11101 P

Action of Sulfanilamide *in vitro* on Aerobic Sporogenic Bacilli.

L. ROSENTHAL.

From the Laboratories of Israel Zion Hospital, Brooklyn, New York.

Experiments dealing with the action of sulfanilamide and its derivatives *in vitro* were performed mostly with those pathogenic bacteria which are susceptible to the drug *in vivo,* namely: streptococci, pneumococci, meningococci, gonococci, *B. coli, B. proteus, Brucella melitensis, et al.*[1] Although there is as yet no complete agreement as to results between experiments *in vivo* and *in vitro,* the belief is growing that under both conditions the drug affects the metabolism or the reproductive mechanism of the bacteria. It seemed of interest to investigate the action of sulfanilamide on bacteria which have a more complex life cycle than those just enumerated. For this purpose, 4 strains of aerobic spore-bearing rods were chosen: 3 of the subtilis-mesentericus group and one of the *B. mycoides.* All strains were isolated in our laboratory.

As sporulation occurs more readily on solid media, a sulfanilamide agar (SA-agar) was prepared by dissolving 23 g of powdered Bacto-Nutrient agar (Difco) in one liter of aqueous solution of sulfanilamide of a desired concentration. The medium was sterilized in the usual way. Cultures of the 4 strains were prepared on SA-agar either on slants or plates and incubated at 37°C. Smears were made daily and stained with warm carbol-fuchsin solution. Cultures on plain nutrient agar (Difco) similarly treated were used as controls. Solutions of sulfanilamide 1 : 500 and 1 :1000 were arbitrarily chosen.

The macroscopic growth of the mesentericus-subtilis strains on SA-agar was approximately the same as in the control cultures while that of mycoides was scantier. Without going into details, which are irrelevant for the main purpose of this paper, we shall describe in a general way the morphology of the bacilli on SA-agar based on the microscopic study of successive daily smears made from the cultures. During the first 10 to 12 hours the growth on SA-agar develops in the same manner as on plain agar. The smears from both media reveal vegetative rod forms uniformly stained. On SA-agar, however, the rods appear longer and sometimes thicker. The *B. mycoides* shows a tendency toward thread-formation. After

[1] See Bibliography, Long, P. H., and Bliss, E. A., *The Clinical and Experimental Use of Sulfanilamide, etc.,* Macmillan, New York, 1939, p. 141.

24 to 48 hours, when the control cultures show almost exclusively naked spores and only few vegetative rods the bacilli do not sporulate at all on SA-agar. No spore formation takes place on this medium even in cultures several weeks old. Occasionally, in the first and second passage on SA-agar, few spores are present and these are very likely the so-called "dormant" spores which failed to germinate.[2] After subsequent passages through SA-agar, even these occasional spores could not be found. The suppression of spore formation by sulfanilamide was confirmed by the following experiment: Suspensions of bacilli in saline solution were prepared from week-old cultures on SA- and plain agar, both were heated at 80°C for 15 minutes to kill vegetative forms and inoculated on plain agar. The controls yielded abundant growth while subcultures from SA-agar either remained sterile or consisted of a few discrete colonies.

Marked changes occur in the morphology of the non-sporulating vegetative rods in the SA-agar cultures. On the second day of growth, the smears begin to show rods irregularly stained. In the next few days the changes are more evident. Many shadow forms with faintly stained cytoplasm appear. Some of the rods are disintegrated into a fine granular debris. Thus, during the early hours of incubation, the sulfanilamide does not appreciably interfere with the bacterial growth. The inhibitive action of the drug becomes manifest only later, after the expiration of the so-called "lag period", a finding noted by previous investigators *in vivo* and *in vitro* in connection with various other microörganisms.[1]

Cultures on SA-agar kept at room temperature for 3 to 4 weeks lose their viability and cannot be subcultured on usual media, while due to spore-formation control cultures are viable for many months.

The bacilli, after repeated passages on SA-agar, when subcultured in the usual way on plain agar, revert to their original type and show spore formation. We succeeded, however, in obtaining 4 strains of hereditary variants of asporogenous bacilli which did not form spores on ordinary media even after repeated passages. The following technic was used: A small inoculum from the bacterial growth on SA-agar was plated on plain agar and after 3 to 4 days' incubation such colonies as were proven by microscopic examination to contain no spores were selected for subculture.

In cultures on agar containing sulfanilamide 1:2000-1:4000 sporulation is not totally suspended as the smears exhibit a few spores scattered among vegetative rods. When the concentration of the

[2] Burke, V., Sprague, A., and Barnes, L., *J. Infect. Diseases*, 1925, **36**, 555.

drug is lowered to 1 :8000-1 :16000 complete sporulation takes place after a retardation of 3 to 4 days.

Neoprontosil solution (one part) added to agar (4 parts) does not inhibit sporulation of the inoculated bacilli. This result can be compared with the observation of Domagk[3] who found that this drug, while effective against streptococci *in vivo,* does not inhibit their growth *in vitro.*

Since the biochemistry of the process of sporulation in general is not well known, the mechanism by which sulfanilamide suppresses it cannot be understood. The significance of a possible destruction of spore-produced catalase (Ruhle[4]) by the anticatalase properties of the oxidation products of sulfanilamide,[5] is only a matter of conjecture.

Conclusion. Sulfanilamide, when added to nutrient media, inhibits spore formation and induces degenerative changes in the bacilli of the subtilis-mesentericus-mycoides group.

11102 P

Effect of Carbon Dioxide on Glucose Metabolism of Trypanosomes.

D. S. SEARLE AND L. REINER.

From the Burroughs Wellcome & Co. U. S. A. Experimental Research Laboratories, Tuckahoe, N. Y.

In the course of the study of the glucose metabolism of trypanosomes it was found that *Trypanosoma lewisi* does not decompose glucose under anaerobic conditions unless the solution contains bicarbonate. This effect has been studied during the last 4 years. Although the mechanism of the carbon dioxide action has not been established definitely, some of the main results are given in this preliminary report.

It is well known that the presence of bicarbonate is necessary for the cultivation of certain bacteria. Werkman and coworkers[1] have

3 Domagk, G., *Deut. Med. Wochnschr.,* 1935, **61**, 250.

4 Ruhle, G. L. A., *J. Bact.,* 1923, **8**, 487.

5 Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

1 Wood, H. G., and Werkman, C. H., *Biochem. J.,* 1936, **30**, 48.