By the cup technique, *L. lactis* shows no response to thymidine, desoxyribonucleic acid, or 0.5% ascorbic acid. Response is obtained with crystalline vitamin B_{12}, vitamin B_{12a} (*7*), liver concentrates, and certain microbiological fermentation products. The fermentation materials frequently require autoclaving to destroy associated antibiotics, or acidification for release of LLD growth-promoting factors from the cells. Insoluble adsorbates, and fermentation residues, often yield full activity following suspension in water and apportioning the suspension directly into assay cups, although supernatant liquors from such suspensions may contain little growth factor.

In order to obtain full LLD activity with some adsorbates, such as APF adsorbates, it is necessary to dilute suspensions to 0.05 μg B_{12} equivalent per ml. For these samples, the check standard solution should also contain 0.05 μg B_{12} per ml.

In the cup assay described, quantitative values are assigned to the growth response of *L. lactis* Dorner (A.T.T.C. No. 10,697) to unknown preparations on the basis of an assumed value of 11×10^6 activity units per mg of vitamin B_{12}. To distinguish from the Shorb LLD unit (*11–13*), the unit of *L. lactis* Dorner activity as determined by the herein-described cup method is designated as the LLDCC unit of LLD type activity. The cup assay shows no LLD response to desoxyribonucleic acid or its constituent nucleosides, whereas the Shorb titrimetric assay shows LLD activity for these substances. Cup and titrimetric assays show somewhat different responses to modified substances, such as vitamin B_{12a}, when assayed against a vitamin B_{12} standard. Impure materials, containing various LLD-active substances, do not necessarily yield equal results by both methods. Shorb's liver concentrate standard, assigned a value of 1,000 LLD units per mg, from which the above-noted LLD activity of B_{12} was determined by titrimetric assay, was found experimentally to contain 1,120 units/mg by the cup assay with crystalline vitamin B_{12} standard.

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Note: Description of a cup assay for the antipernicious anemia factor, in which thymidine and related compounds caused interference, was presented orally by Dr. W. F. J. Cuthbertson before the Biochemistry Society in London, January 22, 1949.

Aureomycin in the Cultivation of *Endamoeba histolytica*[1]

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Three attempts have been made to establish a single strain of *Endamoeba histolytica* in Shaffer-Frye medium (*3*) containing aureomycin in place of other antibiotics. The aureomycin (Duomycin) was obtained from Lederle Laboratories in vials each containing 50 mg of powdered aureomycin. This was diluted in a phosphate buffer, pH 6.9, and used within 2 hr. Final concentrations of aureomycin in the culture tubes ranged from a dilution of 1:1,000 to 1:5,000,000.

In each experiment the inoculum used consisted of 48-hr cultures of the amebae collected in large numbers by centrifuging the buffered saline overlay containing rice starch from flasks of coagulated egg base (*2*). These flasks were inoculated with amebae from Balamuth's culture medium (*1*). Counts were made on the material inoculated into the aureomycin cultures, and a volume of 0.5 ml was inoculated into each tube. Growth of the amebae in culture was estimated by examining them through the wall of the test tube placed under the 16-mm objective of the microscope and recording the relative numbers in terms of 0, ±, +, ++, +++, or ++++.

In the first attempt to establish our human strain XXII in the aureomycin cultures, five dilutions of aureomycin were used with two tubes for each dilution, and four control tubes were set up, two having 10,000 units of streptomycin and 5,000 units of penicillin in 0.5 ml of buffered saline, and two having the same amounts of streptomycin and penicillin in 0.2 ml of physiological saline. The aureomycin dilutions used were 1:1,000, 1:2,000, 1:10,000, 1:100,000, and 1:1,000,000. Transfers of the cultures were made at 48- or 72-hr intervals.

In this first experiment, positive cultures were obtained only in the tubes containing aureomycin diluted 1:100,000 and 1:1,000,000, and in the controls. The two 48-hr cultures of 1:1,000,000 dilution (++++ growth) were pooled and from them tubes containing aureomycin diluted 1:33,000, 1:50,000, 1:1,000,000, and 1:2,000,000 were inoculated. Growth was obtained in all dilutions, but it was decidedly better in the 1:1,000,000 and 1:2,000,000 than in the lower dilutions. The two tubes containing the 1:33,333 dilution remained positive only 48 hr and those containing 1:50,000 remained positive 72–96 hr. One tube containing 1: 2,000,000 was positive 168 hr after inoculation. A third passage was made 72 hr after the previous transfer and positive cultures in dilutions of 1:50,000, 1:100,000 (inoculum 120 hr old), 1:1,000,000, and 1:2,000,000 were transferred. Only two tubes became positive for amebae and they had a ± rating 48 hr after inoculation. After the third transfer the

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control tubes also were negative except for one ± tube 24 hr after inoculation.

