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Effects of Azo-Sulfonamides upon Lysozyme.

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Recent studies of Neter[1] indicate that sulfanilamide, sulfapyridine and sulfathiazole do not interfere with the lytic activity of lysozyme upon *Micrococcus lysodeikticus*. This communication presents the results of the effects of several soluble azo derivatives of the above and related sulfonamide compounds, formed by coupling with acetyl-2R acid (sodium 1-hydroxy - 7 - acetylaminonaphthalene - 3,6 - disulfonate), upon this enzyme system.

The related compounds included sodium sulfadiazine and its corresponding azo-acetyl-2R acid derivative, sulfathiazole-disazo-symmetrical-carbamide-2R acid, 6-sulfaquinoline-azo-acetyl-2R acid and diphenyl-sulfone-disazo-acetyl-2R acid.*

Methods. The test procedures used in this investigation were, with slight modifications, those described by Neter. The effects of 1% (1:100) concentrations of the sulfonamides upon the lytic action of lysozyme were first studied. The sodium salts of sulfapyridine and sulfathiazole were used in these experiments to obtain 1% drug solutions of these compounds. Furthermore, a colloidal gel was formed upon the addition of lysozyme to the quinoline preparation, therefore this compound was tested in a higher dilution as indicated later.

On the basis of preliminary studies a distilled water medium was found to be suitable for testing the various agents in their effects in inhibiting the lytic action of the enzyme. To 2½ cc quantities of sulfonamide solutions were added 2½ cc of a 1:500 dilution of lysozyme (egg white).† Appropriate controls on the activity of the lytic agent upon *M. lysodeikticus* and the effects of several of the sulfonamides alone against this organism were included in the study. The mixtures were incubated at 37°C for 24 hours and to each was added 0.1 cc of the test organism (prepared by suspending the growth from an 18-hour beef-extract agar slant in 4 cc of distilled water).

The inoculated test solutions were placed in a 45°C water bath and observed for visible lysis of the organisms at intervals of 2, 10, and 60 minutes. Following these preliminary observations the test mixtures were placed at 37°C for an additional 23 hours and the results at this time recorded accordingly.

With but two exceptions the compounds studied failed to inhibit the lytic activity of lysozyme in the concentration given. Sulfathiazole-azo-acetyl-2R acid and sulfathiazole-disazo-symmetrical-carbamide-2R acid interfered completely with the lytic action of the enzyme upon the test organism.

A comparison of the relative effects of the 2 lysozyme-inactivating sulfonamides was next investigated. The azo-quinoline analogue of sulfanilamide was included in this study. The results of dilute solutions of these compounds upon the enzyme are presented in Table I.

Sulfathiazole-disazo-symmetrical-carbamide-2R acid in a concentration of 1:10,000 was found to be distinctly effective in inhibiting lysozyme activity. In the presence of 1:60,000 dilution of this preparation the enzyme lysed the test organisms completely only after 10 minutes incubation. The corresponding lysozyme-culture suspension controls showed complete clearing in all instances in less than 2 minutes.

Next in order of effectiveness was the azo derivative of sulfathiazole. While there was a suggestion of anti-lysozyme activity in the 1:20,000 dilution of the compound, definite clearing of the test suspension was noted in the 1:10,000 mixture only after 2 minutes

[1] Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 106.

* The structural formulæ and antibacterial properties of the azo-sulfonamides will be presented elsewhere.

† Kindly supplied by Stein Hall and Company, New York, N.Y.

TABLE I.
Comparison of Effects of Three Azo-Sulfonamides upon Lysozyme.

Compound	Concentration	Water bath 45°C Incubator 37°C — Lysozyme activity 2 min	10 min	1 hr	24 hr	Drug controls No lysozyme 24 hr
Sulfathiazole-disazo-symmetrical-carbamide-2R acid	1:1,000	0*	0	0	0	0
	1:5,000	0	0	0	0	0
	1:10,000	0	0	0	0	0
	1:20,000	0	0	0	4	0
	1:40,000	0	1	4	4	0
	1:60,000	0	2	4	4	0
Sulfathiazole-azo-acetyl-2R acid	1:1,000	0	1	1	3	0
	1:5,000	0	2	4	4	0
	1:10,000	0	4	4	4	0
	1:20,000	2	4	4	4	0
	1:40,000	3	4	4	4	0
	1:60,000	4	4	4	4	0
6-sulfaquinoline-azo-acetyl-2R acid	1:1,000	0	0	0	4	0
	1:5,000	0	1	4	4	0
	1:10,000	2	4	4	4	0
	1:20,000	4	4	4	4	0
	1:40,000	4	4	4	4	0
	1:60,000	4	4	4	4	0
Lysozyme and culture control		4	4	4	4	
Culture control		0	0	0	0	

* 0 = No visible lytic activity.
1-4 = Slight to complete clearing of mixtures.

incubation.

6-sulfaquinoline-azo-acetyl-2R acid proved to be correspondingly effective in dilutions of 1:10,000 and 1:5,000 respectively.

Beef extract agar plates inoculated with a standard loopful of the 1:1000 sulfonamide-lysozyme-organism mixtures of the above drugs at the end of the 2-minute-45°C treatment period, with subsequent incubation of the plates at 37°C for 72 hours, failed to show evidence of growing colonies of the test organism. The corresponding drug-organism controls without the enzyme were relatively ineffective in lowering the bacterial count of the test suspension in the time period given. Furthermore, although complete clearing of the control lysozyme-culture mixture was noted at the end of 2 minutes, this did not indicate that there was a complete loss of viability of the test organisms. The possibility that these results indicate a synergistic action on the part of lysozyme-sulfanilamide solutions acting upon *M. lysodeikticus*, as suggested by Neter has been confirmed. Under somewhat different experimental conditions 1% solutions of the compounds tested, failed to inhibit the lytic effects of *Staphylococcus aureus* bacteriophage.

Summary. Evidence is presented which indicates that certain soluble sulfonamide-azo dyes interfere with the lytic activity of lysozyme upon *M. lysodeikticus*. One of these compounds, sulfathiazole-disazo-symmetrical-carbamide-2R acid, completely suppresses the lytic effects of the enzyme in a concentration of 1:10,000 of the drug. To our knowledge, these are the first sulfonamides found to exhibit anti-lysozyme properties.

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