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"Selection of Mutant Strains of
Neurospora crassa."

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ABSTRACT

Several attempts to isolate mutant strains of Neurospora crassa which were unable to grow on hypoxanthine were made and no mutants were obtained. Transport studies showed that hypoxanthine was transported by only one system in germinated conidia of N. crassa.

INTRODUCTION

The objective of this research is to develop mutant strains of the fungus, Neurospora crassa which lack the ability to transport hypoxanthine into the cell. These strains may be used for examining the mechanisms by which purine bases are transported through the cell membrane. A clear understanding of purine transport could lead to a treatment of human diseases of purine metabolism such as gout and Lesch-Nyhan syndrome.

Both Lesch-Nyhan disease and gout are caused by excessive amounts of hypoxanthine being excreted from cells into the blood stream. This hypoxanthine is changed to urate which is rather insoluble and forms crystals which are deposited in and around the foot joints causing extreme pain. Only about 0.5 grams of uric acid is excreted daily by the normal person, although up to 5 grams of free purines are formed daily. Evidently the greater part of free purines is salvaged by normal individuals. Uric acid is present in blood largely as monosodium urate, however, both the free acid and salts are insoluble in water with the result that in individuals with gouty arthritis, uric acid precipitates and crystallizes in the urine or in cartiliginous tissues, to produce the disease gout. Absence of one of these salvage pathways results in the Lesch-Nyhan syndrome, a rare genetic disorder in which there is a lack of the enzyme hypoxanthine (guanine) phosphoribosyltransferase. If the mechanism of hypoxanthine transport can

be determined, it may be of great value in providing leads for human research in these diseases.

EXPERIMENTAL PROCEDURE

1. General procedure for mutagenesis

The following filtration-enrichment procedure was used for inducing mutations with either UV light (120 sec., 98% killing) or EMS (3%, 40 min., 95% killing) as the mutagen. 2×10^8 Freshly grown conidia were mutagenized in 20 ml of H_2O, added to 200 ml of selective medium, and the colonies which grow were filtered off at 8-12 h intervals for two days. Survivors were then either plated 0.2 ml/plate on non-selective medium, or isolated directly from the original flask after addition of a growth-permissive compound. Each putative mutant was retested for mutant phenotype, and those that passed were crossed to wild type to determine the segregation patterns of progeny.

2. Selection scheme for specific mutants (e.g., mutants lacking ability to transport hypoxanthine).

A number of selection schemes were used to select for mutants deficient in hypoxanthine transport. Because a separate uptake system for adenine is known to exist, ad-1 auxotrophic strains were used that are able to use adenine but not hypoxanthine for growth (i.e., mutagenized conidia that will grow in hypoxanthine will be filtered off, and survivors that grow on adenine will be isolated). Essentially the same scheme was used in wild type (normal). Here mutants were selected for the ability to use adenine but not exogenous hypoxanthine as a nitrogen source.

3. Transport studies

Two ml of germinated conidia (4 x 10^6/ml) were added to 2 ml of a reaction mixture containing a known concentration of labeled compound (and competitor if inhibition was being tested). The mixture was incubated for a time period within the range known to give linear uptake and the contents were collected on a glass fiber filter. The filters were washed and dried thoroughly for radioactivity counting by liquid scintillation methods.

RESULTS

Several attempts to isolate mutant strains by the mutagenesis procedure described above failed to yield any mutants unable to utilize hypoxanthine as a nitrogen source. The time and intensity of U.V. irradiation produced 95-99% killing, generally optimal conditions for producing and recovering mutant strains. It now appears as if more than 500 survivors should have been picked and tested. This would have increased our chances of finding a mutant strain.

Transport studies using conidia from different purine requiring mutants of N. crassa confirmed that hypoxanthine is actively transported. It appears that hypoxanthine is taken up by a single system in ad-1 as in wild type strains. As shown in Fig. 1, ^{14}C-hypoxanthine uptake is inhibited by adenine, guanine and dinitrophenol in the ad-1 strain. However, in ad-8 strains ^{14}C-hypoxanthine is not transported at all but inhibits uptake of ^{14}C guanine.

DISCUSSION

Failure to obtain mutants unable to grow on hypoxanthine as sole nitrogen source was probably due to several factors. Picking about

1000 potential mutants from the filtration medium would have increased chances of finding a mutant several-fold but the amount of work required to test these would be prohibitive in the time allotted for Bich 485. The possibility remains, however, that mutant strains with this phenotype are not obtainable, perhaps because more than one gene is involved in determining protein structure for each step.

Since the initial intent of this study was to select a mutant unable to transport hypoxanthine, we were concerned that more than one defective gene product may be required before hypoxanthine transport is halted. Therefore, we set out to repeat some experiments which had supposedly shown that hypoxanthine is transported into N. crassa cells by a single transport system (1). Our results confirmed this and also suggested that hypoxanthine participated in non-productive binding to the guanine-specific transport system in ad-8.

