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Chemotherapeutic Activity of Combinations of Sulfisoxazole and Oleandomycin. (23147)

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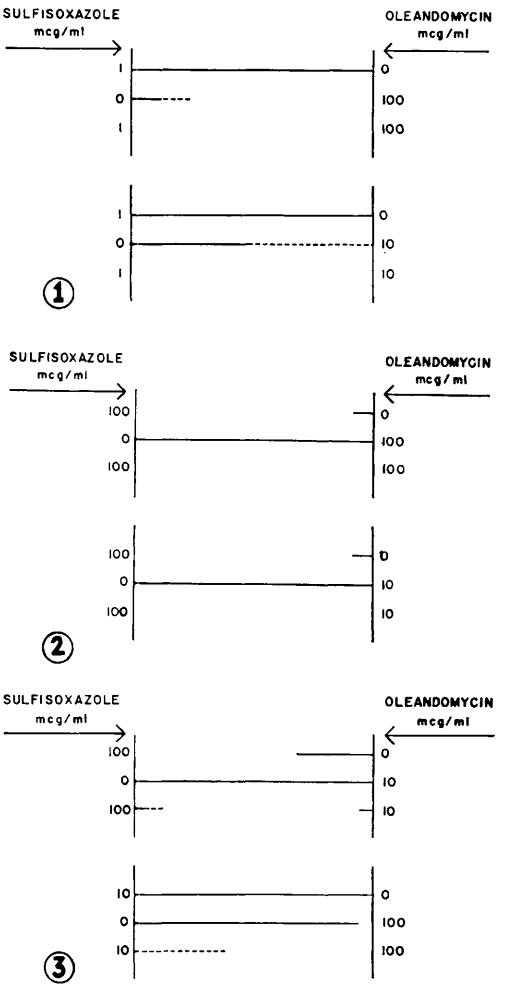
The combined effect of antibacterial agents generally demonstrated by an increased but also occasionally decreased bacteriostatic effect *in vitro* has comparatively rarely been reproduced in animal experiments. This report is concerned with a comparison of combined activity of a sulfonamide and an antibiotic *in vitro* and *in vivo*. The agents used were: (a) sulfisoxazole (3,4-dimethyl-5-sulfanilamido-isoxazole)* which has been described(1) as a substance of low toxicity and marked though not always optimal(2) chemotherapeutic activity *in vitro* and in experimental infections with Gram positive and Gram negative organisms, and (b) oleandomycin, an antibiotic which has been shown by Sobin *et al.*(3) as well as by Fust *et al.*(4) to exhibit *in vitro* and in experimental animals an activity range similar to that of penicillin and erythromycin. Clinically the effect of the antibiotic is particularly marked against Gram positive microorganisms(5).

Materials and methods. The origin of the strains(1) as well as the technics of the broth dilution tests(1) and gradient plates(6) have been described elsewhere. Mueller-Hinton medium(7) was used in all experiments which employed the gradient plate. The methods for the *in vivo* experiments are the same as those given by Schnitzer *et al.*(1) except that the Giorgio strain of *Staphylococcus aureus* (obtained through the courtesy of Dr. R. M. McCune, Jr.) which is of low sensitivity to sulfonamides was used. The infective dose of this organism was 0.5 ml intravenously of a saline suspension containing 250.000.000 cells. Both *in vitro* in the broth dilution method and *in vivo*, sulfisoxazole and oleandomycin were tested alone as well as in the following combinations: (a) a fixed relation of 5 parts sulfisoxazole combined with 1 part oleandomycin, (b) sulfisoxazole in the presence of a constant inactive concentration of oleandomycin, and (c) oleandomycin in the presence of a constant, inactive concentration of sulfisoxazole. *In vitro* the combination of sulfisoxazole and oleandomycin was also tested in the proportion of 1:1.

* Gantrisin®.

Results. Activity *in vitro*. A. Broth dilution method: In the case of *Streptococcus hemolyticus* 4 and *Pneumococcus pneumoniae* 6301, sulfisoxazole showed comparatively low activity (25-50 mg/ml) while oleandomycin was highly effective (0.25-0.5 μg/ml). With *Escherichia coli* J and *Eberthella typhi* F the range of activity was 3.9 to 15.6 μg/ml for sulfisoxazole and 31.2-62.5 μg/ml for oleandomycin. The inhibiting dose against *Staphylococcus aureus* 209 was 3.9 μg/ml for sulfisoxazole and 0.031 μg/ml for oleandomycin. In every case, the combination gave the effect of the more potent component.

