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Sulfanilamide, Sulfapyridine, and Sulfathiazol Therapy of Gonococcal Infection of the Chorio-Allantoic Membrane.

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Until lately no satisfactory experimental animal has been available for testing the effect of drugs on gonococcal infection. The chorio-allantoic membrane of embryo chicks has recently been infected with gonococci and treated successfully with sulfanilamide.[1, 2]

We present here a comparison of the effects of sulfanilamide, sodium sulfapyridine, and sodium sulfathiazol on such infections. We have also tested two sulfanilamide-resistant strains of gonococci for their resistance *in vivo* to these 3 drugs.

Experimental Procedure. Two strains of gonococci which had

[1] Marran, G., and Berry, G. P., *J. Bact.*, 1938, **36**, 280.

[2] Hill, J., and Pitts, A., *J. Urol.*, 1939, **41**, 81.

been made resistant *in vitro* to 80 mg of sulfanilamide were furnished by Dr. Carpenter[3] and were compared with 4 strains isolated from patients who did not respond immediately to drug therapy.

Using the technic of Goodpasture and Buddingh,[4] the chorio-allantoic membrane of chicks 9 to 12 days old was inoculated with one drop of a fresh saline suspension of gonococci from a recently incubated blood plate. The standard suspension contained about 100,000 diplococci per cc, and the ensuing eggs were inoculated with one drop of a suspension of similar turbidity. The eggs were incubated at 36°C for about 12 hours. Discarding dead eggs, varying amounts (see tables) of 1/100 solution of sulfanilamide, sodium sulfapyridine, and sodium sulfathiazol in normal saline were dropped on the chorio-allantoic membranes. The sodium salts of the latter two were used because of the increased solubility of these compounds. That death of the gonococci was not due to temporary alkalinity of the membrane is shown by the facts that gonococci were demonstrable 6 hours after the addition of drug, and that the sulfanilamide-resistant strains survived.

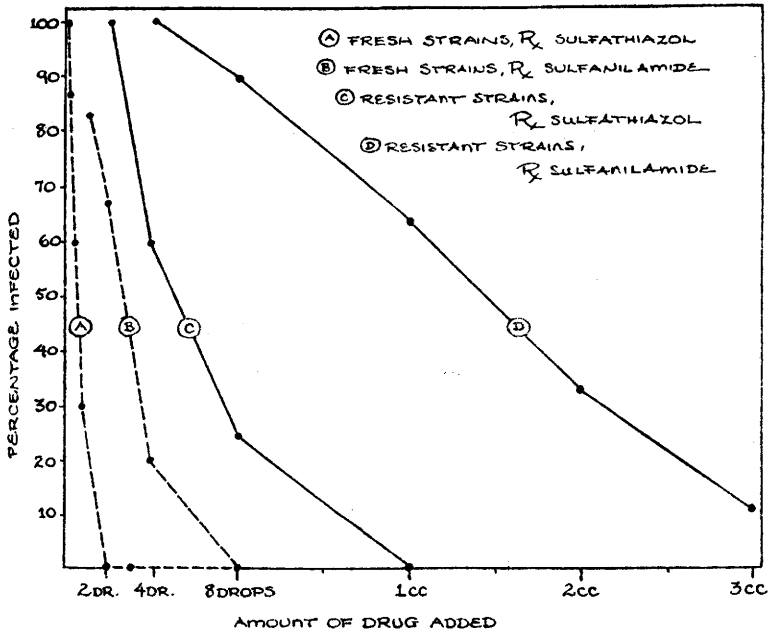

FIG. 1.

The Effect of Sulfathiazol and Sulfanilamide on Freshly Isolated and Sulfanilamide-Fast Strains of Gonococci.

[3] Westphal, Charles, and Carpenter, *J. Bact.*, 1940, **32**, 47.

[4] Goodpasture, E. W., and Buddingh, G. J., *Am. J. Hyg.*, 1935, **24**, 319.

