15175

Effect of Atabrine on *Tetrahymena geleii* (Protozoa, Ciliata).

VINCENT GROUPÉ. (Introduced by Geoffrey Rake.)

From the Division of Microbiology, The Squibb Institute for Medical Research, New Brunswick, N.J.

The development during recent years of pure, bacteria-free strains of protozoa has led to more precisely controlled physiological and nutritional studies. However, the effect of chemotherapeutic drugs on such pure cultures has been little studied. It seemed of interest, therefore, to study the effect of a synthetic antimalarial drug such as atabrine on the ciliate, *Tetrahymena geleii,* whose biochemical characteristics are known.[1]

Methods. Bacteria-free cultures of *Tetrahymena geleii* were obtained through the courtesy of Professor George W. Kidder. All cultures were maintained with strict asepsis on 1.5% proteose peptone at 25°C. Strain H[1] was selected from the 6 strains tested because it was slightly less resistant to atabrine.

Population counts were made using a Howard mold counting chamber on 1.0 ml samples of culture fixed with an equal volume of formalin. Five or more fields were counted per sample using that magnification and dilution resulting in 10 to 50 cells per field. Counts were recorded as the number of intact organisms per ml.

The effect of atabrine on the division rate was determined by adding 3.0 ml of a 24-hour culture of known population diluted 1-10 in 1.5% proteose peptone to duplicate tubes containing an equal volume of a serially diluted aqueous atabrine solution. The number of generations in 24 hours was calculated from the known population at 0 hours and the average population of the duplicate tubes after 24 hours incubation at 25°C. Sterile precautions were observed throughout the entire test.

Serial dilution tests were done as follows: to 0.5 cc of serial 2-fold dilutions of atabrine an equal volume of a 24-hour culture was added. After overnight incubation at room temperature the tubes were examined both microscopically for motility and macroscopically for turbidity. Although it was most important to use bacteria-free cultures it was found possible to disregard sterile precautions in performing the serial dilution test described above without altering the results of the test.

The atabrine dihydrochloride* used in these studies was sterilized by filtration through a chemically clean UF grade sintered glass filter.

Experimental. The effect of atabrine on the division rate of *Tetrahymena geleii* was studied first to determine the concentration necessary to inhibit actively growing cells. It will be seen from the data presented in Table I that a concentration of 100 γ per ml was lethal for all cells and that multiplication was inhibited by 50 γ per ml while little or no inhibition was observed in the presence of 25 γ of atabrine per ml. In tubes containing 50 γ per ml only 25% of the population was motile. This would indicate that a difference in resistance to atabrine exists among the population. Similar results were obtained in another experiment.

Inasmuch as direct observation showed that loss of motility was followed rapidly by swelling and disintegration of the cell in the above experiments, a simple serial dilution test (see methods) was used to determine the effect of age of the culture on the resistance of the cells to atabrine. Population counts, pH, and relative resistance to atabrine were determined on samples taken daily from each of 3 flask cultures over a period of 4 days. Comparable results were obtained on all 3 cultures and the pertinent data from one such culture is presented in Table II. It will be seen that the concentration of atabrine required to immo-

[1] Kidder, G. W., and Dewey, V. C., *Physiol. Zoöl.*, 1945, **18**, 136.

* The atabrine dihydrochloride used was obtained through the courtesy of Dr. Arthur P. Richardson, Head of the Division of Pharmacology of the Squibb Institute for Medical Research.

TABLE I.
Effect of Atabrine on Division Rate.

Atabrine, gamma per ml	Intact organisms per ml		Calculated No. of generations in 24 hr	% of population motile after 24 hr
	0 hr	24 hr		
400	4300	0	—	0
200	"	0	—	0
100	"	0*	—	0
50	"	3,000	−0.5	25
25	"	24,000	2.5	100
12.5	"	24,400	2.5	100
6.3	"	24,000	2.5	100
3.1	"	31,200	2.8	100
1.6	"	33,200	3.0	100
0.8	"	39,000	3.2	100
0	"	37,000	3.1	100

* No growth on subculture.

TABLE II.
Effect of Age of Culture on Resistance to Atabrine

Age of culture, Hr	Population, cells/cc × 1000	pH	Atabrine—γ per ml								
			250	125	62.5	31.3	15.6	7.8	3.9	1.95	0
1	2	6.9	0	0	1+	4+	4+				4+
24	30.6	7.1	0	0	0	1+	4+				4+
48	43.4	7.2	0	0	0	0	0	4+	4+		4+
72	127	7.6	0	0	0	0	0	±	4+	4+	4+
96	114	7.7	0	0	0	0	0	±	4+	4+	4+

0 = No motile cells observed.
± = Occasional motile cell.
1+ = Approximately 25% of cells motile.
2+ = " 50% " " "
3+ = " 75% " " "
4+ = All cells motile.

bilize all cells progressively decreased from 125 γ per ml 1 hour after the flask was inoculated to 15.6 γ per ml 72 hours after inoculation. In order to determine what factors might be responsible for this marked decrease in resistance to atabrine the experiments described below were carried out.

