THE PRODUCTION OF ACETATE FROM FATTY ACIDS BY NEUROSPORA[1]

JOSEPH LEIN AND PATRICIA S. LEIN

Department of Zoology, Syracuse University, Syracuse, New York

Received for publication May 22, 1950

It has been reported that *Neurospora* can use a variety of compounds as a carbon source including both saturated and unsaturated fatty acids (Beadle, 1945). In fact, mutants of this organism have been isolated that differed from the wild type inasmuch as they could not use these compounds. One was unable to metabolize any of the fatty acids tested, whereas another could utilize saturated but not unsaturated fatty acids (Horowitz *et al.*, 1945). Unfortunately these mutants were lost before they could be studied in detail (Beadle, personal communication).

In our laboratory a series of six *Neurospora crassa* mutants that grew in the presence of acetate but not in its absence was isolated. These mutants could also use certain fatty acids as a source of acetate. It was thought that such acetate mutants would be good tools for the study of the metabolic breakdown of fatty acids to acetate, and consequently experiments along these lines were performed. Data obtained with only one of these mutants will be presented, for all the mutants gave essentially similar results.

METHODS

Isolation of the mutant

The mutant designated as S-210 was obtained by use of the selection technique developed by Lein, Mitchell, and Houlahan (1948). It was isolated on minimal medium supplemented with oleic acid. Unlike the true fatty acid mutants, which do not grow in acetate (Lein and Lein, 1949), this mutant grew in acetate. It was considered to be an acetate mutant that could obtain its acetate from degradation of oleic acid. The reasons for this conclusion will be presented below. The mutant was crossed to a wild type of the opposite mating type. Ascospores were isolated in order from 10 asci and tested to determine which had acetate requirements. Typical Mendelian segregation occurred in all asci; consequently it was established that the requirement was associated with a single gene mutation.

Materials and procedures

The unsaturated fatty acids used were obtained from the Hormel Foundation and were of very high purity. The saturated fatty acids were obtained from the Eastman Kodak Company, the stearic and palmitic acids being recrystallized from ethyl alcohol before use.

The fatty acids were added in an emulsion form. The acids were first dissolved

[1] This work was supported by a grant from the Williams-Waterman Fund.

in 100 per cent ethyl alcohol, and these solutions were diluted with appropriate amounts of hot distilled water. The emulsification was performed with a Waring blender.

For the growth studies, one drop of a conidial suspension was used for inoculation. This was added to 125-ml flasks containing 20 ml of minimal medium (Beadle and Tatum, 1945) supplemented with the growth factor. The flasks had previously been sterilized by being autoclaved at 15 pounds for 10 minutes. The inoculated flasks were incubated at 25 C for 7 days; the mycelia were removed, pressed out, dried at 100 C, and weighed.

RESULTS

In order to establish that S-210 was an acetate mutant rather than a fatty acid mutant, an experiment was carried out in which S-210 and a wild type mold, 7A,

TABLE 1

Growth of wild type (7A) and acetate mutant (S-210) with acetic acid or oleic acids as sole carbon source

SUPPLEMENT (25 MG)	STRAIN	GROWTH
		mg
None	7A	0.9
	S-210	0.8
Acetic acid	7A	3.6
	S-210	3.4
Oleic acid	7A	4.1
	S-210	5.3

were grown in oleic acid and acetic acid as the sole carbon sources. If growth occurred with oleic acid as the sole carbon source, then presumably the mold had the necessary enzyme systems to degrade the fatty acid to short chain compounds including acetate. The results of such an experiment are presented in table 1. Small quantities (25 mg) of the carbon source were added so that inhibition phenomena would not mask growth. In this experiment no alcohol was added to dissolve the fatty acid, but the emulsion of the fatty acid was made directly in the Waring blender.

It will be seen from the results that both the wild type (7A) and the mutant can utilize acetic acid and oleic acid as a carbon source. The small amount of growth obtained in the control salt solution was due to small amounts of impurities containing utilizable carbon. These results tended to confirm the view that S-210 was an acetate mutant capable of obtaining its acetate from the breakdown of oleic acid.

Before experiments could be carried out to determine which of the fatty acids would enable the mutant to grow, it was necessary to determine how toxic the various fatty acids were to *Neurospora*. If a particular fatty acid was very toxic, then its ability to enable the mutant to grow might be masked. A series of ex-

periments was carried out to test the toxicity of the even-numbered fatty acids at three different concentrations, 0.1 mg, 0.5 mg, and 1.0 mg per 20 ml of minimal medium. The results are shown in figure 1, where the term "inhibition constant" is equal to the average percentage of inhibition at the three concentrations. Each of the bars represents a different fatty acid. The number before the C indicates the length of the carbon chain, and the number before the double bond indicates the unsaturated fatty acid, i.e., oleic, linoleic, or linolenic acid.