After 2 days, smears and cultures of the membrane and allantoic fluid were made to determine the presence of gonococci. If either of these were positive, the egg was considered infected; if both were negative the egg was adjudged cured. Some of the apparently cured (negative) eggs had died during the 2 days, but these were included in the results since no greater percentage of dead eggs was found in any one group. Controls were used in each experiment, and only one of 30 controls failed to show infection. Histological sections of some of these controls showed that the infection did not penetrate the embryo.

Concentration of Drugs. Colorimetric tests for the presence of the drugs showed that they were distributed throughout the egg, that they were only slightly acetylated.* However, these analyses, taken at the end of the first and second days, accounted for less than half of the drug added. This may indicate that the embryo is able to metabolize the drug.

Results. As shown by Tables I and II, the sulfanilamide-resistant strains grew at much greater concentrations of all 3 drugs than did the recently isolated strains.

TABLE I.
Freshly Isolated Strains of Gonococci.

Drug added Drops to 1-100 sol.	Sulfanilamide		Sulfapyridine		Sulfathiazol	
	Treated	Cured	Treated	Cured	Treated	Cured
⅛ dr					5	0
¼ "					10	3
½ "			2	0	11	5
1 "	2	1	3	1	13	7
2 "	2	1	2	1	3	3
3 "	3	1	3	0	3	3
4 "	2	1				
8 "	3	3	2	2	4	4

TABLE II.
Sulfanilamide-fast.

Drug added Drops to 1-100 sol.	Sulfanilamide		Sulfapyridine		Sulfathiazol	
	Treated	Cured	Treated	Cured	Treated	Cured
2 dr					1	0
4 "	3	0	2	0	3	2
8 "	3	1	6	2	6	4
1 cc	7	3	2	1	4	4
2 "	4	2	3	0		
3 "	3	2	1	0		

* Samples of blood, embryonic fluid, allantoic fluid, amniotic fluid and yolk were analyzed by Miss Dorthea Babbitt of the Department of Pharmacology, Johns Hopkins University.

Sulfathiazol is shown to be more effective than either sulfanilamide or sulfapyridine in treating the resistant strains. This agrees with the clinical reports of the efficacy of sulfathiazol.[5, 6]

Statistical Method. Curves were plotted by accumulating negative and positive results.[7] Curves of sulfapyridine were not included because they closely paralleled those of sulfanilamide.

Summary. Sulfanilamide-resistant and freshly isolated strains of gonococci were tested for their response to sulfanilamide, sodium sulfapyridine, and sodium sulfathiazol on the chorio-allantoic membrane of embryo chicks. The resistant strains retained their resistance *in vitro*. Sulfanilamide and sulfapyridine were about equally efficient in curing the infection. Sulfathiazol was about 3 times more effective therapeutically than sulfanilamide or sulfapyridine.

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The Number of Vitamins Required by the Rat.*

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During their studies of rat dermatitis Richardson and Hogan[1] observed that rats which receive only the recognized water-soluble vitamins are able to survive for long periods of time, and to maintain a moderate rate of growth. Four of the 8 females described in Groups III and IV, Table I. of the report cited, attained an average weight of 176 g in 20 weeks, when it became necessary to discard them. The ration was then changed in various ways in an attempt to secure a more rapid growth rate and 3 of the more important modifications are described in Table I. The response of the animals is shown in Fig. 1.

[5] Mahoney, J. F., Wolcott, R. R., and Van Slyke, C. J., *Am. J. Syph., Gon., and Venereal Dis.*, 1940, 613.

[6] Burkholder, T. M., and Bang, F., *J. Urol.*, 1940, **44**, 541.

[7] Reed, L. J., *Biological Effects of Radiation*, 1936, VI, 227.

* Contribution from the Department of Agricultural Chemistry, Missouri Agricultural Experiment Station, Journal Series No. 680.

[1] Richardson, L. R., and Hogan, A. G., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 583.