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Enhancement of Growth of Certain Fungi by Streptomycin.

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Recent reports[1-7] have shown that the growth of some streptomycin-resistant bacteria may be dependent upon or enhanced by this antibiotic. A primary requisite for this phenomenon was previous exposure of these organisms to this drug. Although it is well known that streptomycin is not effective against fungi, there have been no observations, to our knowledge, concerning the enhancement of growth of fungi by streptomycin. The incorporation of streptomycin into certain selective media for fungi[8] has been instituted for the purpose of inhibition of bacteria. Employing a synthetic liquid medium containing streptomycin, we noted that the growth of certain fungi not previously exposed to streptomycin was more luxuriant than when streptomycin was absent. To further evaluate this observation, the studies described below were conducted on available laboratory strains of fungi which also had not been previously exposed to streptomycin.

Methods. The fungi were assayed in triplicate in a synthetic liquid medium consisting of inorganic salts and 0.5% glucose* to which commercial streptomycin† was added in concentrations of 0.01 to 5.0 mg/ml of medium. The final pH of the medium was 4.0. Inocula were prepared by washing the growth from 3-weeks-old Sabouraud's cultures with sterile saline and adjusting the turbidity to match a No. 3 MacFarland nephelometer standard. The suspensions were homogenized as much as possible by vigorous shaking and pipetting, and the heavier particulate matter allowed to settle. One-tenth ml amounts were then inoculated in triplicate into the test media as well as into control tubes without streptomycin. All cultures were incubated at 28°C and examined daily for comparative growth for 4 weeks.

Results. The growth of all of 6 strains of *Sporotrichum schenckii*, 4 of 4 strains of *Coccidioides immitis*, 2 of 3 of *Histoplasma*

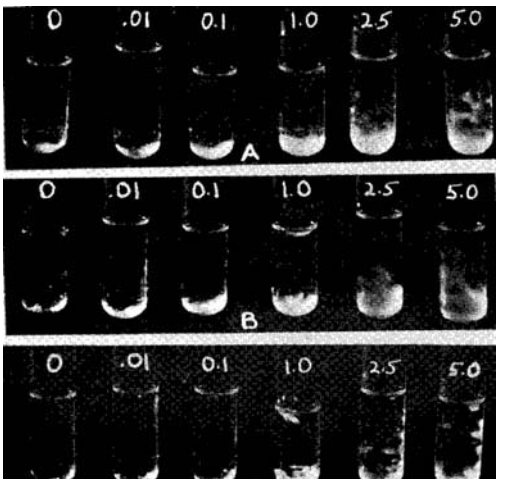

PLATE 1.
Effect of streptomycin on the growth of fungi. Each figure represents mg streptomycin/ml of medium.
A. *S. schenckii*—8th day.
B. *C. immitis*—9th day.
C. *H. capsulatum*—9th day.

* 4.5 g KH_2PO_4, 0.5 g $(NH_4)_2SO_4$, 0.5 g $(NH_4)Cl$, 25 ml M/500 in N/50 HCl $FeNH_4SO_4$, 10 ml 0.4% $MgSO_4 \cdot 7H_2O$, 5.0 g glucose and distilled water to 1000 ml.

† Duplicate studies were done with Merck's streptomycin hydrochloride and Pfizer's streptomycin sulfate.

[1] Miller, P. L., and Bohnhoff, M., *Science*, 1947, **105**, 620.

[2] Kushnick, T., Randles, C. I., Gray, C. T., and Birkeland, J. M., *Science*, 1947, **106**, 587.

[3] Paine, T. F., and Finland, M., *Science*, 1947, **107**, 143.

[4] Vanderlinde, R. J., and Yegian, D., *J. Bact.*, **1948, 56**, 357.

[5] Spendlove, G. A., Cummings, M. M., Fackler, W. B., and Michael, M., *Pub. Health Rep.*, 1948, **63**, 1177.

[6] Iverson, W. P., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., **1948, 69**, 586.

[7] Hobby, G. L., and Dougherty, N., *ibid.*, 1948, **69**, 544.

[8] Thompson, L., *Proc. Staff Meetings of Mayo Clinic*, 1945, **20**, 248; Littman, M. L., *Am. J. Clin. Path.*, **1948, 18**, 409.

capsulatum as well as single strains of *Phialophora verrucosa* and *Trichophyton mentagrophytes* appeared to be increased in the presence of streptomycin. The stimulatory effect was noted as early as 48 hours with *S. schenckii* while similar results were obtained with the other organisms in periods varying from 1-3 weeks. The stimulatory effect was proportional to the concentration of streptomycin up to 2.5 mg per ml, at which level maximum growth was obtained. This effect was noted only with young actively growing cultures and not with old stored suspensions. Plate 1 depicts photographically the results obtained in representative tests.

Since streptomycin is known to be more effective at an alkaline pH, similar studies were made with *S. schenckii* and *C. immitis* in media adjusted to 4.0, 6.0, and 8.0, respectively. Although the amount of growth in the control tubes was considerably less at pH 8.0 than in the acid range, the same stimulatory effect in the presence of streptomycin noted above was observed at all 3 levels.

Summary. Streptomycin incorporated into a synthetic liquid medium enhanced the growth of laboratory strains of *S. schenckii, C. immitis, P. verrucosa, T. mentagrophytes,* and *H. capsulatum* which had not been previously exposed to this antibiotic.

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Phosphoprotein Phosphatase in Rat Young, Embryos, and Placentas.

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Phosphoproteins appear to exist uniquely in food sources for the embryo and young (*e.g.*, casein in milk, vitellin in egg yolk, ichthulin in fish eggs). A clue to the significance of this fact was seen in the observation by Harris[1] that frog eggs contained an enzyme, designated phosphoprotein phosphatase, which released inorganic phosphorus from phospho-proteins without preliminary proteolysis. This was confirmed by Feinstein and Volk,[2] who further showed such an enzyme present also in various mammalian tissues.

The present report presents data regarding the activity of this enzyme in rat placentas, embryos, and young, all of various ages.

Methods. Dated conceptions were obtained by following vaginal smears and introducing the female to the male during an overnight period during estrus. At the desired time after conception, the pregnant female was sacrificed, and the embryos and placentas were removed, separated by rough dissection, quickly frozen in an ether-dry ice mixture, and stored under deep freeze until convenient for assay. Several placentas from each female were pooled for assay, as were 14- and 16-day embryos. Young rats were sacrificed by immersion in the freezing mixture and stored under deep freeze.

For assay, placentas and embryos were homogenized in a glass homogenizer; young rats were blended in a Waring blendor. Further details of the assay and analytical methods have been described.[2]

Results. Fig. 1 presents the phosphoprotein phosphatase activity of whole placentas, embryos, and young rats, all of varying ages.

Fig. 2 presents the activity of this enzyme per gram of tissue and per mg of protein, in

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1 Harris, D. L., *J. Biol. Chem.*, 1946, **165**, 541.

2 Feinstein, R. N., and Volk, M. E., *J. Biol. Chem.*, in press.