinic acid, and the labeled eluate of peak 1 were spotted on Whatman #1 and chromatographed. After chromatography the paper was overlaid with x-ray to locate the radioactive material.

Figure 4 Evidence for the conversion of succinic semialdehyde to succinic acid within the organism.

Radioactive succinic semialdehyde (0.5 μ c) (- - - -) which were extracted and examined for its conversion to succinic acid (————) within the cell (Experimental Results).

precipitated with 2 volumes of ethanol (95%), and centrifuged to remove the precipitate. The supernate was dried and chromatographed in solvent system 3. The labeled compounds were located, eluted from the paper, and counted. The results show that after subtracting the control from the 4 hr fraction, 22.1% of the initial succinic semialdehyde (^{14}C) had been converted to succinic acid.

Purification of Succinic Semialdehyde Dehydrogenase

Since the first step in the metabolism of succinic semialdehyde appeared to be its enzyme mediated dehydrogenation to succinic acid, attempts were made to purify and characterize a succinic semialdehyde dehydrogenase. However, the instability of the enzyme thwarted most attempts to purify it beyond the preparation of an acetone powder. Hence, all enzyme work was done with acetone powder extracts.

1) Inactivation and Stability of the Enzyme The acetone extract was solubilized in 0.05M phosphate buffer, pH 7.0: and reduced with mercaptoethanol (0.005M). While one fraction was used as a control, a second was frozen for 24 hrs, and a third was boiled. The boiled and frozen fractions both lost 100% of the activity of the control when checked for activity.

2) Effect of pH on Activity The standard reaction mixture was employed except that the following buffer systems were used at the designated ranges: phosphate buffer (pH 6.5 - 7.5) and pyrophosphate buffer (pH 8.0 - 9.5). The pH optimum of the enzyme was 8.5 (Fig. 5).

Figure 5 Effect of pH on the activity of the crude extract.

The standard assay procedure was used with the exception that 45 μ moles of the following buffer systems were used at the designated pH ranges: phosphate buffer (pH 6.5 - 8.0), pyrophosphate buffer (pH 8.0 - 9.5). The activity is expressed as millimicromoles of DPNH per 10 min.

3) Coenzyme Specificity The standard reaction mixture was employed except that TPN was substituted for DPN. No activity was observed with TPN.

4) Substrate Specificity The standard reaction mixture was used; however, equal molar amounts of various substrates were substituted for succinic semialdehyde. Succinic semialdehyde dehydrogenase activity was checked on butyraldehyde glyoxylic acid, and glycoaldehyde. No dehydrogenase activity was observed with the substrates.

5) Effect of Substrate Concentration on Enzyme Activity The reaction mixtures used were identical to the standard assay mixture with the exception that the substrate concentration was varied between .025M and .2M. Figure 6 indicates the effect substrate concentration of succinic semialdehyde dehydrogenase activity plotted to the method of Lineweaver and Burke (1934). The K_m value was 2.2×10^{-2} moles/liter.

6) Induction Attempts to induce enzyme activity with γ -hydroxy butyric acid, γ -amino butyric acid, and succinic acid were unsuccessful.

Electrophoretic Forms of Succinic Semialdehyde Dehydrogenase

As previously described an acetone powder extract was submitted to electrophoresis as a further purification step although the enzyme from this procedure was not used for assay work. The results in Figure 7 show that 3 bands corresponded with 3 protein bands indicating that succinic semialdehyde dehydrogenase might exist in three electrophoretic forms.

Figure 6 The effect of substrate concentration on the activity of the crude extract.

The standard assay procedure was used, however, the substrate concentration was varied between .025M and .2M. The units of $1/V$ and $1/S$ are $1/\mu\text{moles}/\text{min}$ and $1/M$ respectively.

Figure 7

Corresponding bands from disc-gel electrophoresis of a crude extract.

1.6 mg protein was submitted to electrophoresis at 2.5 milliamps per gel until the initial boundary was one inch into the small pore gel. One gel was treated with nitroblue tetrazolium, the other with aniline blue black. Both were scanned in a Joyce Chromoscan Densitometer.

DISCUSSION

Strobel (1967) reported that succinic semialdehyde was metabolized in a psychrophilic basidiomycete and preliminary evidence that showed that succinic semialdehyde was oxidized to succinic acid. However, no further work was done to verify this latter observation.

In Fig. 4 there is an increase in succinic semialdehyde (^{14}C) within the cell in conjunction with an increase in incubation time suggesting that succinic semialdehyde was being transported into the cell rather than moving freely through it. After arriving in the cell it appears to be directed to one or more decarboxylating reactions, since 40% of the total ^{14}C administered was collected in the first six hours of incubation, (Fig. 1). In Table I it is evident that the greatest amount of labeling was in the anion and cation fractions. Consequently it is suspected that succinic semialdehyde was being converted to glutamic acid according to the pathway proposed by Strobel (1967) and also directed to the TCA cycle via succinic acid. Since the organism produces HCN it could react with the newly introduced succinic semialdehyde and ammonia yielding 4-amino-4-cyanobutyric acid. However, it appears as though most of the succinic semialdehyde is oxidized to succinic acid (Table I). It should be noted that both pathways contain decarboxylating reactions. The rate of $^{14}\text{CO}_2$ evolution fell off in the fungal mat (Fig. 1) presumably because the labeled succinic semialdehyde in the media was being depleted and the labeled material within the cell was rapidly being directed to anabolic processes since it was the only carbon source available to the organism.

