STUDIES ON A NEUROSPORA MUTANT REQUIRING UNSATURATED FATTY ACIDS FOR GROWTH[1]

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Although many *Neurospora* mutants requiring water-soluble growth factors have been found (Beadle, 1945; Horowitz *et al.*, 1945; Tatum, 1946), there have been no cases reported of mutants requiring lipid-soluble factors. This was thought strange in view of the discovery of fatty acid requirements for various microorganisms (Hutner, 1942; Hutchings and Boggiano, 1947; Williams and Fieger, 1947; Williams, Broquist, and Snell, 1947). It occurred to the authors that such mutants might have been overlooked by the usual techniques of isolation of *Neurospora* mutants (Beadle and Tatum, 1945). A new selection technique was employed with the view of specifically isolating mutants having fatty acids as growth requirements. With such a technique a mutant requiring unsaturated fatty acids has been obtained and its growth characteristics have been studied.

METHODS

Isolation of the mutant. The selection technique used to obtain the mutant was that developed by Lein, Mitchell, and Houlahan (1948). The method consists essentially of picking probable mutants from mixtures of wild type and mutant ascospores germinating on minimal medium. The mutants are readily distinguishable and are placed in minimal medium supplemented with the appropriate growth factor. The cultures that grow are tested to determine whether this growth factor is a necessary requirement.

The details of the procedures used that yielded the mutant are as follows: Conidia from 10 test tube cultures of *Neurospora crassa* (strain 7A, isolated from a cross between 5256A and 5297a) were suspended in distilled water and rayed with a "sterilamp" for a time sufficient to kill 90 to 95 per cent of the conidia. The rayed conidia were added to cultures of the opposite mating type (strain 8a, also isolated from a cross between 5256A and 5297a) that had been growing for 5 days in 20 petri plates on a medium developed by Westergaard and Mitchell (1947). Profuse perithecia were formed and mutants were isolated by the method cited above. A total of 1,201 probable mutants were picked over a 10-day period and placed on a medium consisting of minimal medium (Beadle and Tatum, 1945) supplemented with 500 mg per cent of linoleic acid added in emulsion form. Of the number picked, 201 yielded cultures, and these were tested for a fatty acid requirement. One of these cultures had such a requirement and was designated S-11. The culture was crossed to a wild type of the opposite mating type. Ascospores were isolated in order from 10 asci and tested to determine

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which had lipid requirements. Typical Mendelian segregation occurred, so it was established that S-11 was a true mutant.

Materials and procedures. The unsaturated fatty acids used in determining the growth requirements were obtained from the Hormel Foundation and were of very high purity. The iodine number (Wijs) of the oleic acid was 89.56 (theoretical value 89.87), that of the linoleic acid was 180.70 (theoretical value 181.03), and that of the linolenic acid was 272.5 (theoretical value 273.51). The saturated fatty acids that were used were obtained from the Eastman Kodak Company.

The fatty acids were added in emulsion form. One per cent emulsions were prepared with a Waring blender, and these were diluted to yield the desired concentrations. The solid compounds were first melted before being emulsified. All emulsions were prepared on the day they were used.

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 supplemented with the growth factor. The flasks had been previously sterilized by autoclaving at 15 pounds for 10 minutes. The inoculated flasks were incubated at 25 C for 96 hours; the mycelia were removed, pressed out, dried at 100 C, and weighed.

RESULTS

The mutant S-11 failed to grow in minimal medium or minimal medium supplemented with 1 ml of 1 per cent lauric acid, myristic acid, palmitic acid, or stearic acid. The mutant did grow when minimal medium was supplemented with oleic acid. The mutant also grew in low concentrations of linoleic acid and linolenic acid, but grew slightly or not at all at higher concentrations of these fatty acids. In view of the demonstrated inhibitory effect of unsaturated fatty acids on bacteria (Kodicek and Worden, 1945) it was thought that inhibition might be playing an important role. Polyoxyethylene sorbitan monostearate ("tween 60") was used in an attempt to relieve this inhibition, since Williams and Fieger (1947) reported that certain surface tension depressants render oleic acid nontoxic to lactic acid bacteria.

The results of growth experiments using various concentrations of fatty acids with and without two different concentrations of "tween 60" are presented in figure 1. The lower portion of the figure represents the growth obtained by various amounts of the three fatty acids in the absence of "tween 60." The growth mixture consisted of 20 ml of minimal medium, 1 ml of the concentration of fatty acid listed on the abcissa, and 1 ml of distilled water. The graph shows vividly the inhibition by the higher concentrations of linoleic and linolenic acids. The 1 per cent emulsion of linolenic acid completely inhibited growth. The results of experiments in which 1 ml of 1 per cent "tween 60" was added in place of the distilled water are presented in the middle portion of figure 1. Comparison with the experiment using no "tween 60" shows that there is a marked increase in growth at the higher fatty acid concentrations. This is in all probability due to partial relief of the inhibition caused by these compounds. The upper portion of figure 1 contains the growth curves obtained when 2 per cent "tween 60" is added. The curves of the fatty acids indicate further relief from the inhibitory effects of these compounds.