B. Gradient plate technic. The results of experiments with *Staphylococcus aureus* 209, *Escherichia coli* J and *Eberthella typhi* F,



Graphic demonstration of activity on gradient plates of sulfisoxazole, oleandomycin and combinations of the two substances against:

FIG. 1. *Staphylococcus aureus* 209.
FIG. 2. *Escherichia coli* J.
FIG. 3. *Eberthella typhi* F.

carried out on the gradient plate(6) are graphically depicted in Fig. 1, 2, and 3 respectively. In these figures the arrow indicates the gradient of the substance in a particular layer and the numeral gives the maximum concentration of the substance incorporated. The heavy line between two numerals indicates normal heavy growth, the broken line, light bacterial growth and the absence of a line between two numerals, no growth. Fig. 1, 2 and 3 contain examples of combinations which exhibited an effect superior to that of the single drugs in *Staph. aureus* 209, *E. coli* J and *E. typhi* F respectively. With *E. typhi* F (Fig. 3) at a concentration of 100 μg/ml sulfisoxazole and 10 μg/ml oleandomycin the results show not only the superior effect of the combination but also growth at the highest point of concentration of sulfisoxazole. This growth could be a function of a too heavy inoculum but could also indicate possible interference of minute amounts of oleandomycin with the high concentration of sulfisoxazole under the present experimental conditions. Such an occurrence is feasible since the drug combination balance showing increased or decreased effect is often critical and extremely close in values for a combination of agents.

Chemotherapeutic activity *in vivo*. The activity of the combination of a fixed relation of 5 parts sulfisoxazole and 1 part oleandomycin as compared to the single constituents against the 3 Gram positive (streptococci, pneumococci and staphylococci) and the one Gram negative (salmonella) organisms are given in Table I. From the PD_{50} values (50% protective dose), which were calculated according to the method of Reed and Muench(8) on the basis of animals surviving the 21-day observation period, it can be seen that in the case of streptococci, pneumococci and staphylococci the values obtained for the constituents of the combination are less than that of each substance alone. This can be interpreted as indicating a superior effect of the 5 to 1 combination.

In the case of *Salmonella schottmuelleri* where oleandomycin is completely inactive, the experimental results only show the effect of the active component, sulfisoxazole.

When sulfisoxazole in the presence of a constant inactive concentration of oleandomycin was tested against *Strep. hemolyticus* 4 and *Pn. pneumoniae* 6301, the activity of sulfisoxazole in the combination was essentially the same as that observed when the substance was used alone. The same type of effect was observed against *Pn. pneumoniae* 6301 and *S. schottmuelleri* with oleandomycin in the presence of a constant inactive concentration of sulfisoxazole. However, in an

TABLE I. The *In Vivo* Antibacterial Activity of the Combination of Sulfisoxazole and Oleandomycin in a Fixed 5 to 1 Ratio as Compared with the Single Constituents.

mg/kg/os		*Strep. hemolyticus* 4		*Pn. pneumoniae* 6301		*Staph. aureus* (Giorgio)		*S. schottmuelleri*	
Sulfisoxazole	Oleandomycin	% survival	mg/kg PD_{50}	% survival	mg/kg PD_{50}	% survival	mg/kg PD_{50}	% survival	mg/kg PD_{50}
2000				90	1500	10	>2000		
1000				0					
250								90	
125		80						50	100.6
62.5		40	80.8					50	
31.2		20						10	
	2000							10	>2000
	200			90		90			
	100			50	79.5	50	87.7		
	50	90		20		20			
	25	50	23.3						
	12.5	10							
500	100			70		70			
250	50			50	283.3 + 56.7	30	264.5 + 52.9	90	
125	25	100		10		40		60	92.8 + 18.6
62.5	12.5	60	50.1 + 10			30		40	
31.2	6.25	20						0	
Effect of combination		enhanced		enhanced		enhanced		none	

infection with *S. schottmuelleri,* sulfisoxazole combined with a constant inactive dose of oleandomycin (1000 mg/kg) was more than twice as active (59.1 mg/kg) as it was when given alone (149.7 mg/kg).

