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## Effect of Sodium Oleate on Growth of *Streptobacillus moniliformis.** (18657)

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The effect of oleic and other fatty acids on the growth of various microorganisms is well known. The result may be one of stimulation, inhibition or bactericidal action depending upon the fatty acid and the concentration used. Kodicek and Worden(1) found that *Lactobacillus helveticus* was inhibited for 24 hours by oleic acid, and, for 72 hours or more by linoleic and linolenic acids when used in concentrations of 160 γ per 10 ml of medium. Dubos and Middlebrook(2) have shown that soaps of oleic and other fatty acids exert a marked bacteriostatic and bactericidal action on *Mycobacterium tuberculosis*. Wynne and Foster(4) have more recently reported that sodium salts of oleic and linoleic acids inhibited growth of *Micrococcus pyogenes var. aureus*. The inhibitory effect of oleic and other fatty acids can be neutralized by addition of certain substances such as albumin(2), starch(3), and vegetable lecithin (4) to the medium.

The present study was undertaken in order to determine the effect of sodium oleate on growth of *Streptobacillus moniliformis* in the

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2. Dubos, R. J., and Middlebrook, G., *Am. Rev. Tuberc.*, 1947, v56, 334.

3. Ley, H. L., and Mueller, H., *J. Bact.*, 1946, v52, 4.

4. Wynne, E. S., and Foster, J. W., *J. Inf. Dis.*, 1950, v86, 33.

presence of varying quantities of bovine serum and of animal lecithin.

*Experimental.* Three strains of *Streptobacillus moniliformis*, obtained from Dr. Fordyce R. Heilman, were used. Strains 124 and 1599 were obtained from cases of rat-bite fever. Strain 635 was isolated from a rat. The basal medium was tryptose phosphate broth adjusted to pH 7.6 to 7.8. Stock cultures of the 3 strains were maintained on this basal medium after addition of Seitz-filtered bovine serum in a final concentration of approximately 20%. The same basal medium was used but the concentration of Seitz-filtered bovine serum varied as indicated. In studying the effect of sodium oleate on growth of *Streptobacillus moniliformis*, the sodium oleate was dissolved in water and added to the basal medium. The medium was then tubed and autoclaved and sterile bovine serum was added just before inoculation. The animal lecithin (Nutritional Biochemicals Corp.) was dissolved in petroleum ether and added to each tube of the basal medium before autoclaving, the ether having been driven off in a water bath before the tubes were plugged. Sterile bovine serum was added to the medium before use.

*Results.* Since Wynne and Foster(4) reported that 90 γ of sodium oleate per ml of medium inhibited the growth of *Micrococcus pyogenes var. aureus*, this concentration was selected for use in a preliminary experiment. Tryptose phosphate broth containing 90 γ of sodium oleate per ml and approximately 20% of Seitz-filtered bovine serum was inoculated with 0.1 ml of a 1:50 dilution of a 24 hour stock culture of *Streptobacillus moniliformis*. No evidence of inhibition was observed. Experiments were then conducted to determine whether the toxic effect of sodium oleate could be demonstrated in the presence of smaller amounts of serum. Accordingly 0.25, 0.5 and 0.75 ml of Seitz-filtered bovine serum were added to 5 ml of tryptose phosphate broth containing 90 γ of sodium oleate per ml. These quantities of serum represent approximately 5, 10 and 15% concentrations. Controls containing the same amounts of serum, but no sodium oleate were included. The results of repeated experiments with the 3 strains are shown in Table I. The organism was completely inhibited in the presence of 5% serum while variable results were obtained in the medium containing 10% serum. Growth was consistently observed in the medium containing 15% serum.

