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ACKNOWLEDGMENTS

Supported in part by General Research Support Grant S01 RR05432 from the National Institutes of Health.

The authors thank Mrs. Patti Peterson for the preparation of the manuscript and Merck Sharp and Dohme, Eli Lilly, Pfizer, and Ciba for providing the pure samples of tricyclic antidepressants.

In Vitro Studies on Drug–Antibiotic Interactions I: Analgesics, Antipyretics, Antimalarials, and Tranquilizers

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Abstract □ The antimicrobial effects of some analgesics, antipyretics, antimalarials, and tranquilizers were determined. The phenothiazines were the most active group. The effect of the chosen drugs when combined with a selected number of antibiotics was studied on *Staphylococcus aureus* and *Escherichia coli* to determine the type of interaction. Most analgesics, antipyretics, and antimalarials showed either no effect or a synergistic action. However, some exhibited antagonistic effects. All tested tranquilizers were synergistic. Preliminary studies, using electronic absorption spectrometry, indicated that the antagonistic action may be attributed to a physical interaction.

Keyphrases □ Antimicrobial activity—evaluated in various analgesics, antipyretics, antimalarials, and tranquilizers, effect of combination with various antibiotics □ Drug–antibiotic interactions—various analgesics, antipyretics, antimalarials, and tranquilizers, effect of combination with various antibiotics □ Antibiotics, various—effect of combination with various analgesics, antipyretics, antimalarials, and tranquilizers □ Analgesics, various—antimicrobial activity, effect of combination with various antibiotics □ Antipyretics, various—antimicrobial activity, effect of combination with various antibiotics □ Antimalarials, various—antimicrobial activity, effect of combination with various antibiotics □ Tranquilizers, various—antimicrobial activity, effect of combination with various antibiotics

The bioavailability of drugs at their sites of action can be enhanced or reduced by interaction with other drugs. Several studies concerned the biochemical and pharmacological effects of antimicrobial agents when given with other drugs (1, 2). The type of interactions reported involved competition for renal tubular excretion, displacement from carrier sites, increased metabolism by stimulation of hepatic enzymes, decreased protein synthesis, and increased tissue toxicity (3, 4).

Analgesics, antipyretics, antimalarials, and tranquilizers generally are prescribed along with antibiotics for the treatment of infectious diseases. The pharmacological and

Table I—Concentrations of Drugs and Antibiotics in the Different Tubes

Drug	Antibiotic		
	One-Fourth MIC	One-Half MIC	MIC
One-fourth MIC	1	2	3
One-half MIC	4	5	6
MIC	7	8	9

biochemical actions of these drugs as well as their interactions in humans have been studied thoroughly (1–4).

However, few *in vitro* studies on the effects of these drugs and their interaction with antibiotics on microorganisms have been reported. The antimicrobial effect of chlorpromazine, quinine, and quinacrine was the subject of several studies, and these drugs proved to be synergistic with antibiotics by preventing the emergence of resistant microorganisms (5–9). Their mechanisms of action include complexation of the cationic groups of such drugs with the phosphate groups of nucleic acids, alteration or lysis of the cell wall, alteration of cell permeability, inhibition of spore germination, blockade of RNA synthesis, interference with the cytochrome system, and inhibition of oxygen consumption (10–14).

In this investigation, it was of interest to determine the antimicrobial activity of certain drugs, generally prescribed with antibiotics in the treatment of infectious diseases, when tested alone and in combination with antibiotics. The types of interaction are reported.

EXPERIMENTAL

Stock Cultures and Test Organisms[1]—Cultures of *Escherichia coli, Proteus vulgaris, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis,* and *Candida albicans* were maintained on slants of dextrose nutrient agar or blood agar and stored at 4°. Subculturing was carried out every 2 weeks.

Determination of Minimum Inhibitory Concentration (MIC) of Drugs and Antibiotics—A stock solution was prepared to contain 4 mg/ml of the drug or 1 mg/ml of antibiotic. Compounds that are insoluble in water were first dissolved in small quantities of either 95% alcohol or 50% dimethyl sulfoxide, and then the solutions were diluted to volume with sterile distilled water or 1% phosphate buffer, pH 6–8 (15).

Twofold serial dilution of the stock solutions were carried out in nutrient broth, except with *Str. pyogenes* where dilution was carried out in brain heart infusion; the diluted solutions were distributed in 5-ml quantities in test tubes. Each tube was inoculated with 0.1 ml of the suspension of the test organism ($1–2 \times 10^6$ cells/ml). The inoculated media were incubated at 37° for 18–24 hr, and the MIC was then recorded. Each experiment was performed in triplicate.

