MEDICAL GRAND ROUNDS

Parkland Memorial Hospital

October 2, 1969

"Newer" Antimicrobials and Recent Observations on "Older" Antimicrobials

When the mode of action of sulfanilamide was elucidated by Woods and Fildes, and the theory of bacterial inhibition by metabolic analogues was developed, the way appeared open for devising antimicrobial drugs on a rational basis. Subsequent advances in knowledge of the structure, composition and metabolism of the bacterial cell encouraged hopes still further. This knowledge has been helpful in explaining what drugs do to bacteria, but discoveries have continued to result from purely empirical random trials. Not only is the action of any new drug on individual bacteria still quite unpredictable on a theoretical basis, but so are its effects on the body. Most of the toxic effects of antibiotics come to light only after extensive use. Thus, it seems appropriate and timely both to review aspects of antimicrobial therapy from the vantage of "newer" antimicrobials and to review recent observations, usually evidences of toxicity, relative to "older" antimicrobials.

It is difficult for most physicians today to realize the seriousness of bacterial infections until as recently as 25 or 30 years ago (Figure 1). However, this miraculous medical progress has been achieved at a cost. Adverse reactions as a consequence of diagnosis and therapy pose major problems. There are relatively few documentations of the magnitude of the problem of iatrogenic factors in the infectious disease field. Examples include: In 1945 one of every 4 individuals in the U.S. received penicillin; in 1960 enough chloramphenicol was sold to give 3,730,000 Americans a 10 gram course of therapy (1). Schimmel studied the occurrence of hospital-associated complications in 1,014 patients admitted to a university medical service (2). During the 8-month study, 240 episodes occurred in 198 patients. Of these episodes, 119 (50%) were reactions to drugs, with 35 (15%) associated with antimicrobials (Table 1).

In a recent survey of hospital-associated infections, antimicrobials were given to 30% of the patients, with 13.5% given for prophylaxis (3). The effectiveness of such practices continues to be debated but as data are accumulated, the indications for antibiotic prophylaxis appear to progressively decrease. For example, antibiotic prophylaxis did not decrease the incidence of transient bacteremia in adults at cardiac catheterization (4). Likewise, in a prospective evaluation of antimicrobial prophylaxis in 72 heart surgery patients at Vanderbilt in 1964-66, antibiotic regimens did not alter the frequency of postoperative infection (Table 2) (5).

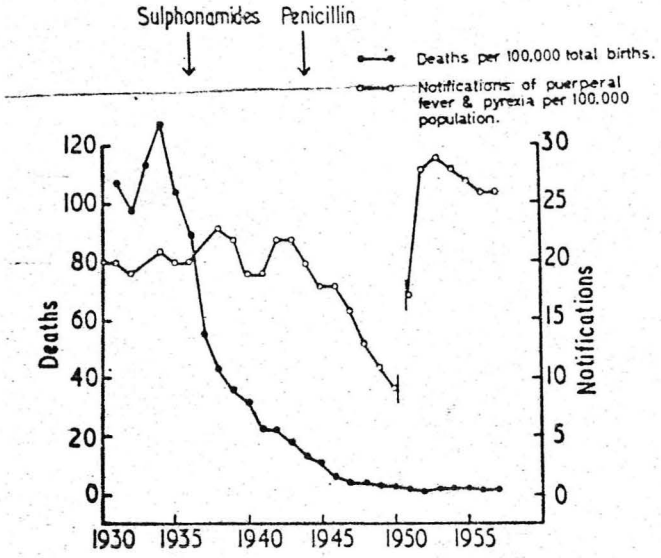


FIGURE 1 Puerperal pyrexia. Deaths per 100,000 total births and incidence per 100,000 population in England and Wales, 1930-1957

TABLE 1

Reactions to Antimicrobial Drugs (2)

Drug	Number and Type of Episodes	
	Toxic	Allergic
Penicillin	1	18
Nitrofurantoin	1	4
Tetracyclines	2	2
Streptomycin	-	3
Amphotericin	1	-
Bacitracin	1	-
Isoniazid	1	-
Neomycin	1	-

TABLE 2

Antibiotic Prophylaxis in Cardiac Surgery Patients (5)

