

## Effects of Fatty Acids on Growth of *Bordetella pertussis* in Defined Medium

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The effects of saturated and unsaturated fatty acids on the growth of *Bordetella pertussis* strain 114 in defined medium were tested. Individual fatty acids were found to be either inhibitory or stimulatory to growth, depending on concentration. Myristic (C<sub>14</sub>), pentadecanoic (C<sub>15</sub>), and palmitic (C<sub>16</sub>) acids were the most inhibitory saturated fatty acids tested. *B. pertussis* 114 was extremely sensitive to the unsaturated fatty acids oleic (C<sub>18</sub>; *cis*-9), elaidic (C<sub>18</sub>; *trans*-9), and petroselinic (C<sub>18</sub>; *cis*-6).

*Bordetella pertussis* is a fastidious, slow-growing bacterium which is difficult to isolate on laboratory medium. Freshly isolated strains are known to be susceptible to a number of inhibitors present in both solid and liquid media, including peptone, sulfur, peroxides, manganese, and fatty acids (3, 10-14, 18). This inhibition has been overcome by the addition of blood, albumen, charcoal, starch, or anion exchange resins to the medium, which serves to neutralize or bind inhibitors (10-15, 17, 18).

Various workers have described the sensitivity of *B. pertussis* to fatty acids (10-12, 15) in complex media. Stanier and Scholte described a defined medium for *B. pertussis* in which the species retains normal biological and immunogenic properties (16). The object of our research was to investigate the effects of specific fatty acids on the growth of *B. pertussis*. We used *B. pertussis* 114 (2, 5, 6, 8, 9) grown in chemically defined medium.

### MATERIALS AND METHODS

**Glassware.** All glassware used in these studies was cleaned in sulfuric acid-dichromate cleaning solution and rinsed extensively in distilled, deionized water to remove growth inhibitors.

**Fatty acids.** Fatty acids were obtained from Sigma Chemical Co., St. Louis, Mo., and were the highest grade available. All fatty acids were in the free acid form, except formic, acetic, and propionic, which were purchased as the sodium salts. Short-chain saturated fatty acids (C<sub>1</sub> to C<sub>5</sub>) were dissolved in Stanier-Scholte (16) medium and sterilized by filtration. All other fatty acids were dissolved in 95% ethanol and used without filter sterilization.

***Bordetella* strain.** *B. pertussis* 114, a well-characterized research strain, was provided by Charles Manclark, Bureau of Biologics, Food and Drug Administration, Bethesda, Md. It was lyophilized in our laboratory and opened as needed to prepare frozen

seed cultures. Strain 114 was routinely grown on home-made Bordet-Gengou medium (4) containing added peptone (15 g/liter; Difco Laboratories, Detroit, Mich.).

**Inocula and culture conditions.** Frozen seed cultures, used to inoculate liquid starter cultures, were prepared as follows: (i) strain 114 was opened from lyophilization, cultured on Bordet-Gengou medium, and incubated for 72 h at 35°C; (ii) growth from these plates was subcultured on Bordet-Gengou medium and incubated for an additional 48 h; (iii) cells were harvested into Stanier-Scholte medium, modified to contain 1.52 g of tris(hydroxymethyl)aminomethane (Trizma base; Sigma Chemical Co.) per liter and 10% glycerol, and adjusted to a turbidity of 175 to 185 Klett units, using a Klett-Summerson colorimeter (540 nm); (iv) each seed suspension was then divided into small samples, quick-frozen in a dry ice-ethanol bath, and stored at -70°C.

One to five milliliters of frozen seed suspension was used to inoculate starter cultures consisting of 50 ml of Stanier-Scholte medium in a 500-ml sidearm flask (Nephelo culture flask; Bellco Glass, Inc., Vineland, N.J.). Starter cultures were incubated for 18 to 24 h at 35°C in a rotary shaker (180 rpm) until late exponential growth was reached (150 to 175 Klett units).

A 5% (vol/vol) inoculum from the starter flask was used to inoculate test flasks (50 ml) containing fatty acids. Control flasks containing ethanol but no fatty acids were inoculated for each fatty acid tested. The concentration of ethanol used did not significantly inhibit growth in the control flasks. Turbidity was measured at intervals for 24 h with a Klett-Summerson colorimeter (540 nm). Results are expressed as percentage of control turbidity at 24 h.

