Coleman Universal spectrophotometer with the wave length dial set at 600 mμ. Comparisons were made against a SG medium blank, which had received the same treatment as the other tubes, though this always remained perfectly clear. This turbidity measurement, of course, gives the total number of organisms present, without regard to their viability.

A composite graph of results in Fig. 1 shows the effect of various substances and combinations of these substances on the growth rate of *E. coli*, as a function of temperature. It will be noticed that the diminution of the growth rate, as effected by sulfathiazole, increases markedly with temperature. This inhibition amounts to 45% at 42°C (108°F), and only to 24% at 37°C. This is in accord with the work of Bang[4] on gonococcus, and of others. The *p*-aminobenzoic acid, in the concentration used, slightly diminished the growth, except at 37°. This concentration, however, overcomes most of the ill effects of sulfathiazole up to about 37°. At this, and higher temperatures, there is an appreciable inhibition of growth, due to the sulfathiazole, or in part, to the *p*-aminobenzoic acid itself. It is also of importance that *p*-aminobenzoic acid diminished the growth rate at temperatures higher than 37° in a manner which increases with temperature. This would suggest that *p*-aminobenzoic acid exerts a sulfonamide-like effect, provided the temperature is slightly raised. Urea alone, in concentrations up to 2%, acts to increase growth except at temperatures higher than the optimum. This increase amounts to about 10% at 37, and thus the effect of the mixture of *p*-aminobenzoic acid, urea and sulfathiazole (UPS) is accounted for. At slightly higher temperatures, urea alone reduces the growth rate sharply. Thus it might be said that at these temperatures, urea would certainly act conjointly with sulfathiazole in diminishing bacterial growth, at least in the case of *E. coli*. The large role which the inoculum size plays in determining the growth rate is brought out by a comparison of the 2 lowest curves, in which the inoculum is varied by a factor of 10.

In summary, the effect of temperature on the bacteriostatic action of sulfathiazole, *p*-aminobenzoic acid and urea, alone and in several combinations, has been studied on *E. coli*. The growth inhibition increases with temperature for all the substances. It is of importance that *p*-aminobenzoic acid inhibits growth at temperatures higher than 37°, and that at these elevated temperatures it is incapable of showing marked antisulfonamide properties.

[4] Bang, Frederik B., *J. Urology*, 1942, **47**, 299.

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Temperature and the Bacteriostatic Action of Sulfathiazole and Other Drugs. II. *Streptococcus pyogenes*.

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Preliminary work carried out on *E. coli* in this laboratory has shown that a study of bacterial growth rates, as influenced by sulfonamides and other drugs at various temperatures, gives interesting information concerning the bacteriostatic action of these substances.[1] In order to extend these observations and to study the generality of the actions, similar experiments have been carried out on *Streptococcus pyogenes*. (Ameri-

[1] Lee, S. W., and Foley, E. J., to be published. See companion article.