Group	No. of Patients	Total Infected Patients (%)	Classification of Infection			
			Major	Wound	Urinary	Pulmonary
Placebo	15	5 (33)	2	3	2	1
Penicillin + Streptomycin	30	8 (27)	2	2	4	2
Oxacillin	27	7 (26)	3	1	4	1

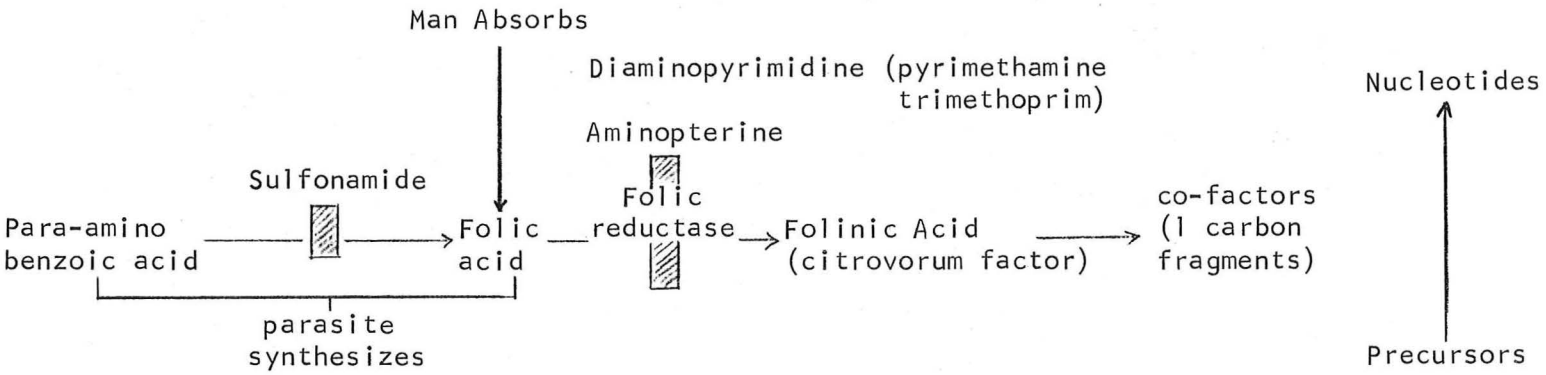
Unfortunately, the limited size of the study precludes a definitive conclusion. The study was interrupted when 2 patients in the placebo group developed pneumococcal endocarditis. Hurwitz and Wade reported on adverse reactions which occurred in a population of 1,268 patients admitted to hospital wards and kept under surveillance by one observer (5a). Drug reactions occurred in 10.2% of the 1,160 patients who received drugs. Most reactions were due to known pharmacological reactions and only 4 were life-threatening in seriousness. Ampicillin administration was associated with a reaction rate of 7.8% (8/103 patients). Of the total of 350 patients who received antibiotics, reactions occurred in 12 (3.4%). Knudsen reports that the frequency of skin reactions associated with ampicillin in the published literature is 383 of 13,638 patients (2.8%) (5b). In attempting to elucidate predisposing factors, Hurwitz found that significantly more patients over 60 years of age and more women than men developed adverse drug reactions (5c).

Against this background of widespread usage, often with imprecise indications, a selected group of more recent antimicrobials will be reviewed considering antimicrobial spectra, pharmacology, toxicity and other attributes.

Trimethoprim (investigational in U.S.): Trimethoprim is a synthetic diaminopyrimidine compound which is a potent inhibitor of dihydrofolic acid reductase (Figure 2). Diaminopyrimidine compounds possess both antimalarial and antibacterial activity, the former being highest in pyrimethamine, the latter in trimethoprim. In the parasite, the diaminopyrimidines act sequentially with sulfonamide in inhibiting the synthesis of folic acid from PABA. As a result this combined action with sulfonamides is synergistic, with about a 10-fold increase in activity when the compounds are given together (6). Trimethoprim has a far lower affinity for the mammalian enzyme and there has been little evidence of interference with folic acid metabolism in animals and man. Trimethoprim has been combined with sulfamethoxazole in a ratio of 1:5, i.e., 80 mg trimethoprim and 400 mg sulfamethoxazole per tablet ("Bactrim"® "Septrin"®) in Britain with early results in urinary tract and other infections including some due to streptococci, staphylococci and neisseria which are encouraging (7-10) (Table 3).

