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Anticatalase Activity of Sulfanilamide and Related Compounds. II. Relation to Growth Inhibition in Pneumococcus.

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In a previous publication[1] we demonstrated the anticatalase property of sulfanilamide and showed that this attribute is markedly increased by the exposure of dilute solutions to ultraviolet light. It was suggested that anticatalase activity may play an important rôle in the therapeutic action of sulfanilamide against such organisms as the streptococcus and pneumococcus, a theory originally promulgated by Locke.[2]

The hypothetical mechanism may be stated as follows: The growing bacterial cell has the power to convert sulfanilamide, presumably through mild oxidation, into a derivative which is a highly active anticatalase.* This reaction produces an accumulation of anticatalase in the immediate vicinity of the cell. The streptococcus and pneumococcus, being active producers of hydrogen peroxide, are able to grow only so long as the peroxide concentration can be kept below a critical level by outward diffusion or destruction. Usually this is accomplished by catalase of which there is ample reserve in the body and in cultures containing blood. However, in the presence of anticatalase, inactivation of catalase takes place in the zone immediately adjacent to the cell with resultant accumulation of hydrogen peroxide to toxic levels. The feasibility of this explanation is enhanced if we recall that catalase must diffuse into the reaction-zone from the outside environment, whereas anticatalase and peroxide, produced by the cell itself, are already present in the critical zone in their maximal concentrations.

This report presents experimental evidence in support of the foregoing hypothesis.

Ten cc of a 0.2% glucose broth were placed in each of a series of 50 cc Ehrlenmeyer flasks. This amount gave a surface-volume

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

[2] Locke, A. P., Main, E. R., and Mellon, R. R., *J. Immunol.*, 1938, in press.

* This might be presumed to be the hydroxylamine derivative or even hydroxylamine itself. In this connection we have succeeded in showing by comparative titrations using hydroxylamine and sulfanilamide that if only 2% of the 8 mg% sulfanilamide were converted to hydroxylamine it would produce an anticatalase effect comparable to that obtained on full radiation by ultraviolet light.

ratio of approximately 1.6.[3] Catalase was supplied through addition of 5×10^{-5} cc of hemolyzed whole rabbit-blood. This amount consistently gave the most clear-cut results with the particular samples of blood and broth used. Additions of as much as 5×10^{-4} cc prevented determinable accumulations of peroxide in the medium obscuring recognition of the much higher concentrations probably existing within the reaction-zone.

Sulfanilamide, as a sterile 500 mg % solution, was added to half the flasks to a final concentration of 8 mg %. All cultures were seeded with a 0.05 cc of an 18-hour broth culture of Type I pneumococcus subjected to prior mouse-passage. The flasks were incubated at 37°C and tested at intervals during the period from 2-6 hours for growth and peroxide-content.

Since the presence of sulfanilamide had been found to encourage chain-formation, it was considered more accurate to estimate growth turbidimetrically against barium sulfate standards rather than by plate-count. Hydrogen peroxide was estimated by adding to 1 cc of the culture a small piece of raw potato as a source of peroxidase, followed by 1 drop of a fresh 1% solution of *o*-tolidine in glacial acetic acid. This reagent develops an intense blue color in the presence of peroxide and is more sensitive that the commonly used benzidine. Concentrations approaching 0.0003% of hydrogen peroxide in broth can be detected.

With the technic employed, detectable peroxide always appeared between 2 and 3 hours in both sulfanilamide- and control-cultures. From the third hour, retardation in growth was apparent in the cultures containing sulfanilamide. The amount of peroxide accumulated in these cultures continued to increase, although the growth, as compared with that of the controls, was becoming increasingly less. Thus the amount of peroxide per unit of bacterial substance became progressively greater in the presence of sulfanilamide. The peroxide-concentrations attained at the 5-hour period in the cultures containing sulfanilamide were of the same general magnitude as is required to cause a corresponding inhibition of growth when added to a similar culture without sulfanilamide (0.001-0.003%).

Because of the limited reproducibility of absolute values inherent in this type of study, attention must be directed to reproducibility of trend. The typically differing trends of peroxide-accumulation and growth in cultures with and without sulfanilamide are shown in Fig. 1. In (A) are shown the peroxide-concentrations reached in

[3] Avery, O. T., and Morgan, H. J., *J. Exp. Med.*, 1924, **39**, 275.