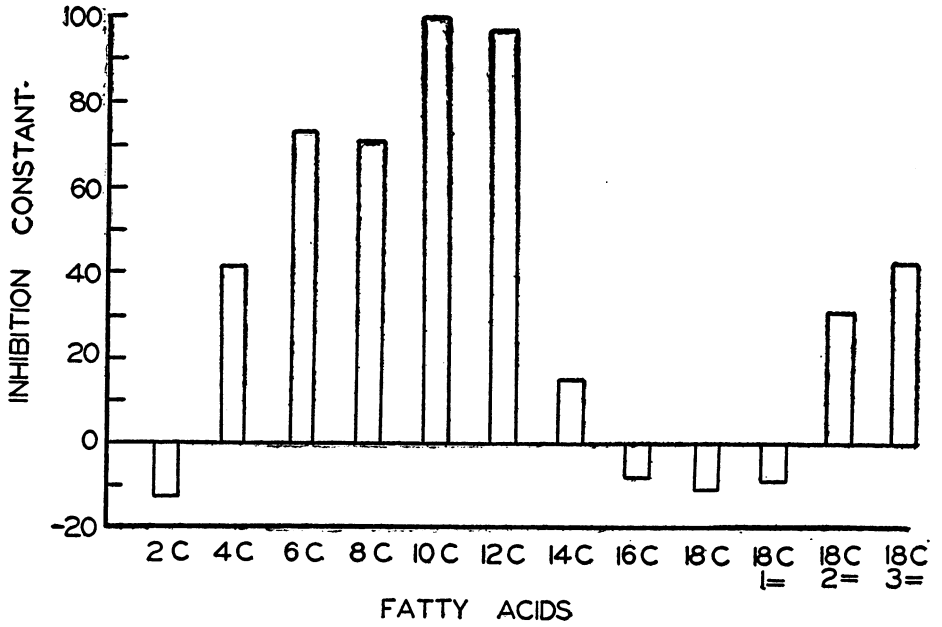

Figure 1. Extent of inhibition of the wild type (7A) by various saturated and unsaturated fatty acids. Number before C indicates carbon chain length; number before = indicates double bond number, i.e., oleic, linoleic, or linolenic acid. Inhibition constant is calculated as average percentage of inhibition at 0.1, 0.5, and 1 mg fatty acid per 20 ml minimal medium.

The data indicate that the short chain saturated fatty acids from butyric acid (C-4) to lauric acid (C-12) are very toxic to *Neurospora*. Also, toxicity increased as the degree of unsaturation was increased in the 18 carbon fatty acids. Because of the toxicity of some of the fatty acids, the latter had to be supplied in very low concentrations when tested for their ability to serve as an acetate source.

Growth studies using the relatively nontoxic fatty acids are presented in figure 2. It will be seen that the mold can use myristic, oleic, linoleic, and linolenic acids for growth. The toxicity of linolenic acid at the highest concentration inhibits growth totally. Stearic acid and palmitic acid were totally ineffective at all concentrations tried although these fatty acids are not at all toxic. This lack of effect cannot be due to a failure of these compounds to get into the cells because if they are supplied to the wild type they increase the amount of growth by about 10 per cent.

The toxic fatty acids were tested at concentrations of 0.01 mg to 1 mg per 20 ml of minimal medium. Although none gave large amounts of growth at these low concentrations, all enabled the mutant to grow at some concentration.

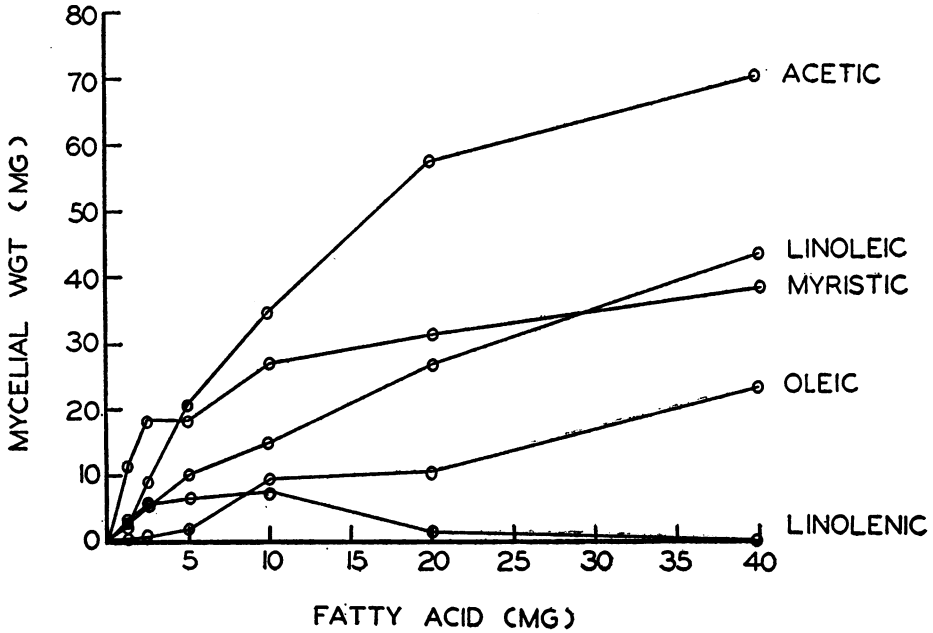

Figure 2. Growth curves of various fatty acids. Stearic and palmitic acids tested at these concentrations gave no growth.