FIGURE 2

Folic Acid Metabolism and Sulfonamides*



* Based upon Hitchings, G.H., Trans. N. Y. Acad. Sci. 23:700, 1961 (6)

TABLE 3

Treatment	No. Patients Treated	Cure* (%) 1 week after therapy	Cure* (%) 4-5 weeks after therapy
Results of Primary Treatment of Urinary Tract Infections (8):			
S-T 5:1	41	85	67
Ampicillin	30	70	52
Sulfadimidine	35	40	15
Results of Secondary Treatment (Patients who had already failed on one drug) (8):			
S-T 5:1	18	89	46
Ampicillin	12	67	17
Sulfadimidine	6	0	0

* Bacteriological

All gram-negative bacilli occurring in urine except Pseudomonas aeruginosa are usually highly sensitive. For some species, MICs are < 1.0 μg/ml, levels comparable to potent antibiotics. It has been suggested that such combination treatment should entirely replace older regimens (10). Unfortunately, but not unexpectedly, in the

same issue of the BMJ, the occurrence of reversible agranulocytosis was reported in a 21-year-old woman who had been on "Septrin" for 23 days (illness characterized by fever, arthralgias, urticarial rash, lymphadenopathy, WBC 2500/mm^3 with 1% neutrophiles) (10a). Because of the ~~antibiotic~~ folic activity, the drug should not be used during pregnancy and used with caution in women of the childbearing age.

This preparation is of additional interest in that it represents a "fixed" combination of antimicrobial agents which appears to fulfill the criteria for efficacy as a combination which exceeds that for either ingredient alone and such should meet the requirements which most currently available fixed combinations do not (11). The broad question of antibiotic combinations will be discussed in a subsequent section.

Sulfamylon: "Burn wound sepsis" has represented the leading cause of late deaths in patients with extensive thermal injury. Almost half of the patients dying of burn wound sepsis showed no evidence of metastatic bacterial spread beyond the limits of the burn wound and immediate adjacent viable tissue (12). The ineffective use of systemic drugs and the avascular nature of the burn dictated some other approach. Lindsey was familiar with the old sulfonamide, mafenide or p-aminomethyl-benzene sulfonamide (Sulfamylon) which was not susceptible to inactivation by purulent secretions and its effectiveness in experimental soft tissue wounds was demonstrated by Mendelson and Lindsey (13). It has subsequently been evaluated extensively in experimental and clinical burns, and the results demonstrate a marked influence on mortality rates (Table 4) (14).

TABLE 4

	Per cent body surface burned										
	0-10	*11-20*	*21-30*	*31-40*	*41-50*	*51-60*	*61-70*	*71-80*	*81-90*	*91-100*	*Total*
No topical therapy											
Patients	39	52	49	36	36	23	14	24	11	6	290
Deaths	0	2	4	17	21	18	11	21	11	6	111
Mortality rate	0	4	8	47	58	78	79	88	100	100	38.3
Sulfamylon											
Patients	226	237	223	185	123	88	59	43	24	10	1218
Deaths	0	1	7	20	33	29	39	38	23	9	198
Mortality rate	0	0.4	3	11	27	33	66	88	96	90	16.3
Silver nitrate*											
Patients		40	19	22	16	10	13	5	8	11	144
Deaths		0	3	6	6	4	9	5	7	11	51
Mortality rate		0	16	27	37	40	70	100	87	100	35.4

*The silver nitrate figures are from Dr. William Monafo. The others are from the United States Army Surgical Research Unit. Simultaneous clinical comparisons of silver nitrate and sulfamylon are available and comparative series must be utilized. Varations in patient groupings make precise comparisons difficult, as do differences in ancillary treatment procedures and methods of determining extent and depth of burn. Both effectively influence mortality rate in burns of loss than 60 per cent. Sulfamylon is more effective in children and considered much easier to use. Modified from Moncrief, J. A.: The status of topical antibacterial therapy in the treatment of burns, Surgery 63:862-867, 1968.