Procedure for Interaction Study—Nine test tubes, each containing 3 ml of dextrose nutrient broth (1.66×), were diluted to 5 ml by adding 1 ml each of the antibiotic and the drug solution. The final concentrations of the drug and the antibiotic in the tubes in terms of the MIC are shown in Table I. For drugs that did not show antimicrobial activity, 100 μg/ml was used instead of the MIC. Each tube was then inoculated with 0.1 ml of the suspension of the test organism and incubated for 18–24 hr. Each experiment was performed in triplicate.

A positive control for growth and a negative control for the MIC of both the drug and the antibiotic were carried out concurrently with each experiment.

The interactions between the drug and the antibiotic were recorded as synergistic (S) when the bacteriostatic action was manifested in tubes 1, 2, and 4 (Table I) and antagonistic (A) when growth was produced in tubes 3 and 5–9.

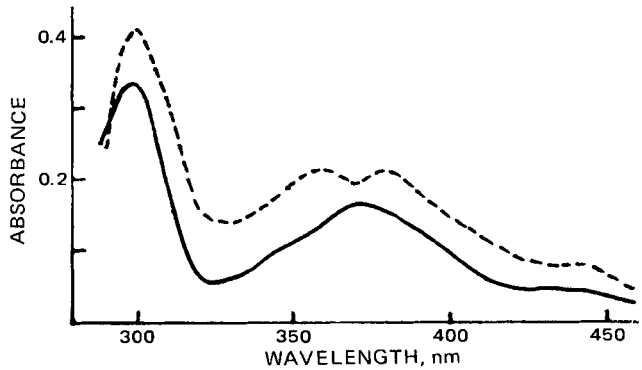

Figure 1—*Electronic absorption spectra of acetanilide–tetracycline hydrochloride aqueous solutions (0.125×10^{-4} M each). Key: ——, experimentally obtained spectrum; and - - -, calculated spectrum.*

Electronic Absorption Spectrometric Studies—For combinations showing an antagonistic effect, preliminary studies were carried out on selected systems using electronic absorption spectrometry. Accordingly, the electronic absorption spectra of the following aqueous solutions at pH 6.8 were determined in the range of 300–600 nm[2]: 1, 0.250×10^{-4} *M* quinine dihydrochloride; 2, 0.250×10^{-4} *M* streptomycin sulfate; 3, 0.125×10^{-4} *M* of both quinine dihydrochloride and streptomycin sulfate; 4, 0.5×10^{-4} *M* chloroquine diphosphate; 5, 0.500×10^{-4} *M* penicillin G sodium; 6, 0.250×10^{-4} *M* of both chloroquine diphosphate and penicillin G sodium; 7, 0.250×10^{-4} *M* acetanilide; 8, 0.250×10^{-4} *M* tetracycline; and 9, 0.125×10^{-4} *M* of both tetracycline hydrochloride and acetanilide.

The data obtained from the spectra of Solutions 1 and 2 were used to calculate the spectrum of Solution 3. The calculated spectrum was compared to the experimentally obtained one. Similarly, the calculated spectra of Solutions 6 and 9 were computed from the data obtained from the spectra of Solutions 4 and 5 and 7 and 8, respectively.

RESULTS AND DISCUSSION

The activities of the tested drugs on the different microorganisms are shown in Table II. The phenothiazines were the most active group; for example, the MIC of trifluoperazine hydrochloride against *Str. pyogenes* was 4.0 μg/ml. The salicylates had low antimicrobial activity (250–1000 μg/ml), and the other analgesics and antimalarials investigated had moderate antimicrobial activity.

The tested Gram-positive microorganisms were generally more responsive than the Gram-negative ones. *Ps. aeruginosa* was the most resistant.

Interaction between Drugs and Antibiotics—Synergism and antagonism between different antimicrobial agents have been studied using various methods (16–18). To study the interactions of the drugs with antibiotics, it was necessary to determine the MIC of the antibiotics against the two strains of *Staph. aureus* and *E. coli.* The types of interactions between the different drugs and antibiotics are shown in Tables III–V.

Aspirin and antipyrine salicylate were synergistic with the antibiotics in Table III except neomycin. With *Staph. aureus,* acetanilide, antipyrine, and dipyrone were antagonistic with tetracycline hydrochloride, nafcillin, and oxacillin, respectively, but no interactions occurred with the other antibiotics.

Quinine dihydrochloride was antagonistic with streptomycins, oxacillin, and nafcillin, while chloroquine diphosphate was antagonistic with penicillin G and penicillin V. However, both showed a synergistic effect with chlortetracycline hydrochloride and demeclocycline hydrochloride. On the other hand, the other antimalarials quinacrine and primaquine were synergistic with most of the tested antibiotics (Table IV).

Most tranquilizers were synergistic with all tested antibiotics (Table V).

