Pimelic acid as a growth stimulant for *Colpidium campylum.*

By

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With 5 figures in the text.

The observations of MUELLER (1937 a, b, c), that pimelic acid stimulates growth of *C. diphtheriae*, suggested the possibility that this substance might exert comparable effects on Protozoa. Accordingly, the effects of pimelic acid on growth of *Colpidium campylum* have been determined in several different culture media.

Material and methods.

The bacteria-free strain of *C. campylum* used in the present investigation is a strain obtained from Dr. T. L. JAHN in 1931 and since maintained in pure culture in our laboratories. Several media were used in the various experimental series: in series I and II, medium A (our stock culture medium for *C. campylum*) containing Difco tryptone (10.0 gm.) and KH_2PO_4 (2.0 gm.) in distilled water (1.0 liter); in series III, Difco granulated gelatin (10.0 gm.) was substituted for peptone in the preceding formula; in series IV, 1.5 % gelatin was used instead of 1.0 %; and in series V both gelatin (15.0 gm.) and Difco dextrose (5.0) were added to the phosphate solution. In view of ELLIOTT's (1935) observations on p_H relationships of *C. campylum*, the experimental media were used at an initial p_H within the range, 5.5—6.5, as specified below.

In determining the effects of different concentrations of pimelic acid, solutions of the acid (Eastman synthetic) were made up in distilled water and added in 1.0 cc. amounts to tubes containing 9.0 cc. of medium. Each control tube received 1.0 cc. of distilled

water. The tubes were sterilized in the autoclave and later inoculated from a stock culture of *C. campylum* in medium A. After fixation of a number of tubes for the initial count (number of ciliates per cubic centimeter) and after determining the initial p_H of each type of medium, the remaining tubes were incubated in darkness at room temperature for the periods indicated below. After incubation, one tube of each type was used for determination of final p_H, and the remainder were fixed for counting (final count).

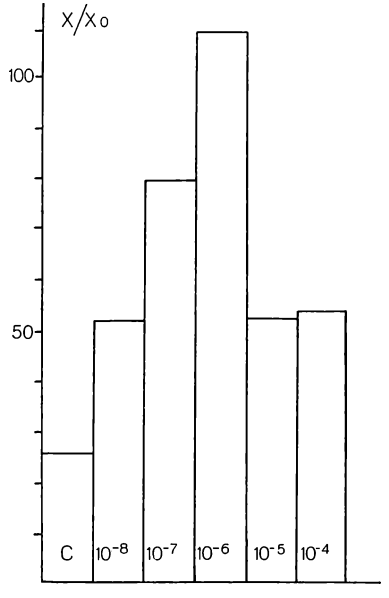

Fig. 1. Growth of *C. campylum* in peptone medium (medium A); C. controls with no pimelic acid; concentrations of pimelic acid ranging from 10^{-8} to 10^{-4} grams per cubic centimeter; x/x_0 is the ratio between initial and final numbers of ciliates per cubic centimeter.

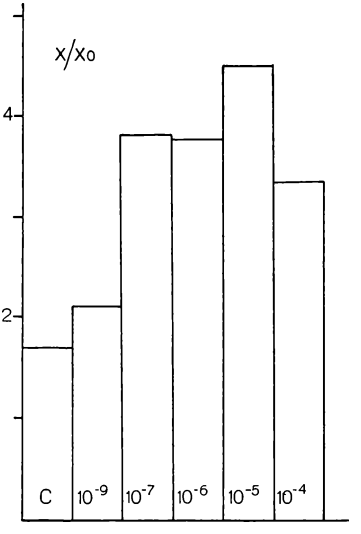

Fig. 2. Growth of *C. campylum* in peptone medium; concentrations of pimelic acid ranged from 10^{-9} to 10^{-4} gm. per cc.; for other explanations, see fig. 1.

All p_H determinations were made with a La Motte roulette comparator. Initial and final counts were made with a Whipple ocular micrometer and a Sedgwick-Rafter counting cell, as described previously (HALL, JOHNSON and LOEFER, 1935).

