

The temperature dependence of the inhibition of growth of a protozoan by antibiotics*

While studying the effect of antibiotics on growth of *Tetrahymena pyriformis*, strain E1, it was decided to extend kinetic experiments to determine the response to varied incubation temperatures. This approach was taken since two of the antibiotics used, chlortetracycline (Aureomycin, Lederle) and oxytetracycline (Terramycin, Pfizer), are structurally similar, while the third drug, chloramphenicol (Chloromycetin, Parke, Davis) is different. It was hoped, therefore, that this type of investigation might help to discriminate between the modes of action of these compounds.

Culture techniques, chemically defined and stock media, solution preparations and the turbidimetric method of growth measurement were the same as those previously reported², with two exceptions: (1) the defined medium was modified by the use of the growth factor, thioctic acid**, in place of protogen A; and (2) experimental cultures were grown at the average temperatures of 28°, 25° (control), 21° and 16° C, respectively, with a variation of ± 1.5 ° C in each case.

Final antibiotic concentrations of $3.1 \cdot 10^{-4}$ M Chloromycetin (CM), $5.8 \cdot 10^{-5}$ M Aureomycin (AM) and $1.5 \cdot 10^{-4}$ M Terramycin (TM) were employed, because these concentrations were found to inhibit growth rate by 50% at the control temperature^{1,2}.

The influence of temperature on growth of *Tetrahymena* is illustrated in Fig. 1. The slope, m , of the growth curve was calculated from the formula: $m = \frac{\log d_1 - \log d_2}{t_1 - t_2}$,

where d = optical density, and t = time in days. It is obvious from this plot, that as temperature decreases (1) growth rate (slope) does likewise, (2) peak growth increases and (3) logarithmic growth phase becomes prolonged. These are the expected effects in invertebrates in general - lowered temperatures for growth usually result in decreased metabolic rate, increased longevity and larger size or body weight.

The relative effect of the normally 50% suppressive levels (at 25° C) of the antibiotics is shown in relation to temperature in Fig. 2. Results are plotted as the calculated slope, m , against the reciprocal of the absolute temperature, T . Chiefly, two results are noteworthy. Firstly, as temperature is lowered, the activity of each of the drugs is decreased until at 16° C, growth rates of control and antibiotic-containing cultures are essentially equal. In this respect, the same general picture is revealed for the action of all three compounds, *viz.*, potency decrease with decrease in temperature, regardless of their respective chemical structures. Secondly, there is an indication from the shapes of the plotted lines, that

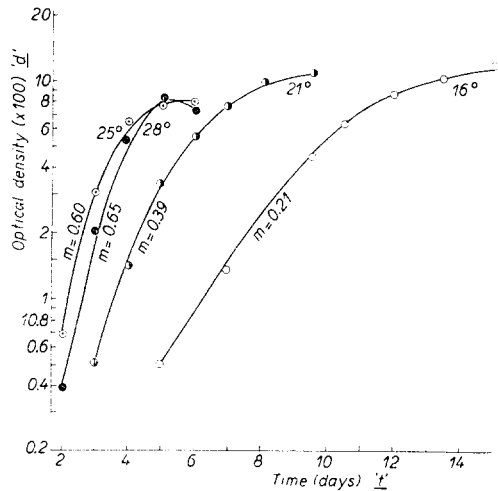


Fig. 1. Growth of *Tetrahymena* in relation to temperature (°C). m = slope (rate of growth); d = optical density; t = days.

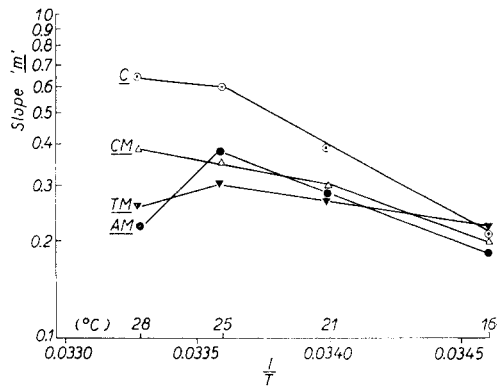


Fig. 2. Interaction of antibiotics and temperature on growth rate of *Tetrahymena*. C = control; CM = Chloromycetin; TM = Terramycin; AM = Aureomycin; T = absolute temperature.

* From a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan, 1953.

