

necessary to determine the approximate concentration of the unknown in a preliminary experiment, so as to have the standard and the unknown of nearly the same concentration. The amount of fluorescent light excited depends both upon the concentration of the riboflavin and on the intensity of the exciting light. Since the riboflavin solution absorbs part of the exciting wavelengths, their intensity becomes less in high concentrations of riboflavin. By having standard and unknown at approximately the same concentration this source of error is avoided.

The following concentrations of riboflavin were observed:

Strain of the bacillus	Amount of riboflavin in culture medium, gamma per cc
R <sub>1</sub>	2.86
BCG	1.07
Bovine	0.85
Human H37	0.50

The bovine strain is the most virulent of the four and the BCG the least virulent, but both produce less riboflavin than the R<sub>1</sub> strain. de Grolier<sup>6</sup> observed greatest pigment production in 2 human strains of reduced virulence, but also in the Kiel strain which was unfortunately virulent. Apparently there exists no close relationship between virulence and pigment production.

*Summary.* Riboflavin was isolated from Sauton medium on which R<sub>1</sub> tubercle bacilli had grown and the amount of riboflavin produced by 4 different strains of tubercle bacilli was determined by a method of fluorescence spectrography described in the paper.

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### Comparison of Bacteriostatic Effects of Sulfanilamide and Sulfapyridine (2 Sulfanilyl Aminopyridine) on Bacteria in Broth Cultures.\*

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Fleming<sup>1</sup> has recently reported that sulfapyridine—2(sulfanilyl aminopyridine)—in concentrations of from 1:256,000 to 1:8,000 inhibited the development of large inocula of type 23 pneumococci

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<sup>1</sup> Fleming, A., *Lancet*, 1938, **2**, 74.



in deleucocytized human blood. In the presence of leucocytes a concentration of 1:32,000 sulfapyridine killed an inoculum of 100,000 pneumococci. The drug had a similar effect upon hemolytic streptococci. Bacteriostasis was obtained in the absence of leucocytes, while in their presence actual killing occurred. A comparison of the efficacy of sulfanilamide and sulfapyridine in whole blood showed that the latter was the more effective against hemolytic streptococci and type 15 pneumococci. Fleming used the slide cell technic which he described some years ago. We<sup>2</sup> have already shown that sulfapyridine is as effective a bacteriostatic agent as sulfanilamide against a strain of *beta* hemolytic streptococcus (C203), and is somewhat more effective against Types I, II and III pneumococci in broth cultures. The present investigation was undertaken with the purpose of comparing the action of sulfapyridine and sulfanilamide against a miscellaneous group of microorganisms.

One strain each of *beta* hemolytic streptococci, Groups B, C, and D, *alpha* hemolytic streptococcus, *Staphylococcus aureus*, *E. coli* and *E. typhi* were tested for susceptibility to 10, 50, and 90 mg % concentrations of sulfanilamide and sulfapyridine in beef infusion broth (2% Neopeptone, 0.075% dextrose). A series of tubes, containing the desired concentrations of the 2 drugs in broth and a drug-free control, were inoculated with 0.5 cc of culture dilution. The cultures had been grown in broth at 37°C for 5 or 6 hours, and the dilutions, made with the same medium, were sufficiently high in most instances to give inocula of less than 100 organisms. The size of the inoculum and the extent of growth in the tests after 20 hours' incubation were determined by pouring plates and counting the colonies which developed. The tests were also examined visually for turbidity and those which showed less than normal growth were returned to the incubator and plates poured again at 44 hours. The results are shown in Table I.

Sulfapyridine was a somewhat more effective bacteriostatic agent than sulfanilamide in broth cultures of *E. coli*, *E. typhi* and Group B *beta* hemolytic streptococci. The two compounds were approximately equally active against Group C *beta* hemolytic streptococci and *alpha* streptococci, and they were equally inactive against Group D *beta* hemolytic streptococci and *Staphylococcus aureus*.

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<sup>2</sup> Long, P. H., Bliss, E. A., and Feinstone, W. F., *Pennsylvania Medical J.*, in press.