[1] Culture collection of the Microbiology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

[2] Pye Unicam SP 8000 recording spectrophotometer and two matched 1-cm fused silica cells.

Table II—MIC of the Analgesics, Antipyretics, Antimalarials, and Tranquilizers against Different Microorganisms

Drug	*E. coli*	*P. vulgaris*	*S. typhi*	*Ps. aeruginosa*	*Staph. aureus*	*Str. pyogenes*	*B. subtilis*	*C. albicans*
Analgesics and antipyretics								
Salicylamide	N[a]	N	N	N	N	N	N	N
Sodium salicylate	N	500	250	N	N	N	1000	N
Aspirin	1000	500	500	1000	500	N	500	N
Sodium gentisate	N	N	N	N	N	N	N	N
Antipyrine salicylate	1000	1000	500	N	500	N	500	N
Acetanilide	N	N	N	N	N	N	N	N
Acetaminophen	N	N	N	N	N	N	N	N
Phenacetin	N	N	N	N	N	N	N	N
Dipyrone	N	N	N	N	N	N	N	N
Antipyrine	N	N	N	N	N	N	N	N
Indomethacin	N	N	N	N	N	N	1000	N
Antimalarials								
Quinine dihydrochloride	N	500	500	1000	N	125	1000	N
Chloroquine diphosphate	N	N	1000	N	N	500	N	N
Primaquine diphosphate	1000	N	25	N	1000	63	1000	1000
Quinacrine	500	N	125	N	1000	16	N	1000
Tranquilizers								
Chlorpromazine hydrochloride	31	16	8	125	125	8	31	31
Promethazine hydrochloride	125	250	125	500	500	31	250	125
Acetophenazine maleate	250	N	250	1000	N	63	250	250
Trifluoperazine dihydrochloride	31	250	125	500	31	4	31	31
Thioridazine hydrochloride	31	63	31	250	63	16	16	31
Triethylperazine dimaleate	63	63	63	250	31	16	31	31
Hydroxyzine hydrochloride	250	500	250	N	500	63	500	1000

[a] N = no effect at 1000 μg of drug/ml.

Table III—Interaction between Aspirin and Antipyrine Salicylate with Antibiotics as Shown by Their Effect on *Staph. aureus* and *E. coli*[a]

Antibiotic	Aspirin		Antipyrine Salicylate	
	Staph. aureus	*E. coli*	*Staph. aureus*	*E. coli*
Kanamycin sulfate	S	S	S	—
Neomycin sulfate	A	A	A	A
Chloramphenicol	—	—	S	—
Oleandomycin phosphate	—	S	—	—
Penicillin G sodium	—	S	S	S
Ampicillin sodium	S	S	—	S
Oxacillin sodium	S	—	S	—
Nafcillin sodium	S	S	—	—
Polymyxin B sulfate	—	S	—	—
Chlortetracycline hydrochloride	S	—	—	—
Novobiocin sodium	—	S	—	S

[a] A = antagonistic; S = synergistic. The dash (—) indicates that the results were found as expected; either *no growth occurred* (the total concentration was equal to MIC or more) or *growth occurred* (the total concentration was less than the MIC).

All synergistic effects (Tables III–V) occurred at one-fourth of the MIC of both the drugs and the antibiotics.

Electronic Absorption Spectrometric Studies—Preliminary studies using electronic absorption spectrometry were done on some combinations that showed antagonistic action, namely quinine–streptomycin, chloroquine–penicillin G sodium, and acetanilide–tetracycline.

With the quinine dihydrochloride–streptomycin sulfate and chloroquine diphosphate–penicillin G sodium combinations, the calculated spectra of their solutions, containing equimolar concentrations, were more or less identical with the experimentally obtained spectra. On the other hand, Fig. 1 shows that the experimentally obtained spectrum of the solution containing 0.250×10^{-4} *M* of both acetanilide and tetracycline hydrochloride was different from the calculated spectrum. This result suggests that, under these experimental conditions, no physical interaction occurs in the first two systems (19). On the contrary, there is some sort of physical interaction between acetanilide and tetracycline.

Further studies on the mechanism of action of drugs showing synergistic and antagonistic effects are in progress. In conclusion, it seems that the indiscriminate administration of drug–antibiotic combinations is questionable and may not be advisable because such *in vitro* interactions may occur *in vivo*.

