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THE TREATMENT OF EXPERIMENTAL BACILLARY INFECTIONS OF MICE WITH QUINOXALINE 1:4 DI-*N*-OXIDE

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Quinoxaline 1:4 di-*N*-oxide (RD 2579) was first described by McIlwain (1943), who drew attention to its bacteriostatic action against *Streptococcus haemolyticus* and *Corynebacterium diphtheriae* in *in vitro* tests. Later Frisk (1946) showed that it possessed tuberculostatic activity, and more recently Jones, Landquist, and Stewart (1953) reported amoebicidal properties, and made incidental reference to its activity against Gram-negative bacteria. Hurst, Landquist, Melvin, Peters, Senior, Silk, and Stacey (1953) found it effective in the therapy of experimental psittacosis and lymphogranuloma venereum (inguinale).

A sample of RD 2579 was supplied by Dr. W. F. Short in August, 1952. We were impressed by its action against Gram-negative bacteria, and particularly by the fact that not only was it bacteriostatic for *Salmonellae, Shigellae,* and coliforms, but also for each of thirteen strains of *Pseudomonas pyocyanea* and of eleven strains of *Proteus vulgaris* against which it was tested. This activity was maintained virtually undiminished in blood-containing media.

METHODS

In vitro *Tests.*—Dilutions in papain digest broth showed that RD 2579 inhibits growth of Gram-negative bacteria at concentrations of about 0.01 to 0.003%.

In vivo–In vitro *Tests.*—The antibacterial activity of RD 2579 in mice was investigated by a modification of the method described by Croshaw and Foley (1954).

The drug was administered to groups of mice and these were sacrificed at intervals. The blood was removed by heart puncture, pooled, and allowed to clot; the separated serum was then diluted in two-fold steps in papain digest broth in test tubes. The tubes were sown with one drop of a diluted 24 hr. broth culture of the test organism and incubated at 37° C. in parallel with dilutions in the same broth of normal mouse serum sown in the same way.

In vivo *Tests.*—A number of tests were carried out to investigate the protection afforded by RD 2579 in experimental infection of mice by *Salmonella typhi, Salmonella paratyphi B, Shigella sonnei,* or *Pseudomonas pyocyanea.*

Albino mice were used throughout and in any one experiment all were of the same sex and strain and, as far as possible, of the same weight. This was usually 25–30 g.

RESULTS

Table I shows typical results of a number of *in vivo–in vitro* tests against a variety of Gram-negative bacteria. The serum of treated mice remained bacteriostatic for at least 2 hr. after an intraperitoneal dose of RD 2579.

TABLE I

IN VIVO–IN VITRO ACTIVITY OF RD 2579 (0.32 MG./G. INTRAPERITONEALLY) AGAINST GRAM-NEGATIVE BACTERIA

Test Bacterium	Time after Administration during which the Serum Remained Bacteriostatic (hr.)	Highest Dilution of Serum at which Activity was Detected
Sh. sonnei	3	× 4
,, *	2 or longer	× 2
Salm. typhi	,, ,, ,,	× 4
Ps. pyocyanea (mixed inoculum of 13 strains) ..	,, ,, ,,	× 8
Prot. vulgaris (mixed inoculum of 11 strains) ..	,, ,, ,,	× 16

* Drug given orally in this test.

Table II shows the effect of the drug upon the survival time of infected mice. In the last experiment in Table II, all local lesions in treated animals cleared in 24–72 hr. At the end of the experiment a pure culture of *Ps. pyocyanea* was obtained from the lesion on the surviving control animal.

This last experiment was modified in accordance with suggestions made by the late Professor F. R. Selbie, who had, at our suggestion, carried

TABLE II

MOUSE PROTECTION TESTS AGAINST VARIOUS GRAM-NEGATIVE BACTERIA

(Intraperitoneal inoculation of organisms in 5% mucin)

Infecting Organism	Dose of RD 2579 and Mode of Administration	Treated Survivors and Duration of Experiment	Untreated Survivors and Time of Survival
Salm. paratyphi B	2×0·13 mg./g. i.p. at 0 and 24 hr. after infection	4/5 6 days	0/5 6 days
Salm. paratyphi B	1×0·32 mg./g. orally 5½ hr. after infection	10/10 8 days	3/10 8 days
Salm. typhi	1×0·26 mg./g. orally 2 hr. after infection	10/10 4 days	0/10 24 hr.
Sh. sonnei ..	1×0·2 mg./g. orally 2 hr. after infection	9/10 5 days	0/10 24 hr.
Ps. pyocyanea	1×0·32 mg./g. orally 2 hr. after infection followed by 1×0·16 mg./g. 4 hr. later	5/10 3 days	1/10 3 days
*Ps. pyocyanea**	3×0·125 mg./g. i.m. right thigh at 2, 24, and 48 hr. after infection	8/10 14 days	1/10 14 days
	3×0·25 mg./g. i.m. right thigh at 2, 24, and 48 hr. after infection	10/10 14 days	
	1×0·5 mg./g. i.m. right thigh at 2 hr. after infection	5/10 14 days	

i.p., intraperitoneal injection. i.m., intramuscular injection.

