rettgeri should be eliminated from the genus *Shigella*. In our opinion *Sh. rettgeri* and type 33111 cultures are the same organism and should be redescribed as *Proteus rettgeri* in the genus *Proteus* or in the appendix of that genus. Because the majority of *Pr. rettgeri* strains were anaerogenic and all strains fermented mannitol some investigators will question the feasibility of placing this organism in the *Proteus* group. *Pr. vulgaris*, *Pr. mirabilis* and *Pr. morganii* produce small gas volumes and old cultures of *Pr. vulgaris*[7,15] not infrequently become anaerogenic and since in the present study some of the strains of *Pr. rettgeri* were aerogenic actual gas production could be considered a flexible criterion for *Proteus*. Despite the fact that Topley and Wilson[16] and St. John-Brooks and Rhodes[17] consider the inability to ferment mannitol a genus characteristic it would seem that ability to attack urea and to swarm are more important genus characteristics. The cultural, biochemical and serological reactions of *Pr. rettgeri* illustrate the difficulty in drawing strict lines of division between related groups of bacteria.

Pr. rettgeri has been described vicariously as a *Bacterium*,[18] *Bacillus*,[12] *Eberthella*,[19] and *Shigella*.[20] This sequence of events in our opinion was not due to carelessness but was brought about by increases in the delicacy and particularly in the number of tests used to differentiate species of bacteria since the isolation of *Pr. rettgeri* in 1909. It seems strange, however, that this relatively rare organism originally isolated from a cholera-like epidemic among fowls should have been isolated from more than 100 gastroenteritis patients or foodhandlers in the past 3 or 4 years.

The taxonomic position of *Pr. hydrophilus* and *Pr. ichthyosmius* has already been questioned.[4,5,17,21] These organisms do not decompose urea, are monotrichate, do not swarm and some strains produce from 20 to 100% gas volumes at 25°C from fermentable sugars, and produce acid and gas slowly from lactose at this temperature. A culture of *Pr. pseudovaleriei* received from Dr. de Assis of Brazil while peritrichate did not attack urea, produced approximately 30% gas in fermentable carbohydrates and appeared to possess all the characteristics of a paracolon organism. For these reasons it is recommended that *Pr. hydrophilus*, *Pr. ichthyosmius* and *Pr. pseudovaleriei* be dropped from the genus *Proteus*.

15 Welch, H., and Poole, A. K., *J. Bact.*, 1934, **28**, 523.

16 Topley, W. W. C., and Wilson, G. S., *The Principles of Bacteriology and Immunity*, 1936, second edition.

17 St. John-Brooks, R., and Rhodes, M., Third International Congress for Microbiology, Report of Proceedings, 1939, 167.

18 Hadley, P., Elkins, M. W., and Caldwell, D. W., Agr. Exp. Sta. Rhode Island State College, 1918, Bull. No. 174.

19 Bergey, *et al.*, *Manual*, 1st ed., 1923, 232.

20 Bergey, *et al.*, *Manual*, 5th ed., 1939, 476.

21 Reed, G. B., and Toner, C. G., *Can. J. Res.*, 1941, **19** D, 139.

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Effect of Temperature on Bacteriostatic Action of Sulfathiazole and Other Drugs. I. *Escherichia coli*.

S. W. LEE AND E. J. FOLEY. (Introduced by Marion B. Sulzberger.)

From the Wallace Laboratories, Inc., New Brunswick, N.J.

As a possible means of throwing light on the mode of action of the sulfonamides, the bacteriostatic effect of sulfathiazole, alone and with other drugs, has been studied as a function of temperature. Other such studies have given revealing information concerning the interaction of these substances with luminous bacteria, as well as isolated enzyme systems.[1]

