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**Influence of Cultured Tissue Fragments on Sulfanilamide-Inhibition of *Beta* Streptococci.\***

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It has been found that sulfanilamide causes a reduction in the size of the colony of sensitive strains of *beta* streptococci grown in tissue-culture media and that it regularly inhibits the development of the diffuse periphery usually seen around such colonies.<sup>1</sup>

A study has been made of the influence of tissue-fragments on these two characteristic responses of *beta* streptococci to the drug. The Maximow culture-technic was used. Details of the technic have been described previously.<sup>2, 3, 4</sup> A culture consists of one drop of heparinized rabbit-plasma and 3 drops of serum-extract of 7-day chick embryos, plus the required amount of sulfanilamide. To the controls a similar amount of saline was added. Sulfanilamide was sterilized by filtration. Streptococci were grown for 24 hours in rabbit-serum extract of 7-day chick embryos to which 5% rabbit erythrocytes were added. A suitable dilution of the bacterial culture made in Tyrode was added to the serum-extract.

A strain of *beta* streptococci, Lancefield group C, from the collection of Dr. F. Heilman, Mayo Clinic, was used in this study.

Cultures were incubated at 37.5°C. Observations were made at intervals during the incubation period. Measurement of colonial diameter was made in the living state (16 mm objective and 6 x ocular) using mechanical stage and ocular micrometer. Photomicrographs were made in the living state. Cultures were then fixed in Carnoy, stained with dilute Delafield's hematoxylin and mounted as whole mounts.

This strain grows rapidly in cultures without tissue and the colo-

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<sup>1</sup> To be published.

<sup>2</sup> King, J. T., *Arch. f. Exp. Zellforsch.*, 1930, **9**, 341.

<sup>3</sup> King, J. T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

<sup>4</sup> King, J. T., Henschel, A. F., and Green, B. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 810.

nies show diffuse peripheries. In cultures without tissue containing 140 mg % of sulfanilamide no diffuse peripheries are seen. In cultures containing tissue-fragments even this very high concentration of sulfanilamide does not suppress the development of diffuse peripheries around those colonies within 1 mm of the fragment. Colonies more than approximately 1 mm distant from the fragment do not show this qualitative "release". The tissues studied were heart, skeletal muscle, liver, and brain. Tissue was derived from 10- to 14-day chick embryos. Heart and skeletal muscle are most effective in causing the development of diffuse peripheries in the presence of high concentrations of sulfanilamide; liver is only moderately effective and brain very slightly effective.

In spite of the fact that brain-fragments exert a very slight effect as compared with muscle in causing qualitative changes in colonies near the fragment in the presence of high concentrations of sulfanilamide, preliminary measurements show that bacteriostasis is much less in cultures containing such fragments than in cultures without fragments. In one series inhibition of colony-diameter was 12.05 and 59.34% respectively at 48 hours. Not less than 100 colonies were measured in each experimental and control set.

It has been found that when a series of cultures is planted from the same inoculated serum-extract the cultures containing tissue-fragments show higher colony-counts. It is known that sulfanilamide is less effective in the presence of a larger number of organisms. However, we know from other series and from other investigations in which this strain has been studied that this factor would be of minor importance in the observed reduction of inhibition.

Quantitative studies are in progress to determine the relative effectiveness of the 4 tissues used in antagonizing the bacteriostatic effect of sulfanilamide under various experimental conditions.

Sections of tissue-fragments cultured under these conditions show extensive cellular damage both on the periphery and in the center of the fragment.

*Summary.* In tissue-cultures, the characteristic effects of sulfanilamide on *beta* hemolytic streptococci are markedly inhibited by the products of tissue-breakdown.