In the "survivors" column the fractions indicate $\frac{\text{survivors}}{\text{number at risk}}$.

* In this test the organisms were injected i.m., in broth, to left thigh.

out a number of experiments with RD 2579 against infections in mice of strains of *Ps. pyocyanea*. In a personal communication Professor Selbie pointed out that RD 2579 not only prevented or delayed death but that it caused marked diminution and ultimate disappearance of local lesions deliberately produced by this technique.

Details of three of Professor Selbie's experiments with three strains of *Ps. pyocyanea* are as follows:

On the basis of *in vitro* bacteriostatic titres of RD 2579 and streptomycin with strains "YDE," "Kilner" and "Sinclair," protection tests were carried out in mice. Eighteen mice were used in each test, and each was infected with 0.2 ml. of a 1 in 5 dilution of an 18 hr. broth culture by injection in the left thigh muscle. Two hours after infection six mice were given RD 2579 (250 mg./kg.) subcutaneously, six received streptomycin (125 mg./kg.) also subcutaneously, and six remained untreated. The size of the local lesion was measured in each survivor 24 hr. after infection by deducting the width of the normal thigh from that of the affected thigh.

Table III shows the results obtained in these tests by Professor Selbie.

TABLE III

COMPARISON BETWEEN RD 2579 (250 MG./KG.) AND STREPTOMYCIN (125 MG./KG.) GIVEN SUBCUTANEOUSLY TO MICE INFECTED WITH STRAINS OF *PS. PYOCYANEA*

Test	Strain of Bacteria	Treatment	Width of Lesions in mm. 24 hr. after Infection in Mouse No.: 1	2	3	4	5	6
1	YDE ..	None	D	D	D	D	D	D
		RD 2579	6·0	4·5	4·5	4·0	3·0	1·5
		Streptomycin	D	D	D	D	8·0	7·5
2	Kilner ..	None	7·5	7·0	7·0	6·5	6·5	5·0
		RD 2579	2·0	2·0	1·5	1·0	1·0	1·0
		Streptomycin	7·5	6·5	6·5	6·0	5·5	5·5
3	Sinclair ..	None	6·5	6·0	5·0	4·5	3·5	2·5
		RD 2579	1·5	0·5	0·5	0·5	0·5	0·0
		Streptomycin	1·5	1·5	1·0	1·0	0·5	0·5

Width of lesion is the difference in mm. between normal and affected thighs.

D, mouse died.

Compatibility with Antibiotics.—A 0.1% aqueous solution of RD 2579 does not suffer appreciable alteration in its antibacterial effect when penicillin, aureomycin, chloramphenicol or oxytetracycline are dissolved in it at a concentration of 0.05% and incubated at 37° C. for 24 hr. The activity of the antibiotics also appears to remain relatively unchanged in the mixture. Agar dilution experiments with strains of *Ps. pyocyanea* and *Prot. vulgaris* have shown that combination of the compound with streptomycin delays the development of resistance to the latter, and there would appear to be a synergistic action which is most marked with strains of *Prot. vulgaris*.

SUMMARY

1. Quinoxaline 1:4 di-*N*-oxide, whether administered orally or by injection, protected mice against experimental infection with *Salmonella typhi, S. paratyphi B, Shigella sonnei,* and *Pseudomonas pyocyanea.*

2. It appears to delay the development of resistance of strains of *Ps. pyocyanea* and *Prot. vulgaris* to streptomycin, and to be synergistic with the latter.

3. It was effective *in vitro* against all strains of *Prot. vulgaris* investigated, but since none of these was pathogenic we have been unable to carry out *in vivo* experiments.

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ADDENDUM

Toxicity of RD 2579

By M. R. Gurd

The main toxic effect of RD 2579 given systemically is on the liver and kidneys (Jones *et al.*, 1953). In our rats, repeated oral doses of 200 mg./kg. daily caused severe cellular degeneration in the liver and less severe damage to the kidneys. Daily oral doses of 50 mg./kg. caused mild liver damage, without discernible damage to the kidneys. When administered subcutaneously mild toxic effects such as inhibition of growth were noted with doses as low as 6 mg./kg. daily.

The compound had little or no irritant effect when injected intrathecally or into the anterior chamber of the eye. The toxicity to leucocytes was also low.

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