COMMENTS AND COMMUNICATIONS

Comments relating to articles which have recently appeared in the Journal of Cellular and Comparative Physiology and brief descriptions of important observations will be published promptly in this Section. Preliminary announcements of material which will be presented later in more extensive form are not desired. Communications should not in general exceed 700 words.

ON THE SPECIFICITY OF SULFANILAMIDE ACTION

WILLIAM D. McELROY
Physiological Laboratories, Princeton, New Jersey

ONE FIGURE

Wood's concept concerning the mechanism of action of sulfanilamide has been utilized as an excellent working hypothesis for the isolation and synthesis of other bacteriostatic agents which are analogues of known growth factors.[1] The use of other sulfonamides with known physical properties whose bacteriostatic activity can in general be predicted, depending upon the dissociation constant and the contribution of the resonating form is further support of the concept. Cowles ('42) and Kumler and Daniels ('43) have suggested that the reason for the maximum in the bacteriostatic activity-pK_a curve found by Bell and Roblin [1] for the N^1 mono-substituted sulfanilamides is due to the greater penetrability of the neutral molecule. This appears to be true for many weak acids. Of interest in this connection is the recent report of Wyss et al. ('44) demonstrating the effect of pH on the availability of p-aminobenzoic acid to the "aminobenzoicless" mutant of Neurospora crassa. The acid is found to be more effective at a low than at a high pH. They also find that pH has no effect on the subsequent synthesis and utilization of p-aminobenzoic for growth. The results are in

[1] See C. B. van Niel ('43) for discussion and references.

support of the suggestion that it is the neutral molecule which is effective in getting to the site of action.

In the past few years numerous reports have appeared concerning the effect of sulfanilamide on enzymatic processes not necessarily involved directly in cellular division. Of particular interest has been the work of Johnson, Eyring and Williams ('42) concerning the effect of sulfanilamide and other inhibitors on bacterial luminescence. The theory developed has been concerned with the broader aspects of inhibitor action on enzymes in general, rather than with a particular site of action. However, all of the supporting data has, at present, been derived from studies on the luminescent reaction exclusively (McElroy, '43). The fact that sulfanilamide inhibition of luminescence can not be reversed by p-aminobenzoic acid is evidence in support of the hypothesis that sulfanilamide may work differently on different enzyme systems. It is not, however, evidence against the original hypothesis proposed by Wood (McIlwain, '42). Likewise, the fact that many other compounds can reverse the effect of sulfanilamide may also be interpreted as an effect on an alternate system which would in no way negate the idea of competitive activity. Kohn and Harris ('43) have emphasized the re-establishing of "secondary reactions" by adding a substrate which would normally have been derived from the reactions involving p-aminobenzoic acid. Such substrates would function as "secondary antagonists".

It would appear, then, that sulfanilamide action should cause different results depending upon the enzymatic reaction studied and its relation to other systems. Therefore the suggestion that sulfanilamide may affect the over-all activity of several enzymatic processes by inhibiting one particular enzyme must be considered. Also, that the inhibition of secondary reactions by p-aminobenzoic acid may be attributed to the stimulation of one of several competing systems, must be kept in mind. The existence of either of these possibilities would in no way affect the analysis presented by Johnson, Eyring and Williams ('42) or Eyring and Magee ('42) on the application of the theory of absolute reaction rates to bacterial luminescence.

The recent studies of Beadle and co-workers on the genetic control of biochemical reactions in Neurospora has opened a new approach to the study of intermediary metabolism. The report by Tatum and Beadle ('42) of a mutant which has lost the ability to synthesize p-aminobenzoic acid is of particular interest in this connection. They studied the effect of different concentrations of sulfanilamide on the growth of this "aminobenzoicless" mutant with a known constant

amount of p-aminobenzoic acid in the culture media. Some interesting comparisons can be made between the data taken from their bacteriostatic activity-concentration curve and the activity-concentration curve of sulfanilamide on the luminescence of luminous bacteria. If log (I_1/I_2-1) is plotted against log molar concentration of sulfanilamide the resulting slope gives the number of inhibitor molecules that combine with the enzyme. I_1 and I_2 are the rates of the control and experimental runs for either growth or luminescence. A similar plot has been employed by Fisher and co-workers ('40). When the plots of the bacterial luminescence of Johnson, Eyring and Williams and the Neurospora growth data of Tatum and Beadle are made as in figure 1, it is evident that the two slopes differ very little, both being approximately 1.6. This value indicates that 1.6 molecules of sulfanilamide combine with the enzyme concerned in the reaction being studied. It is possible that