TABLE I. Effect of Sodium Oleate, Serum and Animal Lecithin on the Growth of *Streptobacillus moniliformis*. 0.1 ml inoculum 1:50 dil. of 24-hr stock culture.

| | Sodium oleate, 90 γ/ml | | | | |
|---|---|---|---|---|---|
| | Serum | | | Lecithin* | |
| Strain | 5% | 10% | 15% | 100 γ/ml | 200 γ/ml |
| 124 | — | ± | + | — | — |
| 635 | — | ± | + | — | — |
| 1599 | — | ± | + | — | — |

* Lecithin tested in the presence of 5% bovine serum.
— = No growth.
± = Variable results.
+ = Growth.

While 90 γ of sodium oleate in the presence of approximately 5% bovine serum consistently inhibited growth of the 3 strains such was not the case when the concentration of sodium oleate was reduced to 45 γ per ml. In this concentration of sodium oleate the results were irregular in that inhibition was observed on some occasions but not on others. These results suggest that the level of sodium oleate required to inhibit the growth of *Streptobacillus moniliformis* in the presence of 5% bovine serum is between 45 and 90 γ.

Because Wynne and Foster(4) reported that the inhibitory effect of 90 γ of sodium oleate per ml of medium for *Micrococcus pyogenes var. aureus* was neutralized by 100 γ of lecithin per ml of medium, experiments were conducted to determine whether the inhibitory effect of sodium oleate on the growth of *Streptobacillus moniliformis* could also be neutralized by lecithin. The results are shown in Table I. The following controls (not shown in the table) were included: (1) Basal medium containing oleate and serum and (2) Basal medium to which only serum was added. The results presented in Table I show that neither 100 γ nor 200 γ of lecithin per ml of medium neutralized the toxic effect of sodium oleate. The difference in results reported here as compared with those reported by Wynne

and Foster(4) may well be due to the lecithin. Wynne and Foster employed vegetable lecithin, whereas in studies reported here animal lecithin was used exclusively.

*Summary.* Sodium oleate in a concentration of 90 $\gamma$ of sodium oleate per ml of medium inhibits the growth of *Streptobacillus moniliformis*. This inhibitory effect was reversed by addition of adequate amounts of bovine serum. Animal lecithin in concentrations of 100 and 200 $\gamma$ per ml of medium failed to neutralize the inhibitory effect of sodium oleate.

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## Effect of Derivatives of L-Cysteine on the Growth of *Leuconostoc mesenteroides* P-60.* (18658)

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Sulfur containing substances play an important role in cellular metabolism. Some microorganisms require organic sulfur compounds for growth while others can utilize inorganic forms of sulfur(1-4). In a nutritional study of *Escherichia coli,* Bargoni(5) reported that alliin (S-allyl-L-cysteine sulfoxide), methionine, and cystine promoted growth of this organism. Work in this laboratory has shown that alliin also stimulates the growth of *Leuconostoc mesenteroides* but to a lesser degree than L-cystine. Since L-cystine is an essential amino acid for the growth of *Leuconostoc mesenteroides*(6,7), the stimulation observed for alliin may be exerted indirectly by participating in a biological synthesis of L-cystine. Because of the close relationship of alliin, L-cysteine and L-cystine and the possible participation of L-cysteine derivatives in the biological synthesis of L-cystine, it was of interest to determine the effect of various L-cysteine derivatives on the growth of *Leuconostoc mesenteroides.*

*Experimental. Preparation of derivatives of L-cysteine.* S-methyl-L-cysteine, S-ethyl-L-cysteine, S-allyl-L-cysteine, S-propyl-L-cysteine, S-ethyl-L-cysteine sulfoxide, S-propyl-L-cysteine sulfoxide, S-allyl-L-cysteine sulfoxide (alliin) and L-cystine disulfoxide were prepared by previously described methods of synthesis(8-11). All compounds were recrystallized from the appropriate solvent until acceptable nitrogen values and melting points were obtained. The properties of these compounds are summarized in Table I.

*Method of assay. Leuconostoc mesenteroides* P-60 was used as the test organism throughout the experiment. Riesen *et al.*(7) found that this organism responded to cystine in a consistent and reproducible manner. The derivatives of L-cysteine were assayed for

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