Table IV—Interaction between Antimalarials with Antibiotics as Shown by Their Effect on *Staph. aureus* and *E. coli*[a]

Antibiotic	Quinine Dihydrochloride		Chloroquine Diphosphate		Primaquine Diphosphate		Quinacrine	
	Staph. aureus	*E. coli*	*Staph. aureus*	*E. coli*	*Staph. aureus*	*E. coli*	*Staph. aureus*	*E. coli*
Dihydrostreptomycin sulfate	A	A	—	—	S	S	S	S
Streptomycin sulfate	A	A	—	—	S	S	S	S
Kanamycin sulfate	—	—	—	—	—	S	—	S
Neomycin sulfate	—	—	—	—	S	S	S	S
Chloramphenicol	—	—	—	—	S	S	S	S
Erythromycin	—	—	—	—	S	S	S	S
Oleandomycin phosphate	—	S	—	S	S	S	S	S
Penicillin G sodium	—	—	A	—	S	S	S	S
Penicillin V potassium	—	—	A	—	S	S	S	S
Ampicillin sodium	—	—	—	—	S	S	S	S
Oxacillin sodium	A	—	—	—	S	S	S	S
Nafcillin sodium	A	—	—	—	S	S	S	S
Gramicidin	—	—	—	—	S	S	S	S
Polymyxin B sulfate	—	—	—	—	S	S	S	S
Tetracycline hydrochloride	—	—	—	—	S	—	S	S
Oxytetracycline hydrochloride	—	—	—	—	S	S	S	S
Chlortetracycline hydrochloride	S	S	S	S	S	S	S	S
Demeclocycline hydrochloride	S	S	S	S	S	S	S	S
Methacycline hydrochloride	—	—	—	S	S	S	S	S
Novobiocin sodium	—	—	—	—	S	S	S	S
Vancomycin hydrochloride	—	—	—	—	S	S	S	S

[a] See Table III.

Table V—Interaction between Tranquilizers with Antibiotics as Shown by Their Effect on *Staph. aureus* and *E. coli*[a]

Antibiotic	Chlorpromazine Hydrochloride		Promethazine Hydrochloride		Acetophenazine Maleate		Trifluoperazine Dihydrochloride		Thioridazine Hydrochloride		Triethylperazine Dimaleate		Hydroxyzine Hydrochloride	
	Staph. aureus	*E. coli*	*Staph. aureus*	*E. coli*	*Staph. aureus*	*E. coli*	*Staph. aureus*	*E. coli*	*Staph. aureus*	*E. coli*	*Staph. aureus*	*E. coli*	*Staph. aureus*	*E. coli*
Dihydrostreptomycin sulfate	S	—	—	—	—	—	S	—	—	—	—	—	—	—
Streptomycin sulfate	S	—	S	—	—	—	S	—	—	—	—	—	—	—
Kanamycin sulfate	S	S	S	—	—	—	—	S	S	—	S	S	—	—
Neomycin sulfate	S	S	S	S	—	—	S	—	S	—	S	—	S	—
Chloramphenicol	S	S	S	—	—	—	S	—	S	—	S	S	S	—
Erythromycin	S	S	S	S	S	—	S	S	S	—	S	S	—	—
Oleandomycin phosphate	S	S	S	S	S	S	S	—	S	—	S	S	S	—
Penicillin G sodium	S	S	S	S	—	S	—	—	S	—	S	—	S	—
Penicillin V potassium	S	S	S	S	—	S	S	—	S	S	S	—	S	S
Ampicillin sodium	S	S	S	S	—	S	S	—	—	S	S	S	—	S
Oxacillin sodium	S	S	S	S	—	S	S	—	S	S	S	—	—	S
Nafcillin sodium	—	S	—	S	—	S	—	S	—	S	—	S	—	—
Gramicidin	S	S	S	S	—	S	S	S	S	S	S	—	—	—
Polymyxin B sulfate	—	S	—	S	—	S	—	S	—	S	—	S	—	S
Tetracycline hydrochloride	S	S	S	—	—	S	—	S	S	S	—	S	—	S
Oxytetracycline hydrochloride	S	—	S	S	—	—	—	—	S	—	—	—	S	—
Chlortetracycline hydrochloride	S	S	S	S	—	—	—	S	S	—	—	S	—	S
Demeclocycline hydrochloride	S	S	S	S	—	S	—	S	S	S	—	S	—	S
Methacycline hydrochloride	S	S	S	—	—	S	—	S	S	—	—	S	S	S
Novobiocin sodium	S	S	S	S	—	S	—	S	S	S	—	S	—	S
Vancomycin hydrochloride	S	—	S	S	—	S	S	—	S	—	S	—	S	S

[a] See Table III.

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ACKNOWLEDGMENTS

The drugs used in this study were supplied by El-Nasr Pharmaceutical Co., Egypt; Swiss-Pharma, Egypt; B.D.H., England; May and Baker, England; Bristol Laboratories, England; Merck Sharp and Dohme; Schering Corp.; Lederle; Pfizer Inc.; Upjohn Co.; and Eli Lilly & Co.

The authors thank Dr. Hassan Y. Aboul-Enein, Faculty of Pharmacy, Riyadh University, for proofreading the manuscript.