In this first attempt to establish the amebae in aureomycin cultures, the tubes containing aureomycin dilutions of 1:1,000 and 1:2,000 were clear but decidedly yellow, due to the high concentration of aureomycin. The other tubes were moderately turbid and became increasingly so with age. After the second transfer all tubes were considerably less turbid than before, but did not become perfectly clear until after the third transfer. When the third passage was made, tubes of brain heart infusion and thioglycolate media were inoculated with the amebic cultures to test for living bacteria. The tubes inoculated from the control cultures remained clear (presumably negative for bacterial growth) for 96 hr, when they were discarded. The tubes inoculated from the aureomycin cultures all became cloudy (positive for bacterial growth), but the inocula containing aureomycin dilutions of 1:33,333 and 1:50,000 showed slower growth of bacteria than did those in dilutions of 1:100,000 and higher.

In a second attempt to establish the amebae in aureomycin cultures, this antibiotic was used in six dilutions: 1:33,333, 1:100,000, 1:333,333, 1:1,000,000, 1:2,000,000, and 1:5,000,000. Four controls with streptomycin and penicillin were set up as before. The first passage of the amebae into the aureomycin medium resulted in positive cultures in all dilutions, but the 1:33,333 dilution was negative after 48 hr. Growth in the 1:100,000 dilution was best at 24 hr and decreased steadily thereafter, while in all the higher dilutions ++++ growth was recorded after 48 hr. All tubes were quite turbid in 24 hr, the 1:5,000,000 dilution having the greatest turbidity.

In this experiment the amebae were maintained through five serial passages made at 48-hr intervals. In one set of tubes, transfer of the amebae was made into tubes containg the same dilution of aureomycin as previously, while in a second set the positive cultures remaining after the third passage were transferred into media containing the next highest dilution of aureomycin in series. In this second set of cultures, four tubes were inoculated containing aureomycin in dilutions of 1:333,333, 1:1,000,000, 1:2,000,000, and 1:5,000,000. The last dilution was turbid in 24 hr and did not become positive for amebae, while the other three cultures were positive after 24–48 hr but were very poor and showed gas production. The four cultures were transferred one more time into the same aureomycin dilutions but only one tube showed growth, the 1:2,000,000, which remained ± for 72 hr before becoming negative.

In the series of cultures which were transferred into the same dilutions of aureomycin, the amebae in the dilution of 1:33,333 did not survive a second passage. The amebae in the 1:100,000 dilution survived four passages before dying out, but did not grow quite as well as those in the higher dilutions. In the 1:333,333 dilution, the amebae grew quite well at first but died out after the fourth passage. Amebae in the dilution of 1: 1,000,000 remained positive through five passages when they were discontinued, since growth at that time was very poor. After the second passage, the tubes having the 1:5,000,000 dilution of aureomycin became very turbid, and the 1: 2,000,000 dilution increased in turbidity. Gas production was evident in the 1: 2,000,000 tubes after the third passage, in the 1:1,000,000 and 1:333,333 tubes after the fourth passage, and in the 1:100,000 tube after the fifth passage. In the control cultures with this series, only two of the four survived the third passage, one the fourth, and none the fifth.

A third attempt was made to establish the amebae in aureomycin cultures, starting this time with four dilutions: 1:100,000, 1:500,000, 1:1,000,000, and 1:2,000,000. Only two controls were run, using 10,000 units of streptomycin and 5,000 units of penicillin in 0.5 ml of buffered saline. The control tubes were negative for amebae after the third passage, and were discontinued. Six serial passages were made with the aureomycin cultures, and the only one which survived them all was the 1:1,000,000 dilution, which became negative 48 hr after the last transfer. The growth of the amebae was poorer in the 1:100,000 dilution than in the higher dilutions and became negative earlier, surviving only four passages. There was little difference in the amount of growth obtained in the other three dilutions; however, the 1:2,000,000 dilution had a tendency to become turbid when kept four or five days. Gas production was never eliminated from those tubes in which the amebae grew.

As in the second experiment, these cultures were transferred following the first passage to media containing the next higher dilution of aureomycin and maintained in the decreased aureomycin concentration. There was no significant difference in the growth or survival of amebae in the dilutions of 1:500,000, 1:1,000,000, and 1:2,000,000; however, when the amebae in 1:2,000,000 were put into a dilution of 1:4,000,000, the medium became so turbid that this dilution was not continued. In the other three dilutions, the amebae survived four and five serial passages.

From the experiments described it seems that there is an optimum range of aureomycin concentration for the growth of *E. histolytica*. Too high a concentration, or a dilution of less than 1: 100,000, appears to inhibit amebic growth, either directly or indirectly, although bacterial growth may be checked or inhibited. Too great a dilution, 1:2,000,000 or more, is insufficient to control bacterial growth and therefore unsatisfactory as a substitute for penicillin and streptomycin. Within a certain range, possibly 1:300,000 up to 1:1,000,000, the amebae grew well but there was evidence that bacterial growth was not inhibited. On the basis of these experiments, it would appear that the strain of amebae tested grows in the aureomycin cultures as well as, if not better than, in the penicillin-streptomycin cultures, although control of bacterial flora was less satisfactory.

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