Discussion. The experimental evidence has shown that under certain conditions both *in vitro* and *in vivo* the combination of the sulfonamide, sulfisoxazole, and the antibiotic, oleandomycin, exerts an effect superior to that of the single constituents. If one attempts to define the conditions which govern the demonstration of the combination effect, it is evident that the serial dilution tests *in vitro* as well as the combination with inactive doses of either agent *in vivo* failed to show increased activity. The more delicate balance maintained in the gradient plates where the abrupt differences of arbitrary dilution steps are avoided demonstrates, however, the enhanced effects of sulfisoxazole and oleandomycin as long as one member exerted at least a partial bacteriostatic activity. The experiments in animals indicate that the fixed relation of doses as given by the 5 to 1 proportion of sulfisoxazole to oleandomycin represents a suitable mixture. It causes a moderate, usually less than a 2-fold, reduction of oleandomycin. The required amount of sulfisoxazole can be considerably lower dependent on the sensitivity of the organism. In infections caused by organisms insensitive to one of the 2 compounds, *e.g. S. schottmuelleri* and oleandomycin, activity of the effective constituent usually prevails. However, even under these conditions an enhancing effect has been observed.

Since these observations do not seem to fit into any of the more or less well defined concepts of synergism, we prefer the expressions "enhanced" or "supplementary" effect to describe the results obtained in the present experimental study. The supplementation of sulfisoxazole consists in the accentuation of its Gram positive quota through combination with oleandomycin. The latter substance is supplemented by the marked influence of sulfisoxazole on Gram negative organisms. Thus, one of the aims of combination therapy, namely the broadening of the antibacterial spectrum, has been accomplished in the sulfisoxazole-oleandomycin mixture.

Furthermore, recent studies on the high frequency of resistant staphylococci, even to penicillin, seem to make it desirable to include substances of potent effect against these or-

ganisms and without cross-resistance to the majority of other clinically useful antibiotics. Oleandomycin is an agent which, with the exception of cross-resistance to members of the erythromycin group, displays these properties.

Summary (1) *In vitro* under the specific conditions of the gradient plate technic, combinations of sulfisoxazole and oleandomycin in specific ratios show an activity superior to that observed with the single constituents. (2) Supplemental activity was also observed *in vivo* in the streptococcal, staphylococcal and pneumococcal infections when the ratio of the combination of sulfisoxazole to oleandomycin was 5 to 1. (3) The same type of effect was also observed in *Salmonella schottmuelleri* infection of mice when activity of sulfisoxazole was determined in a constant, inactive amount of oleandomycin.

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Inactivation of Foot-and-Mouth Disease Virus by pH and Temperature Changes and by Formaldehyde. (23148)

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Methods of tissue culture for production of foot-and-mouth disease virus, type A, and for assay of the virus and its neutralizing antibodies were reported by Bachrach, Hess, and Callis(1). These methods have now been employed for measurements of stability of tissue culture virus under variable environmental conditions. The present report is concerned with rates of inactivation of virus by the action of hydrogen ion, heat, and formaldehyde.

Materials and methods. Virus. Foot-and-mouth disease virus, type A, strain 119 (FMDV-A119), was produced in Roux flask cultures of bovine kidney tissue(1). For preparation of the cultures, cut fragments of bovine kidney tissue were dispersed by incubation in 0.25% trypsin-buffer solution according to Youngner's modification(2) of the Dulbecco and Vogt(3) procedure for monkey kidney tissue. The liberated cells were decanted through 3 layers of gauze, centrifuged at low speed, washed twice in phosphate-buffered saline(3), and suspended at a 1:200 dilution in culture fluid. The fluid consisted of Hanks' salt solution containing 2% bovine serum and 0.5% lactalbumin hydrolyzate (Nutritional Biochemicals Corp.). Penicillin G, streptomycin sulfate, and phenol red were included at final concentrations of 100 units/ml, 0.1 mg/ml, and 0.005%, respectively. Roux flasks were each seeded with 75 ml of the cellular suspension and incubated at 37°C. Fluid changes were made after 3 and 6 days' incubation. By the second fluid change the cultures consisted of confluent outgrowths of epithelial and fibroblastic cells. The flasks containing the cultures were then inoculated with 4 ml of infective fluid from the preceding tissue culture passage and, after