** Thioctic acid, as Na-D,L-6-thioctate, was supplied by Dr. E. L. R. STOKSTAD of Lederle Laboratories, Pearl River, N.Y.

the two tetracyclines, AM and TM, react similarly, while CM, which is structurally dissimilar, acts differently. It appears, therefore, that a study such as that described is able to distinguish between compounds with like and unlike modes of action.

Following the analytical precedent set by JOHNSON *et al.*³, these antibiotics appear qualitatively to fall into the Type II class of inhibitors^{4,5}, *i.e.* their activity declines with decrease in temperature. All observed Type II inhibitors are also known to be biochemically "noncompetitive".

Furthermore, WOOLLEY⁶ has demonstrated, that in *Escherichia coli*, CM acts as a non-competitive inhibitor. The data shown in this report, then, uphold WOOLLEY's contention and in addition indicate that TM and AM function in the same manner. Therefore, it is tentatively concluded that the general mechanism of action of the three antibiotics is the same in that they are Type II inhibitors. However, their specific mechanisms or sites of action seem to be different, and appear to depend upon their chemical structure.

A more critical analysis of these results is untenable at this time. Such analysis must await further studies at intermediate temperatures and varied antibiotic concentrations, as well as growth carried out in constant temperature chambers or water baths of greater sensitivity than those utilized in this investigation.

My gratitude is expressed to Dr. D. E. S. BROWN for suggesting this study, and to Dr. A. M. ELLIOTT under whose direction the investigation was performed.

J. A. GROSS*,**

Department of Zoology, University of Michigan, Ann Arbor, Mich. (U.S.A.)

¹ J. A. GROSS, Ph. D. thesis (1953).

² J. A. GROSS, *J. Protozool.*, 2 (1955) 42.

³ F. H. JOHNSON, H. EYRING AND R. W. WILLIAMS, *J. Cellular Comp. Physiol.*, 20 (1942) 247.

⁴ W. D. McELROY, *J. Cellular Comp. Physiol.*, 21 (1943) 95.

⁵ W. D. McELROY, *Quart. Rev. Biol.*, 22 (1947) 25.

⁶ D. W. WOOLLEY, *J. Biol. Chem.*, 185 (1950) 293.

Received August 23rd, 1955

* Predoctoral Research Fellow of the United States Atomic Energy Commission, 1951-53.

** Present address: Zoology Department, University of California, Los Angeles 24, California, U.S.A.

A sensitive reaction on paper of ninhydrin with hydroxyproline and proline

The sensitivity of the ninhydrin (triketo-hydrindene hydrate) reaction with amino acids separated on two dimensional paper chromatograms has been described by PRATT AND AUCLAIR¹. The reagent has a low sensitivity for both hydroxy-proline and proline due to the formation of a yellow pigment which is difficult to differentiate from the white paper background.

A more satisfactory qualitative test was evolved using the following procedure. The filter paper chromatograms, after development with the solvent system of REDFIELD² and steaming for 10 minutes to remove adsorbed diethylamine, were dipped in a 0.1% (w/v) solution of ninhydrin in benzene containing 0.1% (v/v) collidine. The papers were then placed in dry steam for 1 minute to produce magenta spots from hydroxy-proline and proline, which exhibited an intense red fluorescence in u.v. light. The limit of detection by u.v. fluorescence was 0.008 μg and 0.025 μg amino-nitrogen for hydroxy-proline and proline respectively.

A pigment of similar colour is known to occur as an intermediate in the hydroxy-proline ninhydrin reaction³. This substance, subsequently referred to as pigment A, can be isolated from the aqueous reaction media by extraction with benzene⁴, and is non-fluorescent in u.v. light. It has an absorption spectrum in the 350 to 750 $m\mu$ range identical with that of the fluorescent pigment (pigment B) as measured whilst adsorbed on the paper. This was achieved by placing paper strips carrying the pigment in a glass cuvette containing benzene. Pigments A and B both exhibit an absorption maximum at 570 $m\mu$ and similar strong absorption 260-280 $m\mu$. Pigment B was found to be inextractable from the paper by both polar and non-polar solvents except to a small extent by acetone. The latter gave a magenta-coloured solution, non-fluorescent in u.v. light with absorption spectrum identical with that of pigment A in acetone—each maximal at 550 $m\mu$. This suggests that B is formed by adsorption of A on the paper and this is supported by the observation that addition of A to the paper produced a magenta spot which became fluorescent on steaming for one minute. Moreover, both A and B on prolonged heating yielded a yellow pigment of identical absorption spectra with that published by MOORE AND STEIN⁵.