7. Hoch, F. L., Vallee, B. L., *Nature,* 1955, v176, 256.

8. Reitman, S., Frankel, S., *Am. J. Clin. Path.,* 1957, v28, 56.

9. Reynafarje, B. D., Potter, V. R., *Cancer Res.,* 1957, v3, 241.

10. Hale, H. B., Mefferd, R. B., Vawter, G., Foerster, G. E., Criscuolo, R. M., *Am. J. Physiol.,* 1959, v196, 520.

11. Heroux, O., Gridgeman, N. T., *Can. J. Biochem. Physiol.,* 1958, v36, 209.

12. Selye, H., *The Physiology and Pathology of Exposure to Stress,* Montreal, Acta, Inc., 1950, 605.

13. Constantinides, P., *Endocrinology,* 1951, v49, 512.

14. Knigge, K. M., *ibid.,* 1954, v55, 731.

15. Eranko, O., *Acta Path. Microbiol. Scand.,* 1955, v36, 293.

16. Deane, H. W., Lyman, C. P., *Endocrinology,* 1954, v55, 300.

17. Coxon, R. V., Korenchevsky, V., Lawrence, A., *J. Path. and Bact.,* 1956, v72, 613.

18. Light, A., Tornaben, J. A., *J. Nutrition,* 1953, v49, 51.

19. Stenram, U., *Exp. Cell Res.,* 1952, v3, 147.

20. Mandel, L., Jacob, M., Mandel, P., *Compt. Rend.,* 1951, v145, 1075.

21. Knigge, K. M., *Proc. Internat. Symp. Temp. Acclim.,* Leiden, 1962, in press.

22. Popovic, V., *Bull. Mus. Comp. Zool.,* 1960, v124, 105.

23. Chaffee, R. R. J., Hoch, F. L., Lyman, C. P., *Am. J. Physiol.,* 1961, v201, 29.

24. Burwell, R. G., *J. Path. Bact.,* 1955, v20, 387.

25. Potter, V. R., *Fed. Proc.,* 1958, v17, 1060.

26. Vignais, P. V., Vignais, P. M., *J. Biol. Chem.,* 1957, v229, 265.

27. Reynafarje, B., Chaffee, R. R. J., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 225.

28. Smith, R. E., Panel on Intermediary Metabolism and Electron Transport, *Proc. Internat. Symp. Temp. Acclim.,* Leiden, 1962, in press.

29. Beyer, R. E., Alterations of Electron Transport and Related Reactions During Cold-Acclimation, *ibid.,* 1962, in press.

30. Klain, G., Alterations of Protein Metabolism During Cold-Acclimation, *ibid.,* Leiden, 1962, in press.

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Stimulation of *Turbatrix aceti* by Antibiotics. (28295)

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The mechanism of action of antibiotics in increasing growth rates, food efficiency, and other parameters of biological systems has been debated with varying degrees of evidence(1) since the discovery of growth stimulation(2). One of the main difficulties is the inadequacy of methods. Problems with vertebrate experimentation involve space, time, complexity of the organism, and variability. The main difficulty with the bacterial tool is the separation of mutation from adaptation. Consequently an invertebrate species was sought in an effort to combine the speed and power of bacteriological methods with the significance and ease of separation of adaptation from mutation presented by metazoan species. Of several invertebrates considered, the vinegar eel, *Turbatrix aceti,* has many assets: its small size, its availability in large numbers, ease of handling as a suspension, and ease of obtaining sterile material. The data presented here indicate that different antibiotics stimulate this species over a wide range of concentration.

Methods. Turbatrix aceti from unprocessed vinegar obtained from a vinegar factory, Établissements Lourtie at Theux, Belgium, were maintained in vinegar adjusted to pH 5.0 at room temperature. Procaine penicillin, oxytetracycline Cl, chloramphenicol, and staphylomycin were dissolved in vinegar at pH 5.0. The staphylomycin was first dissolved in a small quantity of methanol. In each experiment antibiotics were added in graded levels to flasks containing vinegar at

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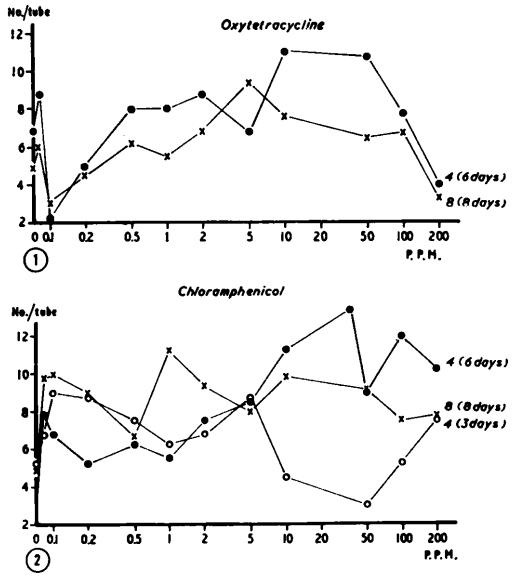

FIG. 1. Effect of oxytetracycline upon total number of *Turbatrix aceti*. The points give avg number in 4 replicas at 6 days and 8 replicas at 8 days.