TABLE 2

Growth of wild type (7A) and acetate mutant (S-210) with palmitic or stearic acids as sole carbon source

SUPPLEMENT (25 MG)	STRAIN	GROWTH
		mg
None	7A	0.9
	S-210	0.8
Palmitic acid	7A	0.3
	S-210	0.5
Stearic acid	7A	0.5
	S-210	0.4

The results indicate that of all the fatty acids tested, only stearic and palmitic acids do not enable the mutant to grow. These fatty acids presumably are not degraded into acetate. In order to test this further, experiments were carried out in which stearic acid and palmitic acid were supplied as the sole carbon source in a concentration of 25 mg per 20 ml of minimal medium. The results are presented

in table 2, and comparison should be made with the results in table 1, in which similar experiments using acetic acid and oleic acid as carbon sources are presented. Confirming the growth studies, the carbon source experiments indicate that stearic and palmitic acid cannot serve as carbon sources and presumably are not degraded.

A peculiarity of the S-210 mutant is its susceptibility to inhibition by two amino acids, arginine and lysine. Experimental results showing this are presented in table 3. The results indicate that very small concentrations of L-arginine and L-lysine cause a considerable inhibition of growth, but increasing the concentration never completely suppresses it. Lysine is more efficient in producing this inhibition. Resistance to inhibition occurs in a small number of flasks, possibly through mutation. The mechanism of the inhibition produced by the two amino acids has not been determined.

TABLE 3

Inhibition of S-210 by L-*arginine and* L-*lysine*

(Duration of experiment, 5 days; acetic acid concentration, 20 mg per 20 ml minimal medium)

AMINO ACID	MYCELIAL WT	
	Arginine	Lysine
mg	*mg*	*mg*
0.0	42.1	42.1
0.125	10.3	2.6
0.25	6.8	1.8
0.5	36.7	1.6
1.0	8.3	1.7
2.0	10.8	1.7

DISCUSSION

It is interesting that the ability to produce acetate from particular fatty acids and the ability to use the fatty acid for a carbon source are associated with each other. It suggests that the organism, in utilizing fatty acids for a carbon source, breaks down the fatty acid to acetate, which then can be used for anabolic reactions as well as serve as an energy source. Although the term "acetate" has been used extensively in referring to the degradation product of fatty acids, it is of course possible that the actual product itself is not acetate but a substance closely related to it such as acetyl phosphate. There is no way to determine this from the data.

Although palmitic and stearic acids have been termed catabolically inert in relation to the formation of acetate, this does not mean that they cannot be used by the organism. Growth experiments with the wild type indicate that stearic and palmitic acid increase the mycelial weight by as much as 10 per cent. Obviously, these compounds are being used for some metabolic process. They are, however, inert in relation to the formation of acetate in *Neurospora*. There are

reports in the literature that these fatty acids are metabolized by other microorganisms. Thus, Oginsky *et al.* (1950) have reported that palmitic and stearic acids increased the oxygen consumption of resting cells of *Mycobacterium tuberculosis*, whereas myristic acid did not. This contrasts with *Escherichia coli*, which could oxidize both stearic acid and myristic acid. These results, with those presented in the present paper, indicate that the metabolic breakdown of the various fatty acids may be mediated by different enzymes. The absence of the particular enzymes involved in such reactions may be characteristic of certain microorganisms.

SUMMARY

A *Neurospora* mutant was isolated that could not grow unless it was supplied with acetate. This mutant could obtain its acetate from the degradation of fatty acids. It was used to determine which fatty acids could be degraded to acetate by *Neurospora*, and it was found that all of the naturally occurring fatty acids tested except palmitic and stearic acid could be used as acetate sources.

REFERENCES

BEADLE, G. W. 1945 Genetics and metabolism in *Neurospora*. Physiol. Revs., **25**, 643–663.

BEADLE, G. W., AND TATUM, E. L. 1945 *Neurospora*. II. Methods of producing and detecting mutations concerned with nutritional requirements. Am. J. Botany, **32**, 678–686.

HOROWITZ, N. H., BONNER, D., MITCHELL, H. K., TATUM, E. L, AND BEADLE, G. W. 1945 Genic control of biochemical reactions in *Neurospora*. Am. Naturalist, **79**, 304–317.

LEIN, J., AND LEIN, P. S. 1949 Studies on a *Neurospora* mutant requiring unsaturated fatty acids for growth. J. Bact., **58**, 595–599.

LEIN, J., MITCHELL, H. K., AND HOULAHAN, M. B. 1948 A method for selection of biochemical mutants of *Neurospora*. Proc. Natl. Acad. Sci. U. S., **34**, 435–442.

OGINSKY, E. L., SMITH, P. H., AND SOLOTOROVSKY, M. 1950 The action of streptomycin. IV. Fatty acid oxidation by *M. tuberculosis*, avian type. J. Bact., **59**, 29–44.