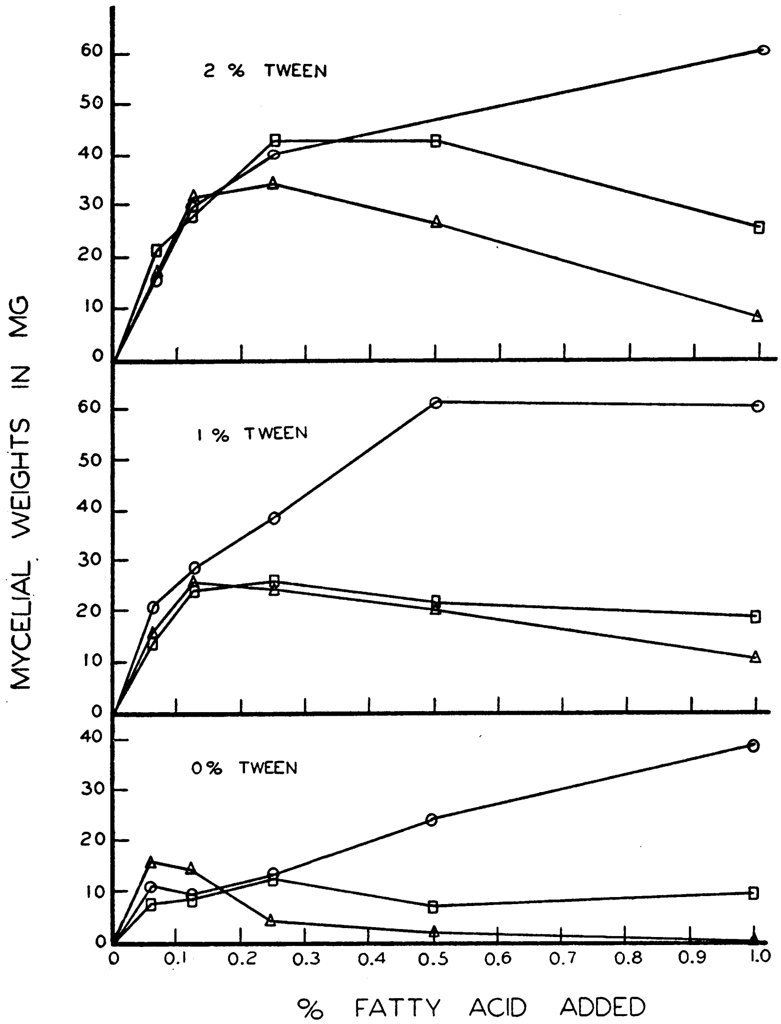

Figure 1. Growth of S-11 mutant in minimal medium supplemented with various concentrations of fatty acids and 0, 1, and 2 per cent "tween 60." Circles represent oleic acid values; squares, linoleic acid; and triangles, linolenic acid.

DISCUSSION

In the case of mutant S-11 we have an example of a substance that enables the mutant to grow and also acts as an inhibitor of its growth. It is improbable that these two effects on the organism are related since they can be dissociated through the use of polyoxyethylene sorbitan monostearate. The results also indicate that the degree of inhibition by the unsaturated fatty acids depends on the number of unsaturated bonds. Thus, judging from the extent of growth one obtains at the higher concentrations of the fatty acids in the absence of the surface tension depressant, the oleic acid is less inhibitory than linoleic acid and linoleic acid is less inhibitory than linolenic acid. The mechanism of this inhibition and its relief by a synthetic detergent is as yet unknown.

The fact that S-11 will grow when it is given oleic acid, linoleic acid, or linolenic acid indicates that the syntheses of these three compounds are related. The simplest scheme of manufacture of these compounds would be the synthesis of linoleic acid from oleic acid and linolenic acid from linoleic acid by dehydrogenation. This view has for support the finding in the rat that linoleic acid and linolenic acid but not oleic acid will prevent nutritional disorders produced by diets devoid of fats (Burr *et al.*, 1929, 1930, 1932). Evidently the rat can make its oleic acid but not linoleic or linolenic acids. This is further supported by the isotope work of Bernhard and Schoenheimer (1940). The rat, therefore, seems to have a metabolic block between oleic acid and linoleic acid, but in the *Neurospora* mutant studied the block lies before oleic acid. This is represented in the following scheme:

$$\xrightarrow{\text{S-11}} \text{oleic acid} \xrightarrow{\text{Rat}} \text{linoleic acid} \longrightarrow \text{linolenic acid}$$

Though the growth experiments indicate that unsaturated fatty acids play a necessary role in some cellular process, the results do not enable us to determine what this role is. The work of Axelrod *et al.* (1947), Williams and Fieger (1947), and Williams, Broquist, and Snell (1947) indicates that there is a relation between biotin and oleic acid since oleic acid can replace the biotin requirement in certain bacteria. Since the minimal medium for *Neurospora* contains biotin, the mutant studied must have some need for an unsaturated fatty acid other than that related to its biotin requirement.

SUMMARY

A *Neurospora* mutant has been isolated which requires for growth either oleic acid, linoleic acid, or linolenic acid. It does not grow when supplied with saturated fatty acids. The unsaturated fatty acids that make growth possible have an inhibitory effect on growth, the amount of inhibition varying directly with the degree of unsaturation of the fatty acids. This inhibition is relieved by the synthetic detergent polyoxyethylene sorbitan monostearate. The growth studies are consistent with the view that linoleic acid is synthesized from oleic acid and that linolenic acid is synthesized from linoleic acid. In the mutant studied, the metabolic block lies before the synthesis of oleic acid.

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