It is evident that over the four hr period the ^{14}C skeleton was used through the metabolic processes of the organism since ^{14}C was found in the four major functions. The heavy labeling found in the organic acid fraction suggests that succinic semialdehyde was rapidly being converted into one or more organic acids. Previous "in vitro" work by Nirenberg and Jakoby (1960) and Strobel (1967) suggested that succinic acid γ -hydroxybutyric acid were the immediate products of succinic semialdehyde metabolism. Attempts to find labeled γ -hydroxybutyric acid in cultures fed succinic semialdehyde (^{14}C) were unsuccessful. However, the identification of succinic acid as the major labeled organic acid (Fig. 3) supports the finding of Strobel (1967) of a succinic semialdehyde dehydrogenase in this organism.

The studies with cell free extracts revealed that the conversion of succinic semialdehyde was an enzymic process carried out by one or more dehydrogenases. It was also demonstrated that the enzyme would not stabilize according to the methods used by Jakoby and his workers (1960). The enzyme is specific for DPN and succinic semialdehyde whereas two succinic semialdehyde dehydrogenases found by Nirenberg and Jakoby (1960) would react with TPN. However, it has an optimum of 8.5 that agreed with the pH optimum of the Pseudomonas enzymes.

The electrophoretic studies showed that either there are several succinic semialdehyde dehydrogenases or that there is one isozyme that broke down into active subunits (Fig. 7).

The results of this study support the hypothesis that succinic semialdehyde is metabolized by a psychrophilic basidiomycete via succinic acid and other reactions. This report is the first, to the author's knowledge, in which succinic semialdehyde has been implicated as a bona fide substrate in the overall metabolism of a microorganism.

SUMMARY

A psychrophilic basidiomycete was found to metabolize succinic semialdehyde (^{14}C). The ^{14}C was found in cation, anion, and neutral fractions of the fungus. Further studies showed that the major portion of succinic semialdehyde was oxidized to succinic acid. Using a crude extract a soluble succinic semialdehyde dehydrogenase was characterized as to stability, pH optima, K_m , substrate specificity, coenzyme specificity, and induction. Electrophoresis of the crude extract showed that either there are several enzymes or several forms of the same enzyme in the fungus that will oxidize succinic semialdehyde.

LITERATURE CITED

- Albers, R. W. and R. A. Salvador. 1958. Succinic semialdehyde oxidation by a soluble dehydrogenase from the brain. *Science* 128: 359-360.
- Aronoff, S. 1956. Techniques of Radiobiochemistry, Iowa State University Press. pp. 119.
- Bessman, S. P., Rossen, J., and Layne, E. C. 1953. γ -Amino butyric acid-glutamic acid transamination in brain. *Jour. Biol. Chem.* 201: 385-391.
- Green, D. E., Westerfield, W. W., Vennesland, B., and Knox, W. E. 1942. Carboxylases of animal tissue. *Jour. Biol. Chem.* 145: 69-84.
- Kretovich, V. L., Karyakina, T. I., Lyubimova, N. V. and Neronova, A. N. 1966. Succinic semialdehyde - A precursor of glutamine in plants. *Koklady Akademii Nauk SSSR*, Vol. 176, No. 5, pp. 1212-1215.
- Lowry, O. H., N. J. Rosebrough, A. L. Fon, and Rose J. Randall. 1951. Protein measurement with the Folin phenol reagent. *Jour. Biol. Chem.* 193: 265-275.
- Nirenberg, M. W. and Jakoby, W. B. 1960. Enzymic utilization of γ -hydroxybutyric acid. *Jour. Biol. Chem.* 235: 954-960.
- Ochoa, S. 1944. α -ketoglutaric dehydrogenase of animal tissues. *Jour. Biol. Chem.* 155: 87-100.
- Shemin, D. and Wittenberg, J. 1951. Mechanism of protoporphyrin formation. *Jour. Biol. Chem.* 192: 315-334.
- Scott, E. M. and Jakoby, W. B. 1958. Pyrrolidine metabolism soluble γ -amino butyric transaminase and semialdehyde dehydrogenase. *Science* 128: 361.
- Strobel, G. A. 1967. 4-Amino-4-cyanobutyric acid as an intermediate glutamate biosynthesis. *Jour. Biol. Chem.* 242: 3265-3269.
- Trevelyan, W. E., Procter, D. P., and Harrison, J. S. 1950. Detection of sugars on paper chromatograms. *Nature* 166: 444-445.
- Ward, E. W. B., and Lebeau, J. B. 1962. Autolytic production of hydrogen cyanide by certain snow mold fungi. *Can. J. Bot.* 40: 85-88.

MONTANA STATE UNIVERSITY LIBRARIES
3 1762 10013421 0

N378 Davies, P.N.
D288 The metabolism of
cop.2 succinic semialdehyde
by a psychrophilic
basidiomycete

NAME AND ADDRESS

N378
D288
cop.2