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Growth of Bacteria in Media Containing Colchicine.

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Colchicine is toxic for plant and animal cells, and seems to be specifically a mitotic poison, preventing normal division of the chromosomes and thus causing mutation.[1] True bacterial cells differ from the cells of the higher plants and animals, in that so far as is known they do not contain chromosomes or well defined nuclei.

If the above statements are true then we might expect that colchicine would have either no effect or a different effect on bacterial cells than on the cells of the higher plants.

This work was designed to study the effect of colchicine on several different types and species of bacteria with respect to the character of growth, colony characteristics, cell morphology, biochemical reactions, and acceleration or inhibition of multiplication of the organisms.

Methods. A 4% solution of colchicine in plain meat infusion broth was sterilized by filtration through a Berkefeld N filter. This solution of colchicine was then diluted with broth to the desired concentrations. All media used were adjusted to pH 7.2.

Serial Transfers in Plain Broth and Colchicine Broth. One standard 4-mm loopful of a 24-hour broth culture of Staphylococcus H was inoculated into 5 cc of a 2% colchicine broth and into the same amount of plain broth. A culture of *B. typhosus* 109 was inoculated in the same manner. All cultures were incubated at 37°C. Serial transfers were made daily into fresh media of the same kind (colchicine into colchicine, and plain broth into plain broth) for 12 days and every 48 hours thereafter until a total period of 22 days had elapsed.

At the time of each transfer subcultures were made on plain agar plates in order to observe colony morphology and also smears made and stained by Gram's method. On the 8th and 16th transfer all the cultures were inoculated into various carbohydrate media, milk and gelatin for observations of their biochemical reactions.

Old Cultures. Cultures of *Staphylococcus aureus* H and *B.*

[1] Nebel, B. R., and Ruttle, M. L., *J. Heredity*, 1938, **29**, 2.

typhosus 109 were made in 2% colchicine and plain broth as before but instead of serial transfers the original cultures were incubated at 37°C for 22 days during which time subcultures were made every 4 days and treated in the same manner as described above.

Serial Transfers on Plain and Colchicine Agar. Plain agar and 2% colchicine agar plates were inoculated with a 24-hour broth culture of *Staphylococcus aureus* H and *B. typhosus* 109 in such a manner as to obtain isolated colonies. After 48 hours' incubation single colonies were picked and re-streaked on fresh plates. The colonies from the colchicine agar plates were streaked on colchicine agar and also on plain agar plates, and the colonies from the plain agar plates were streaked on plain agar and also on colchicine agar plates. These transfers were made every 48 hours over a total period of 22 days. Smears were made of the colonies and stained by Gram's method.

Other Organisms. A second series of colchicine broth tubes was inoculated using in this series varying concentrations of colchicine (2%, 1%, 0.50% and 0.25%). The organisms used were *Staphylococcus aureus* 1038, *Staphylococcus aureus* L, *Streptococcus hemolyticus, M. catarrhalis* and *B. megatherium.* Control tubes of plain broth were inoculated at the same time. Incubation was at 37°C for 22 days with the following observation at 48-hour intervals for the first 6 days and at 4-day intervals thereafter. Subcultures were made on plain agar plates for observations of colonies, smears made and stained by Gram's for morphology, and inoculation into various carbohydrate media, gelatin and milk to note changes, if any, in cultural reactions. Comparison was made of the amount of growth in the colchicine broth with that in plain broth controls as an indication of any inhibiting or accelerating action of the colchicine on the growth of the organism.

Results. The type of growth of *Stapylococcus aureus* H in 2% colchicine broth was markedly different, being a coarse granular growth which settled out rapidly, in contrast to the diffuse turbid growth of the plain broth controls. On 2% colchicine agar the colonies were wrinkled, waxy, and of a peculiar greenish-yellow color. The cell morphology in the colchicine broth and agar differed from the controls in that the organisms were larger and arranged in tetrads and had the appearance of a stained preparation of sarcinæ. These changes in growth, colony and cell morphology occurred on the first transfer to media containing colchicine and could be obtained only when grown in the presence of colchicine. On transfer to plain media the organisms immediately reverted to normal.

There was no change in the biochemical reactions. All the other organisms used, including 2 other strains of *Staphylococcus,* showed no variation from normal when grown in the presence of colchicine. The growth of *Streptococcus hemolyticus* was inhibited by 1% of colchicine; 0.50% and 0.25% had no apparent effect. There was marked but not complete inhibition of growth of *M. catarrhalis* in 2% colchicine, some inhibition in 1% but none in 0.50% and 0.25%. The staphylococci and *B. megatherium* grew equally well in all concentrations of colchicine used. There was no evidence of any stimulating effect on the growth of any of the organisms.

Blakeslee[2] says that colchicine affects only the cell division of the chlorophyl-bearing plants and that the fungi including the bacteria are not affected. Jennison[3] could detect no change in the rate of reproduction or colony morphology of bacteria in the presence of colchicine. With the exception of one strain of Staphylococcus which showed a temporary variation in cell and colony morphology when grown in the presence of colchicine, our results are in accord with these observations but further indicate that colchicine does not affect the cell metabolism of bacteria.

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The Liver and Endogenous Androgens.*

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It is well known that most androgens and estrogens are either inactive when administered orally, or are less potent than when administered subcutaneously. In the case of the estrogens, experimental evidence has been offered to explain this phenomenon. All workers agree that the lack of effectiveness with oral administration is due to inactivation of estrogens in the liver. When natural estrogens are incubated *in vitro* with liver,[1,2] their potency is lost.

2 Blakeslee, A. F., *Science,* 1939, **89**, 10.

3 Jennison, M. W., *J. Bact.,* 1940, **39**, 20.

* Supported in part by a grant from the Josiah Macy, Jr., Foundation.

1 Zondek, B., *Skand. Arch. f. Phys.,* 1934, **70**, 133.

2 Heller, C. G., *Endocrin.,* 1940, **26**, 619.