1 Johnson, Frank H., *Science*, 1942, **95**, 104.

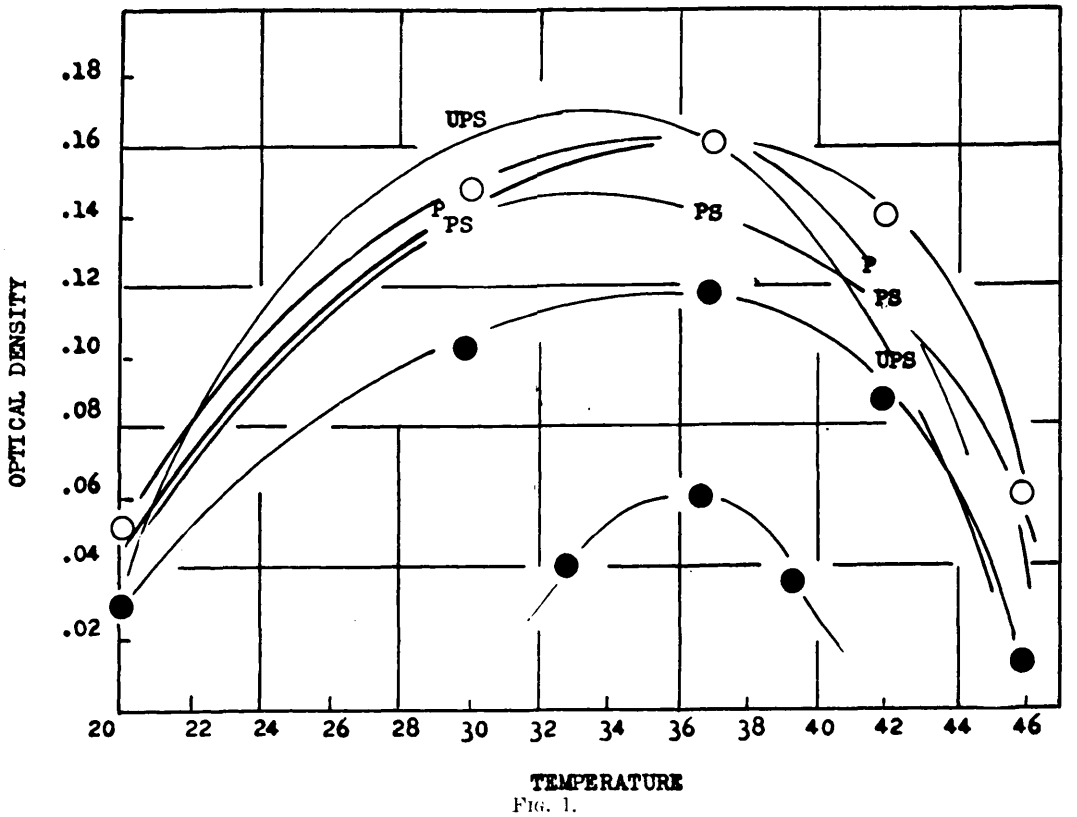

FIG. 1.

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

Open circles: Organism in medium SG (controls). Constant inoculum in all cases of about 300 million organisms.

Solid circles: Organism in medium SG and 10^{-4} M sulfathiazole.

P: Organism in SG and *p*-aminobenzoic acid 10^{-5} M.

PS: Organism in SG, PAB 10^{-5} M and sulfathiazole 10^{-4} M.

UPS: Organism in SG, urea 1.5%, PAB 10^{-5} M and sulfathiazole 10^{-4} M.

Readings taken after six hours.

Solid circles, lowest curve. Organism in medium SG and 10^{-4} sulfathiazole.

Inoculum, about 30 million. Control for this curve same as for the remainder.

With the work of Kohn and Harris[2] as a guide, several experiments have been carried out on *Escherichia coli*. This organism was chosen because of its susceptibility to the drugs in question, and because it grows well over a rather wide temperature range. Included in this report are data concerning the rate of growth of *E. coli*, as a function of temperature, and to some extent, of the concentration of the added rate-influencing substances. The substances chosen are among those of current interest, sulfathiazole, *p*-aminobenzoic acid and urea.[3] These have been studied separately, and in some of the possible combinations. In general, the first results are in line with what one would predict.

In the method chosen, massive inocula of *E. coli* were added to the water clear SG (salt and glucose) medium,[2] or to this medium containing the indicated quantities of added substances. The large inocula were chosen to give, in a day's time, large turbidity differences, which were measured with a

2 Kohn and Harris, *J. Pharm. and Exp. Therap.*, 1943, **77**, 1.

3 Tsuchiya, Tenenberg, Clark and Strakosch, PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 262.

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.