A comparison between silver nitrate and sulfamylon is presented in Table 5.

TABLE 5

	Silver nitrate	*Sulfamylon*
Availability	Readily available	Investigative drug, FDA approved and marketed
Chemistry	Inorganic silver salt	Methylated sulfonamide
Method of use	0.5% solution in distilled water, use to wet dressings q 2 h. Dressings changed b.i.d. Debridement with dressing changes	10 per cent of drug in water-miscible base applied once or twice daily without dressings Wash off drug daily and debride wound
Mode of action	Probably dependent upon free silver ions	Unknown
Spectrum of activity	Entire spectrum Bacteriostatic	Entire spectrum Primarily bacteriostatic
Local and systemic effects	Poor penetration Rapidly precipitated in local tissue as AgCl Very little absorption No argyria	Penetrates tissue well in active drug form Locally nontoxic Rapidly broken down in blood to inactive form of acid salt and excreted in urine No crystalluria
Biochemical changes	Hyponatremia due to absorption of distilled water and leaching of sodium Hypochloremia due to AgCl precipitation Methemoglobin elevation Correction by oral and intravenous NaCl	Acid salt breakdown product provides heavy acid load Carbonic anhydrase inhibition with HCO_3 excretion and chloride retention Compensation by hyperventilation and CO_2 depression
Advantages	Sensitivity not present Painless in application No antagonists No resistant organisms Wide spectrum	Active penetration allows delayed therapy to be effective Wound readily visible Easy to use and clean Nontoxic Wide spectrum Not inactivated by local substances Resistance does not develop Allows full joint motion
Disadvantages	Poor penetration prevents successful control of deep tissue and delay of therapy not tolerated Biochemical changes particularly in children Pain on changing dressings Messy and requires much work to use properly Dressings impede joint mobility Discoloration obscures the wound	Biochemical changes Pain of varying intensity on application 5 per cent sensitivity manifest by rash

Both substances, if properly used, can effectively control bacterial growth and eliminate burn wound sepsis as the major cause of death. From Moncrief, J. A.: The status of tropical antibacterial therapy in the treatment of burns, Surgery 63: 862-867, 1968.

The concept of controlling local infection by means of an agent which penetrates well into avascular areas and which has broad spectrum of antimicrobial activity, e.g., clostridia and pseudomonas, may be of importance in a variety of medical problems such as decubitus ulcers, ischemic gangrene of extremities and cold injuries (frostbite).

Carbenicillin (Pyopen) (investigational in U.S.): Carbenicillin was reported in 1967 as unique from all other penicillins by its degree of activity against Pseudomonas aeruginosa, most strains being inhibited in vitro by 25 to 200 μg/ml (15). Structurally it is disodium alpha carboxybenzyl penicillin (Figure 3).

FIGURE 3

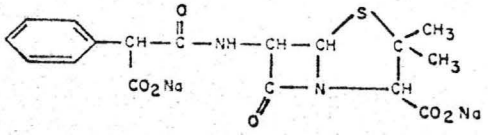


TABLE 6

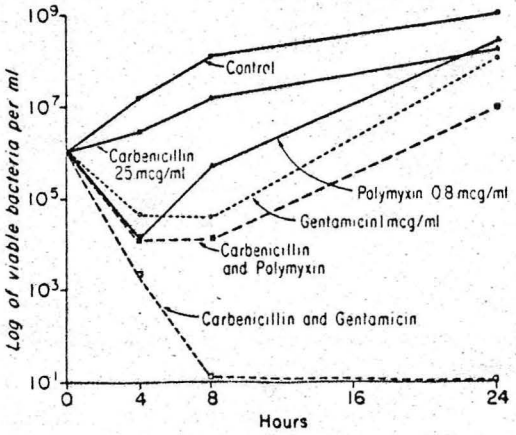
In Vitro Antimicrobial Activity (25)