Effects of pimelic acid in different concentrations.

Series I. The initial p_H was 6.2 and the initial count was 152 ciliates per cc. Inoculations were made from a 24-hour stock culture in medium A. The period of incubation was 100 hours. Growth

rates, expressed as x/x_0 (ratio of final to initial concentration of ciliates per cc.) are described graphically in figure 1. It will be noted that growth of *C. campylum* was accelerated by concentrations of pimelic acid ranging from 10^{-8} to 10^{-4} grams per cubic centimeter; the maximal effect was produced in a concentration of 10^{-6}.

Series II. This series, which was similar to series I, was started at an initial p_H of 6.1, with an initial count of 875, and was incubated for 95-hours. The series was inoculated from a 48-hour stock culture

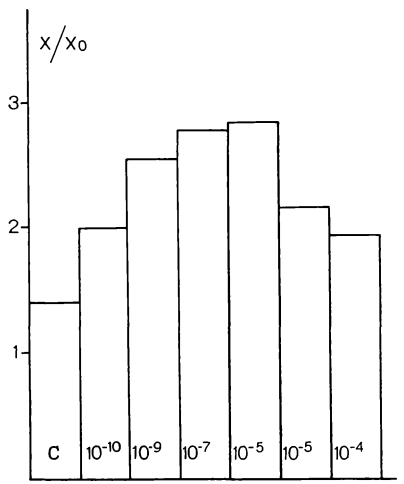

Fig. 3. Growth of *C. campylum* in 1.0% gelatin medium; pimelic acid concentrations ranged from 10^{-10} to 10^{-4} gm. per cc.; for other explanations, see fig. 1.

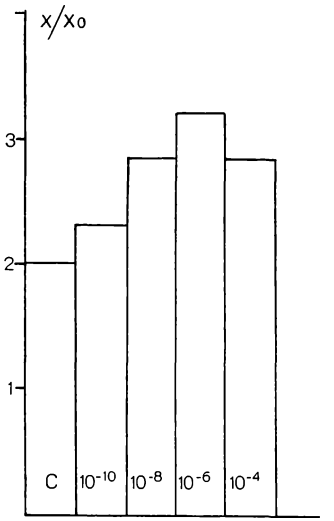

Fig. 4. Growth of *C. campylum* in 1.5% gelatin medium; for other explanations, see Fig. 1.

in medium A. Comparison of growth rates in the different media (Fig. 2) shows accelerating effects in concentrations of pimelic acid ranging from 10^{-7} to 10^{-4} gm. per cc., with an insignificant effect at 10^{-9}. In this series, the maximal effect was noted in a concentration of 10^{-5}.

Series III. This series in gelatin medium was started at p_H 5.7 with an initial count of 693, and was incubated for 114 hours. The tubes were inoculated from a 24-hour culture in medium A. The results (Fig. 3) indicate maximal acceleration of growth in pimelic acid concentrations of 10^{-6} and 10^{-7}, and less marked effects in lower and higher concentrations.

Series IV. In this series, initial p_H was 5.5; initial count, 1705; and the period of incubation, 10 days. The series was inoculated from a 72-hour stock culture in medium A. The results (Fig. 4) are comparable to those obtained in series III.

Series V. This series was started at p_H 5.5, with an initial count of 1633, and was incubated for six days. The series was inoculated from a 96-hour stock culture in medium A. In this case, the medium contained dextrose but was similar in other respects to that used in series IV. The results (Fig. 5) indicate that no significant effect was produced by pimelic acid in any of the concentrations used. It may be noted that x/x_0 (approximately 15.0, as compared with 2.0 to 3.4) and densities of population (approximately 25,000, as compared with 3500—5500) were much higher than in series IV. Comparable results have been obtained in paired series in dextrose-gelatin medium, one with pimelic acid (10^{-6}) and one without. In one pair, the final densities of population were 15,366 and 15,133 per cc., respectively: in another pair, 16,203 and 16,105; and in a third, 15,952 and 16,360, respectively. It may be concluded, therefore, that in the presence of dextrose any accelerating effects of pimelic acid are masked by effects of the sugar itself.