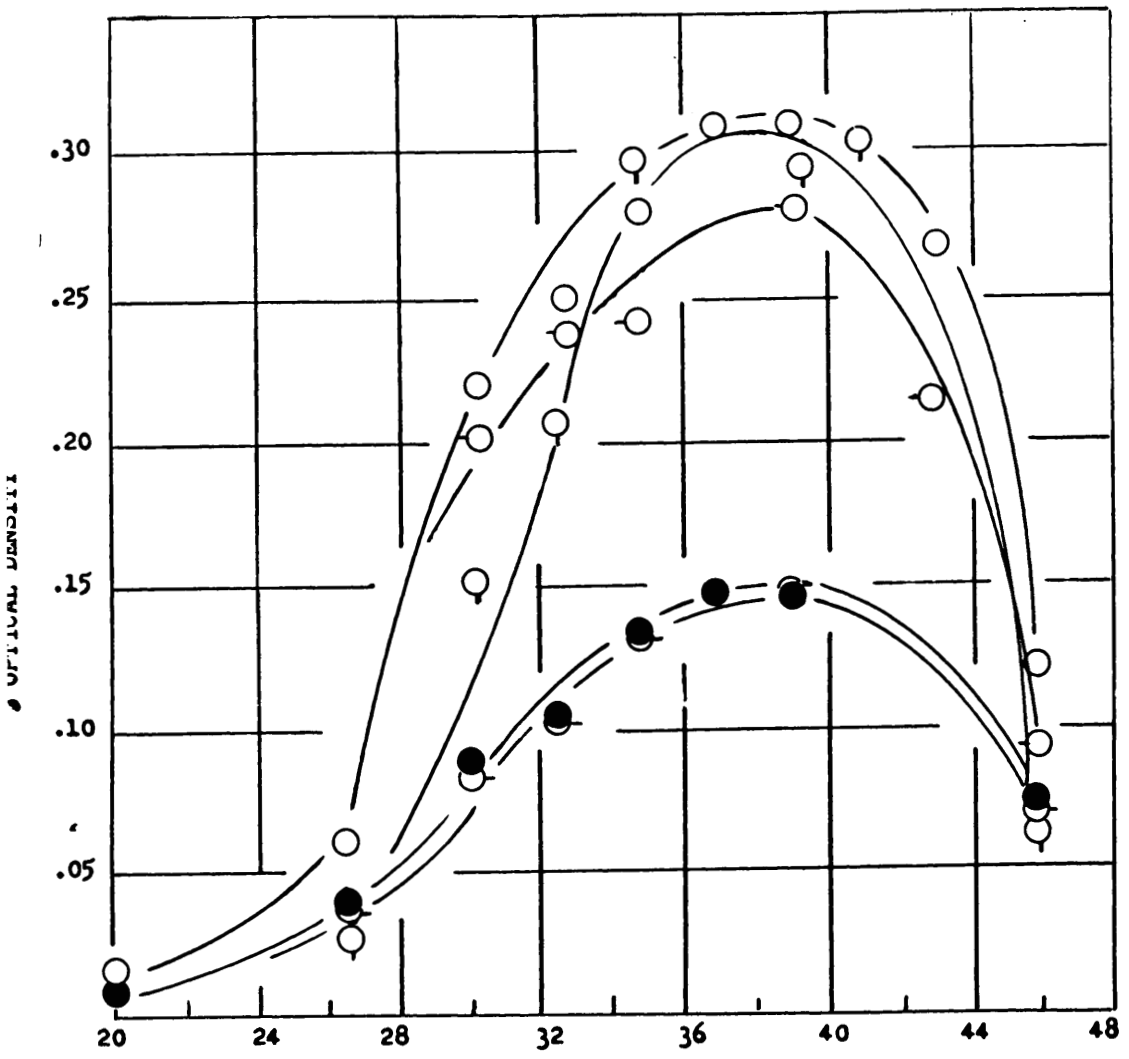

FIG. 1.

The Effect of Various Substances on the Growth Rate of *Streptococcus pyogenes*, as a Function of Temperature.

○ Control, no drug.
–○ *p*-aminobenzoic acid 10^{-5} M, and sulfathiazole 10^{-4} M.
○ Urea, 1%.
●
● Sulfathiazole, 10^{-4} M.
○– Sulfathiazole, 10^{-4} M and urea, 1%.

can Type Culture Collection number 8668).

The methods used in this series of experiments are essentially those of the earlier work.[1] Inocula which gave an initial population of about 50 million organisms per ml were added to the simplest medium which could be found to support active and rapid growth. This procedure has been advocated as a valid method for studying the action of the sulfonamides.[2] The medium used consisted of 75 parts of the basal medium of

[2] Bell, P. H., and Roblin, R. O., Jr., *J. Am. Chem. Soc.*, 1942, **64**, 2905.

Kirby and Rantz[3] and 5 parts of Leventhal blood filtrate, prepared from equal parts of the above basal medium and horseblood by boiling and filtering through cheese cloth and a Seitz funnel. This medium was also found to be relatively free of sulfonamide inhibitors, using as a guide the action of low concentrations (10^{-5} and 10^{-6} M) of sulfathiazole on *E. coli*. The growth measurements were taken at times during which the increase in population was approximately linear (period of retarded growth) as determined by separate experiments. With the inocula used, this time was usually from 4 to 6 hours. The drugs chosen for study were again sulfathiazole, *p*-aminobenzoic acid and urea. Urea is of interest as a potentiator of the sulfonamides. In kind, the results paralleled those obtained on *E. coli*.

Fig. 1 shows most of the experimental results. Optical densities, as obtained from the drum of a Coleman Universal Spectrophotometer, are plotted against temperature. In the case of this organism, the density is directly proportional to population, and a density of 0.20 corresponds to a population of about 2.7 billion organisms per ml. The action of *p*-aminobenzoic acid alone has not been plotted, but at a concentration of 10^{-5} M it increased the growth rate up to the optimum growth temperature, and at higher temperatures exerted a marked inhibition. From the figure, it is clear that up to about 40°, *p*-aminobenzoic overcomes most of the bacteriostatic effects of sulfathiazole. At higher temperatures, the bacteriostatic action of this pair becomes increasingly pronounced. Again, the sulfathiazole shows greater bacteriostatic activity at the higher temperatures. Urea alone greatly diminishes growth at elevated temperatures, but, with this method of study, shows no synergistic action with sulfathiazole, except at the highest temperature.

Summary. The effect of sulfathiazole, *p*-aminobenzoic acid and urea, separately and in combinations, on the growth rate of *Streptococcus pyogenes* has been studied as a function of temperature. The bacteriostatic action of all these substances has been shown to increase with temperature. The sulfonamide-like action of *p*-aminobenzoic acid at higher temperatures is considered to be of particular significance. With the method used, urea shows no synergistic action with sulfathiazole on this organism. These results with *Streptococcus pyogenes* have been found to be analogous to those obtained with *E. coli*.

[3] Kirby, W. M., and Rantz, L. A., *J. Exp. Med.*, 1943, **77**, 29.

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An Antibiotic Substance Produced by Submerged Cultivation of *Aspergillus flavus*.

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The strain of *Aspergillus flavus* used by White[1] and by Jones, Rake and Hamre[2] for the production of aspergillic acid has been grown in submerged culture with the production of an entirely different antibiotic substance. Erlenmeyer flasks containing a basic Czapek-Dox medium with the addition of adjuvant substances were inoculated with a heavy spore suspension of the mold. The flasks were subjected to constant, moderate agitation at a temperature of approximately 25°C. Within 48 hours there was heavy mycelial growth, and as this progressed, the

[1] White, E. C., *Science*, 1940, **92**, 127.

[2] Jones, H., Rake, G., and Hamre, D. M., *J. Bact.*, 1943, **45**, 461.