FIG. 2. Effect of chloramphenicol upon total number of *Turbatrix aceti*. The points give the avg number in 4 replicas at 3 and 6 days and 8 replicas at 8 days.

pH 5.0; then equal quantities of the suspension of vinegar eels were added and the results studied with respect to time at 30°C. Duplicate flasks were used in each experiment, and 2-4 one ml aliquots were used for observation from each flask. The tubes were shaken and total population counted in each aliquot. The 1 ml tube was placed under a 3-fold magnification in a bright light at such an angle that the Tyndall effect made individual living eels easily observed due to their most characteristic feature of constant action. Results were presented using the average of the values obtained at each concentration. Statistical validity of the major points was established using the formula devised for small samples.

Results and discussion. The data in Fig. 1 and 2 give the total population per 1 ml tube at different times with different concentrations of the antibiotics. In general the data taken before 3 days were less significant than those at 3-8 days, while the data taken beyond 2 weeks seem to be less reproducible. Any values found above that of the tube containing no antibiotic represent an increased population and may be interpreted as a stimulation from the antibiotics. As was expected, stimulation may be obtained at one concentration and depression at another. This effect may change with time as shown by the different curves. With each antibiotic there were one or more areas where definite stimulation was found, and in the case of chloramphenicol and staphylomycin this stimulation was seen over a wide range. The results with procaine penicillin resembled those of chloramphenicol, except that few areas of stimulation above 0.5 ppm were noted.

The stimulation was verified by 3 repetitions of the experiment. Statistical evaluation of the data (Table I) indicates that the major points on the graphs are valid for general representation. The increased population found in presence of antibiotics was generally not seen in one to 2 days, and the number of organisms in the tube with no antibiotics did not significantly increase during the experiment. Although the specific action of the antibiotics upon the organism is not known, it must be an effect upon hatchability, growth and/or survival. The effect may be antibacterial, although few micro-organisms grow luxuriantly at pH 5 and the cultures never looked cloudy. Nevertheless, the experiments should be repeated with axenic nematodes.

The fact that these free-living nematodes do react to antibiotics makes them of great interest for further use in the study of the mechanism of action of antibiotics. Specifically they will be subjected to different forms of stress to determine whether or not the hypothesis of antibiotic action on adaptibility is valid(3); heat is presently being used by the author as the stressing agent for this purpose(4). It is of interest to note that the application of the proposed hypothesis to the present results would suggest that the animals used in this study were under stress.

Summary. *Turbatrix aceti* have been found to be stimulated by different concentrations of procaine penicillin, oxytetracycline, chloramphenicol, and staphylomycin after 5 days

TABLE I. The Effect of Antibiotics upon *Turbatrix aceti.*

	Chloramphenical								Oxytetracycline								Procaine penicillin				Staphylomycin			
	3 days				8 days				7 days				8 days				7 days				7 days			
ppm	Avg*	n†	σ‡	P§	Avg	n	σ	P	Avg	n	σ	P	Avg	n	σ	P	Avg	n	σ	P	Avg	n	σ	P
0	5.6	8	2.12	—	5.43	7	1.06	—	5.9	14	.875	—	4.43	7	.940	—	3.67	3	.892	—	7.67	3	.471	—
.05	6.6	7	1.05	.33	10.61	7	2.10	.001	7.5	8	.889	.08	6.00	8	2.00	.095	8.0	4	3.50	.134	8.67	3	1.24	.33
.1	8.33	6	1.00	.009	9.88	8	1.39	.001	5.4	8	.875	.59	2.88	8	1.75	.07	10.5	4	1.75	.001	10.0	3	1.33	.056
.2	8.6	8	1.69	.006	9.57	7	1.22	.001	7.3	7	.0	.067	4.50	8	1.125	.9+	9.0	4	.500	.001	—	–	—	—
.5	7.9	8	2.37	.082	6.0	7	1.71	.51	7.9	8	.440	.023	6.13	8	1.75	.047	6.75	4	1.75	.065	9.5	4	4.96	.20
1	6.6	7	1.51	.36	11.25	8	2.56	.001	9.25	8	.0	.001	3.83	6	.833	.25	6.75	4	2.75	.175	18.0	3	1.80	.001
2	5.9	7	.912	.76	8.29	7	1.47	.002	8.5	8	.440	.011	5.57	7	1.51	.15	5.75	4	3.74	.455	10.3	4	1.41	.08
5	7.4	8	2.45	.186	8.00	8	2.25	.003	7.0	6	.440	.26	9.38	5	1.88	.001	8.0	4	2.50	.032	8.5	4	1.78	.66
10	5.9	8	.928	.75	9.87	8	3.13	.001	6.5	6	.90	.46	7.68	8	1.38	.001	4.67	3	.892	.30				
50	5.4	8	1.80	.9+	9.13	8	1.78	.001	7.0	6	2.75	.24	6.50	8	2.75	.082	7.67	3	.443	.001				
100	6.4	8	.825	.39	7.50	8	2.25	.06	5.7	6	.672	.82	7.43	7	1.49	.001	8.25	4	1.25	.007				
200	6.4	8	1.13	.41	7.4	5	.518	.008	5.0	6	.672	.36	3.25	8	1.00	.008	10.0	3	.667	.001				