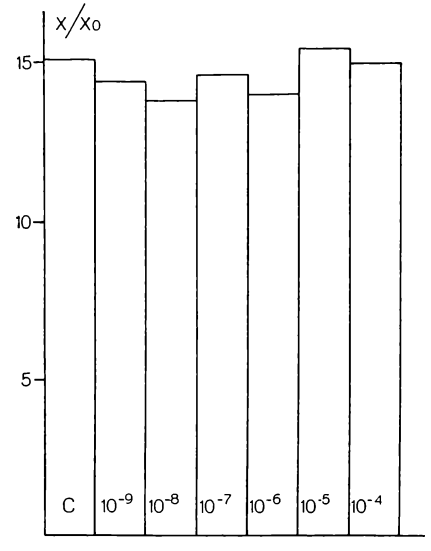

Fig. 5. Growth of *C. campylum* in gelatin-dextrose medium; for other explanations, see Fig. 1.

Discussion.

The results obtained with *Colpidium campylum* agree in general with those reported by Mueller for the diphtheria bacillus. For the latter, maximal acceleration of growth was produced by pimelic acid in a concentration of 10^{-7} gm. per cc. In cultures of *C. campylum* maximal effects were noted in concentrations ranging from 10^{-5} to 10^{-7}, and slight acceleration was noted in dilutions of 10^{-9} and 10^{-10} in several cases. *C. diphtheriae* appears to be sensitive to concentrations at least as low as 10^{-8}. For *Colpidium campylum* in

peptone medium, the maximal increase in population was of the same order as that reported for the diphtheria bacillus — within the range of 200—300 %. In gelatin medium, however, the ciliate population was increased by only 60—75 %.

The significance of this accelerating action of pimelic acid on *C. campylum* is uncertain, and whether or not this substance should be considered a growth factor in the usual sense remains to be determined. Some preliminary results (HALL, 1938) indicate that pimelic acid may exert significant effects in gelatin-dextrose medium, although growth rate and density of population are not increased. A strain of *C. campylum* has been established in gelatin-dextrose medium containing pimelic acid (10^{-6} gm. per cc.), and has now reached the tenth serial transfer. The controls, in the same medium without dextrose, failed to grow after the second transfer, and previous attempts (HALL and ELLIOTT, 1935) to grow this ciliate in gelatin medium had also proven negative. Although the preliminary findings appear suggestive, a definite conclusion regarding the "growth factor" status of pimelic acid is not yet warranted.

On the basis of present evidence, it may be assumed that pimelic acid exerts some sort of catalytic effect on the metabolism of *Colpidium campylum*. Such an effect is apparent, in peptone and gelatin media, as an increase in the growth rate and in density of population. The effect may be masked, or perhaps eliminated, by the addition of dextrose to the medium.

Literature cited.

ELLIOT, A. M. (1935): Effects of certain organic acids and protein derivatives on the growth of Colpidium. Arch. Protistenkde **84**, 225—231.

HALL, R. P. (1938): Pimelic acid as a growth factor for the ciliate, Colpidium campylum. Anat. Rec. **72** (Suppl.), 110.

HALL, R. P. and A. M. ELLIOTT (1935): Growth of Colpidium in relation to certain incomplete proteins and amino acids. Arch. Protistenkde **85**, 443—450.

HALL, R. P., D. F. JOHNSON and J. B. LOEFER (1935): A method for counting Protozoa in the measurement of growth under experimental conditions. Trans. amer. micr. Soc. **54**, 298—300.

MUELLER, J. H. (1937a): Pimelic acid as a growth accessory factor for a strain of the diphtheria bacillus. Science (N. Y.) **85**, 502—503.

— (1937b): Studies on the cultural requirements of bacteria. X. Pimelic acid as a growth stimulant for C. diphtheriae. J. Bacter. **34**, 163—178.

— (1937c): Pimelic acid as a growth accessory for the diphtheria bacillus. J. of biol. Chem. **119**, 121—131.