3 comparable series,† plotted as the differences between concentration found in the culture containing sulfanilamide and that found in the control culture containing no sulfanilamide. (B) shows a similar plotting of difference in population in the same cultures. The significant portion of the curves begins at about the fourth hour. After this point is reached, the peroxide- and population-curves diverge.

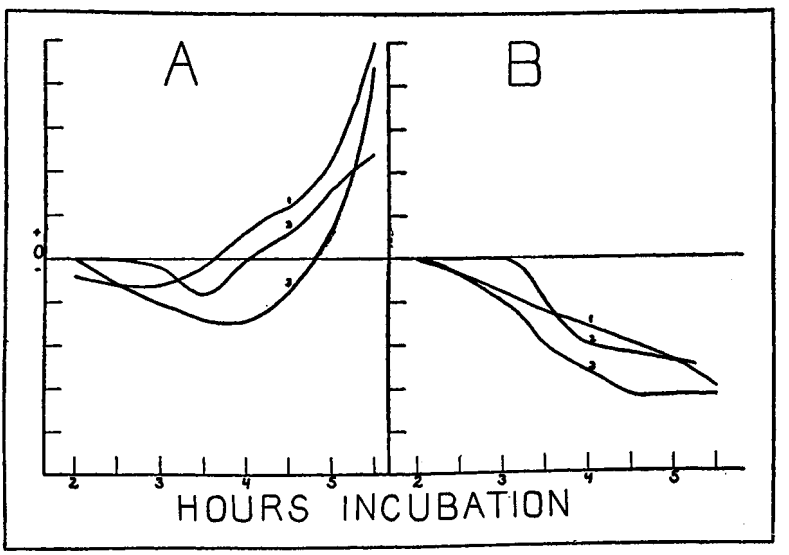

FIG. 1.

A. Curves representing the preponderance of peroxide in cultures containing sulfanilamide over peroxide in cultures not containing sulfanilamide.

B. Curves representing the preponderance (negative) in population of cultures containing sulfanilamide over that of cultures not containing sulfanilamide.

Curves 1, 2, and 3 refer to 3 separate comparable experiments.

No values are attached to the ordinates because the limited reproducibility of absolute magnitudes deprives them of real significance. The point to be illustrated is the direction of the trend.

Theoretically, such divergence would be expected. The presence of sulfanilamide permits the accumulation of peroxide, which, through its toxic effect, retards growth of the organisms. The depression in growth would, in turn, be expected to lower the rate of peroxide-formation. However, peroxide accumulates in spite of the reduced growth, in amounts even larger than those associated with a normal growth-rate.

The negative value assumed by the peroxide-difference curves dur-

† The cultures, in these series, received additions of 5×10^{-5} cc of blood. Series both without added catalase and with catalase added in higher amount gave results corroborative in direction but of magnitudes of difference less satisfactory for graphic representation.

ing the early period is possibly due to the fact that the points defined are by necessity limited to estimations of the amount of peroxide that has diffused out into the surrounding medium rather than the amount present in the effective zone immediately about the organism. If a diffusion-lag of approximately one hour is assumed, the time of average upturn of the peroxide-curves corresponds closely to the point of beginning fall in the population-curves. This would relate the depressed values for peroxide to the very early growth-period regarding which little information is available other than that stimulation by sulfanilamide during this period takes place.[4]

Regardless of the interpretation placed on this feature, it is nevertheless clear that under the conditions of the experiment growth is inhibited by sulfanilamide and this inhibition is accompanied by an increase in peroxide as compared with control cultures. This we consider to be supportive evidence for the theory that the mechanism of sulfanilamide action is in part through the inactivation of catalase.

The reported strong activity of sulfanilamide against highly virulent strains as compared to those of low virulence may find explanation in the anticatalase-hypothesis. Highly virulent strains, particularly in the streptococci, are usually heavily encapsulated. In the pneumococcus, loss of capsule means loss of virulence. Assuming that the conception of an anticatalase-mechanism is correct, it is not inconceivable that the extent and nature of the capsule about the organism may play a definite rôle. Any factor that restrains the inward diffusion of catalase and the outward diffusion of peroxide and anticatalase should necessarily increase the degree of peroxide-accumulation. The capsule may function in this way by creating a stabilized zone in which diffusion-rates are reduced.

[4] Locke, A. P., personal communication.