### RESULTS

The effects of saturated fatty acids of carbon chain lengths C<sub>1</sub> to C<sub>17</sub> on the growth of *B. pertussis* 114 are presented in Fig. 1. Individual saturated fatty acids were found to be either inhibitory or stimulatory, or both, depending on

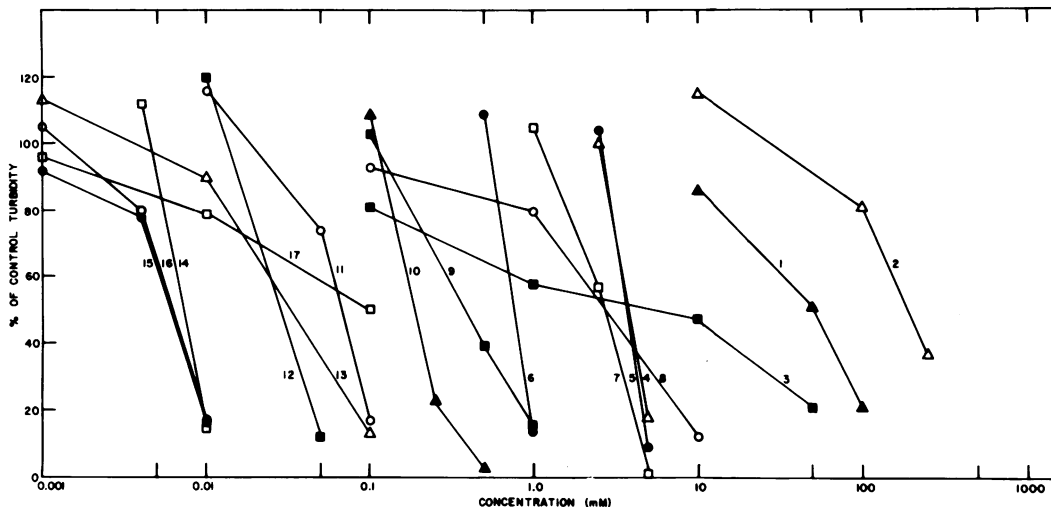


FIG. 1. Effects of saturated fatty acids ( $C_1$  to  $C_{17}$ ) on the growth of *B. pertussis* 114. Fatty acids and their salts are designated by number as follows:  $C_1$ , formic acid, sodium salt;  $C_2$ , acetic acid, sodium salt;  $C_3$ , propionic acid, sodium salt;  $C_4$ , butyric acid;  $C_5$ , valeric acid;  $C_6$ , caproic acid;  $C_7$ , heptanoic acid;  $C_8$ , caprylic acid;  $C_9$ , nonanoic acid;  $C_{10}$ , capric acid;  $C_{11}$ , undecanoic acid;  $C_{12}$ , lauric acid;  $C_{13}$ , tridecanoic acid;  $C_{14}$ , myristic acid;  $C_{15}$ , pentadecanoic acid;  $C_{16}$ , palmitic acid;  $C_{17}$ , heptadecanoic acid. Symbols are used only to designate individual lines. The number adjacent to each line indicates the carbon chain length of the fatty acid.

concentration. The steep slopes seen with several fatty acids were particularly striking. In many cases, small changes in concentration resulted in shifts, from complete inhibition to stimulation of growth (e.g., caproic acid [ $C_6$ ] in Fig. 1). In general, a pattern of inhibition was seen in which the concentration required to inhibit growth decreased as the chain length of the fatty acid increased. Exceptions to this pattern were seen most notably with acetic ( $C_2$ ), propionic ( $C_3$ ), and heptadecanoic ( $C_{17}$ ) acids. Saturated fatty acids of chain lengths  $C_{18}$  (stearic),  $C_{19}$  (nonadecanoic), and  $C_{20}$  (arachidic) were also tested, but they neither inhibited nor stimulated growth. A similar pattern of inhibition by saturated fatty acids was recently reported for *Neisseria gonorrhoeae* (7).