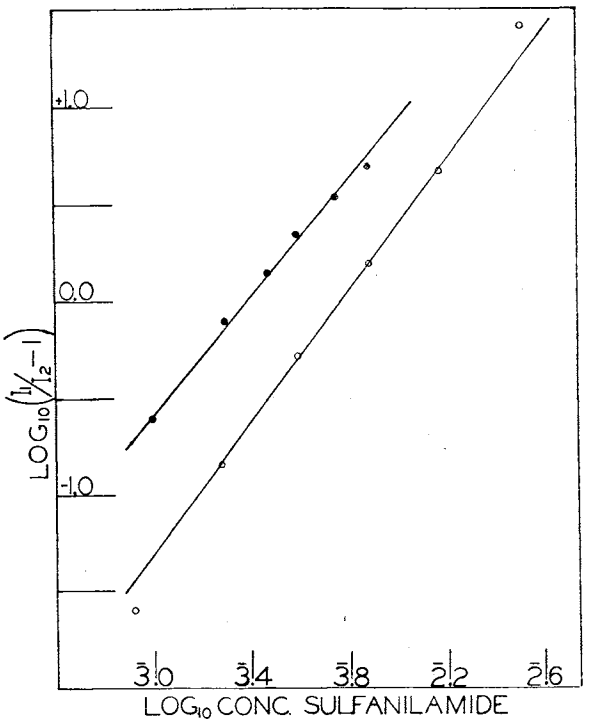

Fig. 1 Plot of log (I_1/I_2-1) against log molar concentration of sulfanilamide. I_1 and I_2 are the rates of the control and experimental runs for either growth or luminescence. The slope of the line gives the number of inhibitor molecules that combine with the enzyme. The bottom curve is taken from the report of Tatum and Beadle on the growth of Neurospora at 25°C. Slope is 1.7. The top curve is taken from the report of Johnson, Eyring and Williams on the luminescence of Photobacterium phosphoreum at 8°C. Slope is 1.5.

activity (a) corrections would change the slope slightly. Therefore comparisons can be made only with the same compound under these conditions. If one assumes two "active regions" on the enzyme at which sulfanilamide can combine, it would be possible by "partial" dissociation to get a value of 1.6. It is interesting that Fox and Rose find that 1 molecule of p-aminobenzoic acid will antagonize 1.4 ions of sulfanilamide. Also, Kohn and Harris calculated from Wood's data, using the average dissociation constants supplied by Schmelkes et al. and Fox and Rose, that 1 molecule of p-aminobenzoic antagonises 1.6 ions of sulfanilamide.

The comparison of the luminescence and growth data is of interest, since the results would be compatible with the hypothesis that sulfanilamide is acting at a similar group on the enzymes concerned in the two cases. Assuming that the interpretation of the slopes is correct, it would appear that there are certain chemical groups of both enzymes which are similar, or at least react equally well with the sulfanilamide molecule. Johnson, Eyring and Williams state that a straight line relationship in figure 1 indicates that a single enzyme is being affected. The results would, therefore, be in agreement with the suggestion that the enzymes are identical in the two cases, with the secondary effects differing from each other only because of the nature of the end reactions being studied.

LITERATURE CITED

EYRING, H., AND J. L. MAGEE 1942 Application of the theory of absolute reaction rates to bacterial luminescence. J. Cell. and Comp. Physiol., vol. 20, pp. 169–177.

FISHER, K. C., AND R. OHNELL 1940 The steady state frequency of the embryonic fish heart at different concentrations of cyanide. J. Cell. and Comp. Physiol., vol. 16, pp. 1–13.

JOHNSON, F. H., H. EYRING AND R. W. WILLIAMS 1942 The nature of enzyme inhibitions in bacterial luminescence: sulfanilamide, urethane, temperature and pressure. J. Cell. and Comp. Physiol., vol. 20, pp. 247–268.

KOKN, H. I., AND J. S. HARRIS 1943 On the mode of action of sulfonamides III Purines, amino acids, peptones and pancreas as antagonists and potentiators of sulfonamide in E. Coli. J. Pharm. and Exp. Therap., vol. 77, pp. 1–16.

KUMLER, W. D., AND T. C. DANIELS 1943 The relation between chemical structure and bacteriostatic activity of sulfanilamide type compounds. J. Am. Chem. Soc., vol. 65, pp. 2190–2196.

MCELROY, W. D. 1943 The application of the theory of absolute reaction rates to the action of narcotics. J. Cell. and Comp. Physiol., vol. 21, pp. 95–116.

MCILWAIN, H. 1942 The biochemical specificity of sulfanilamide and of other anti-bacterial agents. Science, vol. 95, pp. 509–511.

TATUM, E. L., AND G. W. BEADLE 1942 Genetic control of biochemical reactions in Neurospora: an "aminobenzoicless" mutant. Proc. Nat. Acad. Sci., vol. 28, pp. 234–243.

VAN NIEL, C. B. 1943 Biochemistry of Microorganisms. Ann. Rev. Biochem., vol. 12, pp. 551–586.

WYSS, O., V G. LILLY AND L. H. LEONIAN 1944 The effect of pH on the availability of p-aminobenzoic acid to Neurospora crassa. Science, vol. 99, pp. 18–19.