It was concluded that respiratory obstruction was primarily responsible for the lung changes observed, and that the reaction of smaller animals to bilateral vagotomy, in this particular respect, differed in no way from that of the larger animals, but that it was less readily demonstrated because of a smaller airway which easily became occluded.

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In vitro Studies on the Action of Sulfapyridine.

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The use of sulfapyridine (2-sulfanilyl aminopyridine) in experimental pneumococcus pneumonia,[1] together with favorable clinical reports in the treatment of lobar pneumonia, led Fleming[2] to conduct *in vitro* studies on the action of the drug on the pneumococcus. He observed a marked bacteriostatic effect in deleukocyted blood, using his "slide-cell" technic. His criterion of bacteriostasis was the relative colony size of drug-treated cultures as compared with untreated controls. Whitby[1] reports a degeneration and final disappearance of the capsule in the peritoneum after 4 hours' growth in drug-treated mice. Fleming[3] has not been able to confirm this in his *in vitro* work.

The present studies were made to obtain data regarding the growth curve of the pneumococcus under the influence of the drug, which would give a quantitative measurement of bacteriostasis. In addition, morphological studies were made at regular intervals during the growth curve.

The organism used was a Type II pneumococcus which had been carried in mice for over a year by the method of Neufeld and Handel.[4] Eight-hour cultures, made by adding a drop of heart's blood from a freshly dead mouse to 20 cc of veal infusion broth, were used in all experiments. A culture of this age was chosen to prevent the appearance of a lag phase, which would result in exposure of resting

1 Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

2 Fleming, A., *Lancet*, 1938, **2**, 74.

3 Fleming, A., *Lancet*, 1938, **2**, 564.

4 Neufeld, F., and Handel, L., *Berl. Klin. Woch.*, 1912, **49**, 680.

cells to prolonged action of the drug. This is in accordance with previous work by Chesney,[5] showing that subcultures made during or immediately following the logarithmic growth phase exhibit little or no lag.

Two cubic centimeters of a 1:1000 solution of sulfapyridine were added to 18 cc of the menstruum to be tested. This made a final concentration of 1:10,000 of the drug. Two cubic centimeters of saline were added to the controls. Each tube received ½ cc inoculum of the whole pneumococcus culture.

Each hour 1 cc samples of the cultures being examined were diluted, pipettes being changed with each dilution. Plates were made with 3% rabbit-blood agar. Each calculated point on the curve represents the average of 10 plates.

The growth studies presented are representative ones chosen from the experiments. Fig. 1 shows curves obtained by growing the organism in 2% peptone (Parke-Davis) veal infusion broth, with and without the drug. No significant difference is to be observed due to the presence of the drug.

Lockwood[6] has shown that the action of sulfanilamide upon streptococci is inhibited in the presence of peptone. Fig. 2 shows the curves obtained by growing the organisms in peptone-free veal infusion broth. A significant bacteriostatic action by the drug is seen; the number of organisms in the treated culture rises more slowly, and at 8 hours, is approximately half that of the control.

Measurements were made of the number of organisms growing in uninactivated rabbit serum. Samples were sealed and rotated according to the method of Todd.[7] Fig. 3 shows curves obtained under these conditions. Marked bacteriostasis is observed, much greater than in the peptone-free broth, although the serum control rises to a higher point than the veal-infusion control.

When peptone sufficient to make a final concentration of 2% was added to serum, the effect of the drug was largely lost, as is shown in Fig. 4.

It has been shown that in the absence of peptone, sulfapyridine in a concentration of 1:10,000 exerts a pronounced bacteriostatic effect upon growing pneumococci. This is especially apparent in the whole uninactivated serum.

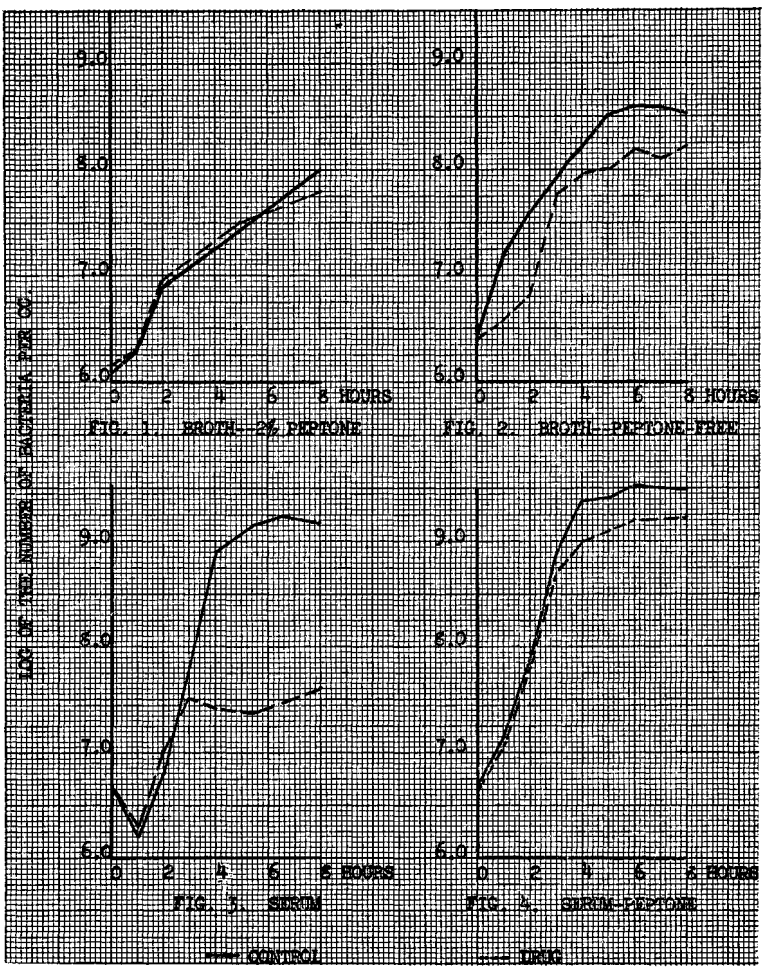
Slides made at hourly intervals were stained by Maneval's[8] method

[5] Chesney, A. M., *J. Exp. Med.*, 1916, **24**, 387.

[6] Lockwood, J. S., *J. Immun.*, 1938, **35**, 155.

[7] Todd, E. W., *Brit. J. Exp. Path.*, 1927, **8**, 1.

[8] *Stain Technology*, 1928, **4**, 21.



for the demonstration of capsules. No inhibition of capsule formation could be seen in the drug-treated cultures. In peptone-free serum cultures, the organisms growing in the presence of the drug showed a marked tendency to grow in long chains of 10-18 cocci, whereas in the controls, the diplococcus form was predominant, with some short chains of 4 or 6 cocci.

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