* Avg = Average number of living eels per tube.

† n = No. of individual tubes involved.

‡ σ = Standard deviation = $\sqrt{\frac{\Sigma\,(\text{deviation from mean})^2}{\text{individuals}}}$

§ P = Probability calculated from the statistic t using the formula of P. M. Nastucoff (Dept. of Mathematics, Univ. of Notre Dame): $t = \frac{M_1 - M_2}{\sqrt{n_1 \sigma_1^2 + n_2 \sigma_2^2}} \times \sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{n_1 + n_2}}$

incubation with vinegar containing graded amounts of antibiotics. The major effects were not seen after 2 weeks. The specific action is not known; the stimulus was noted by counting the total population at different periods. Further work is under way using this system to study the effects of antibiotics in adaptation to heat stress.

It is a pleasure to acknowledge with thanks the gift of staphylomycin from its discoverer, Dr. P. de Somers, Professor of Virology, Univ. of Louvain, and oxytetracycline (terramycin chloride) from Pfizer, S. A. Brussels.

1. Luckey, T. D., *Antibiotics, Their Chemistry and Non-Medical Uses,* H. D. Goldberg, Ed., D. Van Nostrand, Princeton, N. J., 1959, p174.

2. Moore, P. R., Luckey, T. D., Everson, G. A., McCoy, E., Elvehjem, C. A., Hart, E. B., *J. Biol. Chem.,* 1946, v175, 437.

3. Luckey, T. D., *Recent Progress in Microbiology,* Almquist and Wiksell, Stockholm, 1959, p340.

4. ———, *Nature,* 1963, v198, 263.

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Effect of Podophyllin (P) and Podophyllotoxine (PT) on the Rat Litter *in utero*. (28296)

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In toxicity studies of Podophyllin (P) and Podophyllotoxine (PT)(5), it was shown that the LD_{50} for rats was approximately 15 mg/kg, i.p. Both compounds arrest cell mitosis in metaphase(2,4) and are used in treatment of condyloma accuminata. As the compounds resemble in their action on mitosis the effects of colchicine(2,4), their possible effect on the rat embryo was of interest. Didcock, Jackson and Robson(3) reported intra-amniotic and intraplacental injection of rat fetuses with PT on the 17th day of gestation (g.d.). The drug was lethal to the fetuses in doses of 20 mg by the intra-amniotic route and had an LD_{50} for the fetuses of 100 mg by intraplacental administration. Wiesner and Yudkin(7) destroyed 3 successive litters in Swiss mice in the same mothers with subcutaneous doses of 0.25 mg of PT, given at least 3 days after fertilization.

Controls. Thirty-six control rats, treated with a placebo, produced 98.3% normal fetuses, each 5 g in weight and 4.6 cm in length, and 1.7 $\pm$ 2.4% of all fetuses were resorbed, indicating that more than 4.1% resorption of fetuses is abnormal.

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Procedure. Both compounds were given to groups of pregnant rats, prepared as described in detail previously(6). Both compounds were given dissolved in distilled water, intraperitoneally. Female rats were paired with males. On findings of massive vaginal sperm, the females were isolated, weighed and injected with P or PT. The animals were sacrificed on the day before littering. The uteri were removed in toto; their contents, fetuses, placentas, implantation sites and sites of fetal resorption, recorded. Surviving fetuses were fixed, cleared and stained with Alizerin. The maternal internal organs were microscopically studied, as were the sites of fetal resorption, and surviving placentas, ovaries and breast tissues.

Results with Podophyllotoxine (Table I). A 2 mg/kg dose, given on the 7th and 8th g.d. (that is, after implantation), led to the loss of 17.5% of all fetuses at term, and stunting of 5.7% of the survivors. Twenty-one percent of the placentas of the resorbed fetuses were found at term together with other live fetuses in the same uterus. Only one animal had its entire litter destroyed in this group and here 13 placental remnants, each 0.3 cm in diameter, were found at term *in utero*. The surviving fetuses were normal in size and weight