A PRELIMINARY REPORT ON THE EFFECTS OF COLCHICINE ON BACTERIA[1]

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The phenomenal success (Eigsti 1941) plant breeders have enjoyed in recent years through the application of varying amounts of colchicine and its derivatives to the higher plants has led to speculation as to whether or not the drug will show like influences on bacteria and other lower forms.

An investigation of the literature reveals only one series of experiments on the effect of colchicine on the bacterial cell. These experiments showed no positive results of the $1/10^3$ concentration used. A review of the literature and association with the investigations being conducted with higher plants at the University of Oklahoma and its cooperative plant breeding project have led the writer to wonder whether a concentration of $1/10^3$ might not be lethal.

With this question in mind a series of investigations was planned to see if smaller concentrations of colchicine would give a better understanding of the effects of the drug on bacteria. This paper is a preliminary report of the result obtained in the early stages of the investigation.

The test organism used was a large, spore-forming bacillus, believed to be *B. mesentericus*. A solution consisting of 3 gm beef-extract, 5 gm peptone, and 8 gm sodium chloride, dissolved by boiling in 1000 cc of distilled water, was used as the basic medium. This medium was adjusted to $1/10^3$, $1/10^4$, $1/10^5$, and $1/10^6$ concentrations of colchicine. These solutions, together with a control consisting of the basic medium, were used as the test media. All results thus far are from liquid media.

Media containing colchicine at these four concentrations and the colchicine-free control medium were inoculated with *Bacillus* sp. and incubated at 37.5° C. The weight of the organisms grown in each of the test media was ascertained at hourly intervals from the sixth to the sixteenth hour by colorimeter reading of turbidity, a Bausch and Lomb No. 4 standard 50 mm Duboscq colorimeter being used. The turbidity standard was made by suspending one gram of fuller's earth in one liter of the basic medium and comparing portions of this standard with equal portions of the test solutions. This method made possible the selection of a standard time required for the growth of one gram of bacterial cells in each of the test solutions. To determine the approximate number and weight of bacteria per liter, plates of each test solution were poured at that age of the culture when the turbidity was equal to that of one gram of bacteria per liter.

After a preliminary series of tests had been made, the solution of $1/10^3$ concentration was eliminated from the experiment, since it had been definitely shown to have a retarding influence on growth. At the same time the study of the solution of $1/10^6$ concentration was discontinued, as the results were near enough to those from the control to indicate that this concentration was too small to have any noticeable influence on growth.

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[1]Contribution from the Botanical Laboratory of the University of Oklahoma, n.s. No. 73.

At this point there were promising indications of a marked influence of the $1/10^4$ and the $1/10^5$ solutions on the rate of growth as regards number and weight of individual cells and the time required to reach given turbidity levels. These solutions, together with the colchicine-free control, were used in additional tests, with results as shown in table I.

TABLE I

Variation in rate of bacterial growth with concentration of colchicine.

Age of culture	Concentration of colchicine				
	1:1,000[1]	1:10,000[2]	1:100,000[2]	1:1,000,000[1]	Control[2]
hrs.	[3]	[3]	[3]	[3]	[3]
6	0.750	0.920	1.016	0.872	0.872
7	.714	0.973	1.001[4]	.847	.926
7.5	.758	1.002[4]	1.071	.949	.974
8	.750	1,050	1.114	.949	.926
8.5	.790	1.067	1.172	.949	.943
9	.798	1.076	1.115	.949	.993
9.5	.802	1.122	1.139	.943	.999[4]
10	.815	1.091	1.131	.989[4]	1.013
11	.825	1.080	1.114	1.034	1.027
12	.828	1.080	1.140	1.020	1.041
13	.837	1.095	1.142	1.034	1.056
14	.825	1.022	1.055	1.071	1.056
15	.936	1.106	1.155	1.079	1.071
16	.959	1.150	1.230	1.079	1.083
21	1.013[4]	1.333	----	----	----

[1]Based on 32 tests. [2]Based on 221 tests. [3]Grams of bacteria produced per liter. [4]Plates poured from these cultures.

The results tabulated permit the following conclusions: (1) Colchicine in the most concentrated solutions ($1/10^3$) retards growth of the bacillus. (2) Solutions containing very low concentrations of colchicine ($1/10^6$) are too weak to have much effect on growth. (3) The period of most rapid growth is reached much earlier in the solutions containing colchicine in concentrations of $1/10^4$ and $1/10^5$ than in the colchicine-free control or the $1/10^3$ or $1/10^6$ solutions. Thus the arbitrary standard of turbidity was equalled by bacterial growth in 6 hours in the $1/10^5$ solution, 7.5 hours in the $1/10^4$ solution, 9.5 hours in the colchicine-free control and the $1/10^6$ solution, and only after 21 hours in the $1/10^3$ solution of colchicine.

Young cultures, transferred from organisms grown in solutions of colchicine for several weeks, were stained with nigrosin. Microscopic examination of slides showed that colchicine had an influence on bacterial size as indicated in table II.

The results here conform with those from the colorimeter turbidity tests. Measurements of the individual cells revealed that: (1) the $1/10^3$ solution of colchicine, while limiting the number of organisms produced, seems to increase the size of those which are able to grow; (2) the $1/10^4$ solution of colchicine produces an increase in both width and length of ap-

proximately 30 percent; (3) the $1/10^5$ concentration of colchicine shows the greatest influence, producing an increase in cell length from approximately 3 microns to approximately 6.4 microns, the increase in width being about 40 percent.

TABLE II

Size of Bacillus sp. as affected by colchicine in the nutrient medium at various concentrations

Colchicine concentration	Length[1]	Width[1]
1:1,000	4.575	.990
1:10,000	4.915	1.355
1:100,000	6.365	1.385
Control	2.905	.990

[1]Measurements in microns.

LITERATURE CITED

Eigsti, O. J. 1941. Research with colchicine in retrospect. The Biologist 22 (4).