The effects of several unsaturated fatty acids on the growth of 114 are shown in Fig. 2. As with saturated fatty acids, unsaturated fatty acids were found to be inhibitory or stimulatory or both, depending on concentration. However, two distinct concentration ranges giving inhibition were evident. The monomeric unsaturated fatty acids, petroselinic (*cis*-6-octadecenoic;  $C_{18}$ ), oleic (*cis*-9-octadecenoic;  $C_{18}$ ), elaidic (*trans*-9-octadecenoic;  $C_{18}$ ), and palmitoleic (*cis*-9-hexadecenoic;  $C_{16}$ ), were highly inhibitory. The corresponding unsaturated fatty acids containing multiple double bonds, linoleic (*cis*-9,*cis*-12-octadecadienoic;  $C_{18}$ ) and linolenic (*cis*-9,*cis*-12,*cis*-

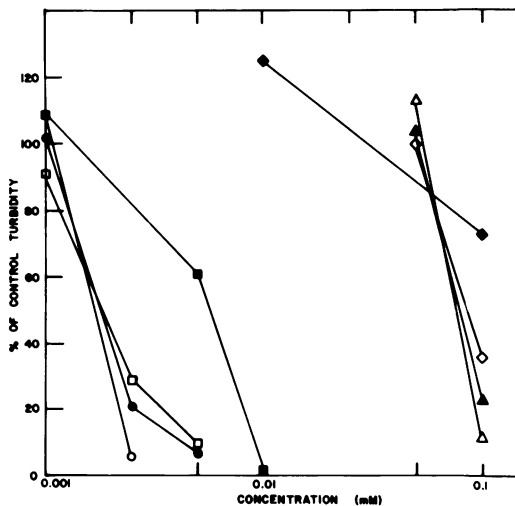


FIG. 2. Effects of unsaturated fatty acids on the growth of *B. pertussis* 114. Unsaturated fatty acids tested included petroselinic (○), oleic (●), elaidic (□), palmitoleic (■), docosahexaenoic (◆), linolenic (◇), and arachidonic (▲).

15-octadecatrienoic;  $C_{18}$ ), were about 50 times less inhibitory. Arachidonic (5,8,11,14-eicosatetraenoic;  $C_{20}$ ) and docosahexaenoic acids were also less inhibitory than the monomeric acids. Two additional monomeric unsaturated fatty acids, erucic (13-docosenoic;  $C_{22}$ ) and nervonic (15-tetracosenoic;  $C_{24}$ ), were stimulatory to the

growth of strain 114 at concentrations of 0.01 to 0.1 mM (data not shown).

### DISCUSSION

These studies conclusively demonstrate that the growth of *B. pertussis* 114 is inhibited by low concentrations of certain saturated and unsaturated fatty acids. Preliminary results with fresh clinical isolates of *B. pertussis* indicate similar patterns of inhibition (L. H. Field and C. D. Parker, in C. R. Manclark and J. C. Hill, ed., *Symposium on Pertussis*, in press). Individual fatty acids can be either inhibitory or stimulatory to growth, depending on concentration. Small changes in the concentrations of individual fatty acids seem to result in large changes in sensitivity. Myristic (C<sub>14</sub>), pentadecanoic (C<sub>15</sub>), and palmitic (C<sub>16</sub>) acids were the most inhibitory saturated fatty acids tested. A concentration of 0.01 mM of any of these three fatty acids completely inhibited the growth of strain 114.

Bundeally and Rao reported that acetate stimulated the growth of *B. pertussis* in Cohen-Wheeler medium (1). Our results also suggest that acetate may be stimulatory to growth. However, low concentrations of other saturated fatty acids also appeared to stimulate growth in our studies.

In 1947, Pollock reported that a strain of *B. pertussis* which grew on 10% blood agar was inhibited by long-chain unsaturated fatty acids (11). However, the inhibitory concentration could not be determined, since the strain he used required the presence of albumen for growth. Our results have demonstrated the exquisite sensitivity of *B. pertussis* to unsaturated fatty acids. A concentration of 0.005 mM of the monomeric unsaturated fatty acids oleic, elaidic, or petroselinic acids was inhibitory to the growth of strain 114. Of interest was the observation that fatty acids of the same chain lengths but with multiple bonds were 50-fold less inhibitory to growth.

*B. pertussis* has always been difficult to isolate on laboratory media. For many years it was assumed that this must be due to its complex nutritional requirements. It is now known that *B. pertussis* has simple growth requirements, and its poor growth can be explained by its sensitivity to various inhibitors present in isolation medium. Both saturated and unsaturated fatty acids are ubiquitous on laboratory glassware and in medium components (including agar). Since only small concentrations are re-

quired to inhibit growth, fatty acids must be considered as a major problem in the routine study of *B. pertussis*.

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