LITERATURE CITED

1. Magill, J. M. and C. W. Magill. 1975. Purine base transport in Neurospora crassa. J. Bacteriol. 124:149-154.

Fig. 1 a. Transport of hypoxanthine into germinated conidia of ad-1 strain

● ^{14}C hypoxanthine alone

○ ^{14}C hypoxanthine + 1mM adenine

△ ^{14}C hypoxanthine + 1mM guanine

□ ^{14}C hypoxanthine + 1mM 2,4 DNP

Fig. 1 b. Transport of guanine into germinated conidia of wild type strain, 74A, and ad-8.

○ 74A (wild type)

● ad-8

Fig. 2 a. Transport of ^{14}C guanine into germinated conidia of ad-8 strain.

● ^{14}C guanine alone

Fig. 2 b. Transport of ^{14}C guanine into germinated conidia of ad-1 strain.

○ ^{14}C guanine alone

Fig. 1 Hypoxanthine uptake in germinated conidia.

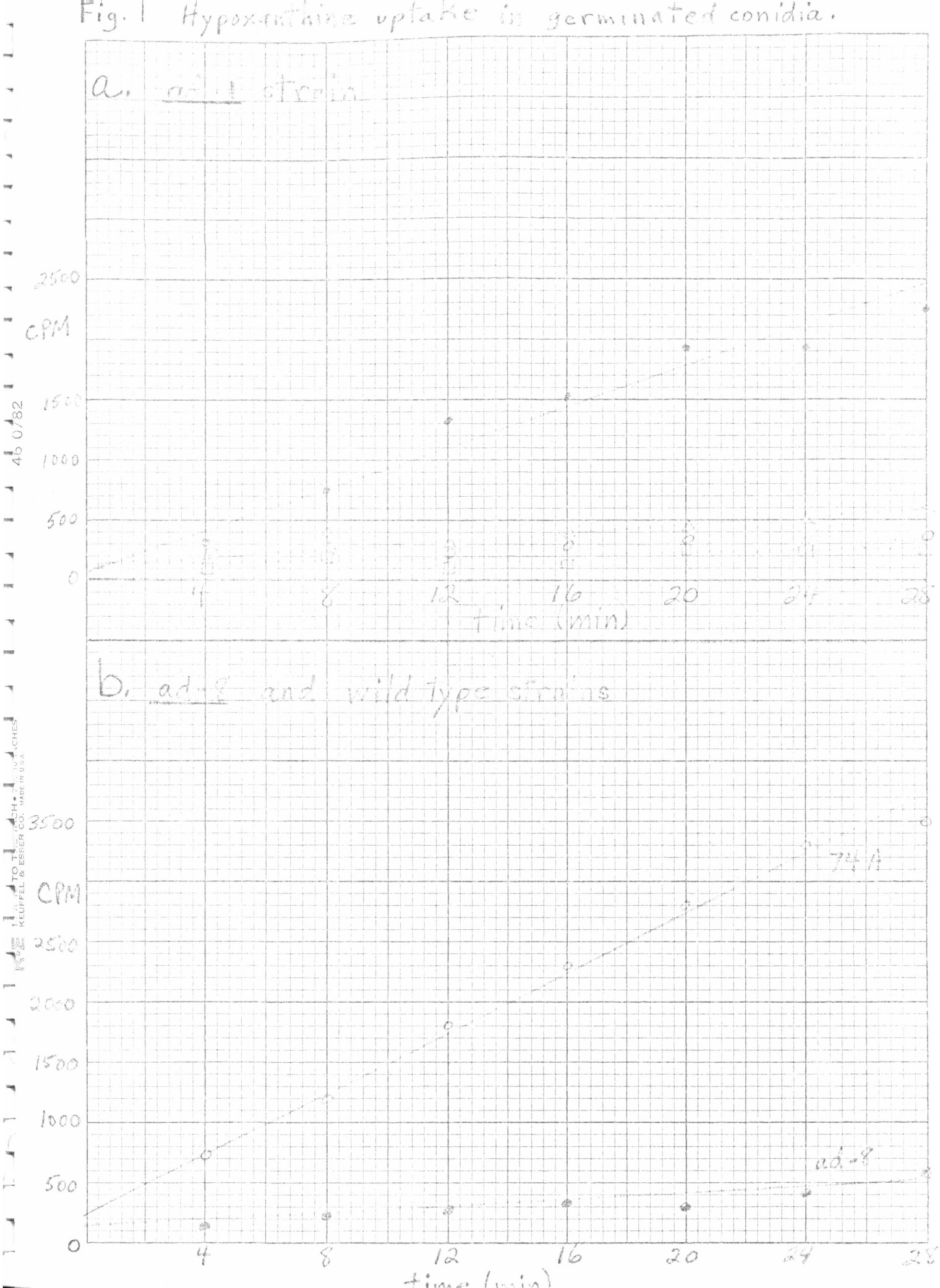

Fig. 2. Guanine uptake in germinated conidia