Organism	No. Strains	% Inhibited at Various Concentrations of Carbenicillin (μg/ml)									
		1	2.5	5.0	12.5	25	50	75	100	150	200
Pseudomonas	40	-	-	-	-	22	72	85	90	98	-
Proteus mirabilis	17	88	100								
P. rettgeri	6	66	83								100
P. morganii	18	61	83	83	89	94	-	-	-	-	-
P. vulgaris	6	33	-	-	50	-	68	-	-	83	-
Providence	3	68	100								
E. coli	21	-	19	71	90	95	-	-	-	-	-
Enterobacter	14	-	7	50	71	86	-	-	93	-	-
Klebsiella	24	-	-	-	-	-	-	4	-	12	21
Serratia	12	8	42	68	75	83	-	-	-	-	-

Williams also observed that klebsiella were resistant, but also found only 20% of serratia (47 strains) inhibited at concentrations of 500 μg/ml (27). Bodey, et al. also found it ineffective against serratia (28). Carbenicillin is not effective against penicillinase-producing staphylococci.

Synergism: The combination of carbenicillin and gentamicin has shown marked synergism against pseudomonas (16,24,25) (Figure 4).

FIGURE 4



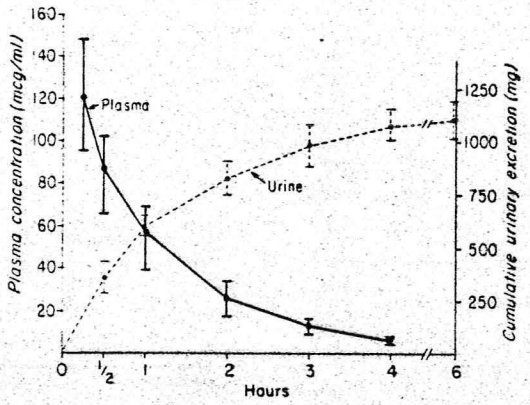
Development of Resistance: Resistance develops rapidly in vitro (21). Lowbury, et al. in 1969 noted the appearance of highly resistant (MIC > 4000 μg/ml) carbenicillinase-producing strains which quickly displaced all other strains of pseudomonas from the ward (23). Standiford, et al. noted resistance in one of 5 patients.

Reversion to sensitivity was enhanced when strains were exposed to acriflavine, suggesting that an extrachromosomal factor was responsible for resistance. The transfer of this factor could account for the nearly simultaneous emergence of resistance in different types of pseudomonas (23). The phenomenon of extrachromosomal resistance factors will be discussed subsequently.

The emergence of highly resistant strains makes it essential to reserve this antibiotic for treatment of severe infection and suggests that it should be used in combination with gentamicin.

Pharmacology: Protein binding is 49% at 20 μg/ml. Concentrations in plasma and urinary excretion after the IV injection of 1.0 gram are shown (Figure 5) (25):

FIGURE 5



With normal renal function it is necessary to give 1.0 gm IV every hour with probenecid to give levels of 100 μg/ml. Bodey, et al. administered 5.0 gm IV over a 2-hour period, every 4 hours, for a total dose of 30 grams daily, in addition to probenecid 0.5 gm four times daily.

With severe renal failure (Cl_{CR} < 5 ml/min) the serum half-life was 12.5 hours. 2.0 gm every 8 hours IV will maintain serum levels of approximately 100 μg/ml. Hemodialysis reduced the t/2; 2.0 gm IV q4h is recommended. Peritoneal clearance was poor, 6.8 ml/min in 2 patients, and a dose of 2.0 gm IV q6h is recommended (18). Standiford, et al. administered 32 mg in 8 ml of saline intraventricularly twice daily (levels 1070 μg/ml) without incident.

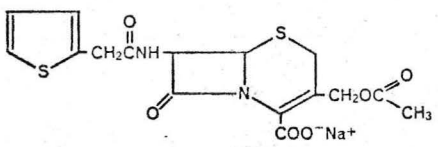
Therapeutic Results: Bodey, et al. in patients with acute leukemia and metastatic cancer observed a response rate of 91% in 23 episodes of pseudomonas and 58% of 12 E. coli infections (28). The majority of patients responded in 48 hours. Carbenicillin was ineffective against serratia and klebsiella. Similar results are reported by others.