Size of the population did not appreciably affect resistance to atabrine since a 32-fold dilution of washed cells from a 24-hour culture had little or no effect on the concentration of atabrine required to immobilize the cells. However, as shown in Table II, the pH increased from 6.9 to 7.6 during 72 hours of incubation. Since an increase in pH resulted in more effective inhibition of *Escherichia coli* by atabrine,[2] it was of interest to study the effect of the pH of the culture on the resistance of *Tetrahymena geleii* to atabrine.

It will be seen (Table III) that the resistance of a 24-hour culture to atabrine decreased at least 1,000-fold when the pH of the culture was increased from pH 6.35, where the cells survived a concentration of 5,000 γ or more of atabrine per ml, to pH 7.48 where only 4.9 γ per ml were required to immobilize all cells. In each of 2 additional experiments resistance to atabrine was strikingly decreased when the pH was increased although it was not possible to predict the lowest inhibitory concentration of atabrine from the pH of the culture. It was also found that when a 9-day-old culture at pH 7.8 was adjusted to pH 6.9 the resistance to atabrine only increased from 4 to 16 γ per ml.

It was of interest to determine whether, aside from the increase in pH, the medium was altered during growth by the accumulation of metabolic products or cellular debris in such a way as to decrease the resistance of the cells. The results of a single experiment pre-

[2] Silverman, M., and Evans, E. A., *J. Biol. Chem.*, 1944, **154**, 521.

TABLE III.
Effect of pH of Culture on Resistance to Atabrine.

pH of culture	Atabrine—γ per ml													
	5,000	2,500	1,250	625	313	156	78	39	19.5	9.8	4.9	2.5	1.3	0
6.35	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
6.60	1+	1+	1+	2+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
6.80	0	0	0	0	0	0	4+	4+	4+	4+	4+	4+	4+	4+
6.95	0	0	0	0	0	0	0	±	4+	4+	4+	4+	4+	4+
7.18	0	0	0	0	0	0	0	0	0	0	4+	4+	4+	4+
7.48	0	0	0	0	0	0	0	0	0	0	0	3+	4+	4+

TABLE IV.
Effect of Conditioned Medium and Frozen Cells on Resistance to Atabrine.

24-hr culture plus an equal volume of:	pH	Atabrine—γ per ml							
		500	250	125	63	31	16	8	0
Fresh medium	7.0	0	0	0	1+	4+	4+	4+	4+
Conditioned medium*	7.0	0	0	0	0	0	4+	4+	4+
Frozen cell suspension† (cellular debris)	7.0	0	0	0	0	0	2+	4+	4+

* Cell-free supernate from 6-day-old culture adjusted to pH 7.0.
† 24-hour-old cells resuspended in ⅓ volume of fresh medium then frozen and thawed.

sented in Table IV show that at pH 7.0 the resistance of a 24-hour-old culture was significantly decreased by the addition of an equal volume of conditioned medium (obtained by adjusting the cell-free supernate from a 6-day-old culture to pH 7.0) or a frozen cell suspension containing only cellular debris.

That the decreased resistance of cells from older cultures is the result of environmental changes is shown by the fact that when 24-hour- and 9-day-old cells were resuspended in fresh medium to contain 122,000 and 140,000 cells per ml respectively at pH 6.9 a concentration of 63 γ of atabrine per ml immobilized approximately 50% of the population in each case.

Although, as shown in the experiments described above, the resistance of *Tetrahymena geleii* to atabrine was influenced by many factors it was possible to obtain quite reproducible results using 24-hour bacteria-free cultures in the logarithmic phase of growth at pH 6.9 to 7.0. A test similar to that used for the quantitative determination of antibiotic activity[3] was used and performed as follows. Two ml of culture was added to a series of Wassermann tubes each of which contained 20% less atabrine than the preceding tube. After overnight incubation at 25°C the endpoint was recorded as the highest dilution of atabrine in which no macroscpic turbidity was observed (although motile cells were present). Providing a bacteria-free culture was used the endpoint was not affected when sterile precautions were disregarded in performing the test. In a series of 14 replicate titrations performed over a period of one month, according to the technic described above, an endpoint of 40 to 43 γ of atabrine was obtained in every case. Bacteria-free cultures of *Tetrahymena geleii* are particularly well suited for such studies because conjugation does not occur and endomixis has not been observed.[4]

Summary. Multiplication of *Tetrahymena geleii* was inhibited by 50 γ of atabrine per ml. Resistance of the cells to atabrine was progressively decreased by the environmental changes occurring during growth of the culture and was strikingly decreased when the pH of the culture was increased.

[3] McKee, C. M., Rake, G., and Menzel, A. E. O., *J. Immunology*, 1944, **48**, 259.

[4] Kidder, G. W., personal communication.