Toxicity: In the studies of Bodey, et al. (56 trials in 34 patients)(28), superinfection due to serratia or klebsiella developed in 7 patients (13%). The incidence of toxicity was low; two patients had nausea and vomiting, 3 developed generalized urticaria, 4 of 22 had increases in SGOT and SGPT (highest values 364 and 370 u) (28). Thrombophlebitis was not a problem.

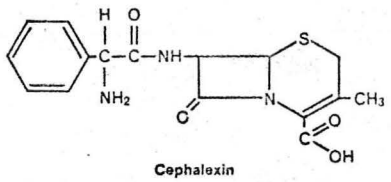
Cephalexin ("Keforal"®):

Cephalexin is a new cephalosporin antibiotic which is chemically related to cephaloglycin ("Kafocin"®) but which is better absorbed orally (Figure 6).

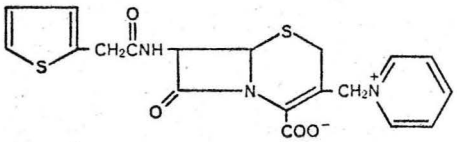
FIGURE 6



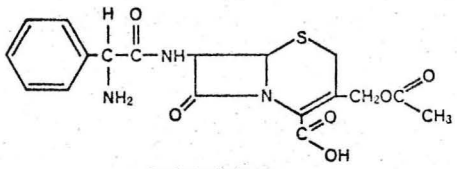
Sodium Cephalothin



Cephalexin



Cephaloridine



Cephaloglycin

In Vitro Antimicrobial Activity: Studies of its in vitro activity indicate its spectrum is comparable to that of the existing cephalosporins, cephalothin, cephaloridine and cephaloglycin (29). Cephalexin appears to be less active against staphylococci, especially penicillinase-producing staphylococci, than cephalothin or the synthetic penicillinase-resistant penicillins, e.g., only 40% of penicillin resistant strains were killed by 100 μg/ml of cephalexin, whereas 70% of penicillin susceptible isolates were killed at 100 μg/ml (29). At a concentration of cephalexin generally attained in serum with 500 mg of cephalexin po (15 μg/ml), the antibiotic inhibited 90% of strains of E. coli, Proteus mirabilis and klebsiella. Most strains of enterobacter (aerobacter) were resistant to at least 100 μg/ml. It also had essentially no antibacterial activity at clinically useful concentrations against the indole positive Proteus spp. (rettgeri, morganii and vulgaris), enterococci or pseudomonas. In vitro susceptibility studies based upon tube dilution studies are illustrated in Table 7.

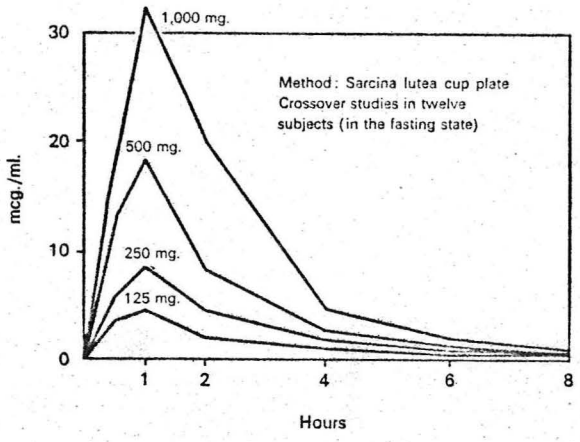
TABLE 7

Organism	Number of Isolates	<0.1	0.5	1	1.56	3.12	6.25	12.5	25	50	>50*
Streptococcus (group A)	53	6%	62%	88%	94%	100%					
Pneumococcus	18	17%	39%	77%		100%					
Streptococcus (viridans)	21		5%	9%	28%	52%	90%	100%			
H. influenzae	21					52%	71%	85%		90%	10%
Staphylococcus (+) (−)	287		3%	14%	28%	65%	89%	99%	<1%		<1%
Salmonella	35			6%	9%	26%	88%	94%			6%
Shigella	40					15%	57%	82%	90%	100%	
Pr. mirabilis	31					3%	16%	55%	94%	97%	3%
Proteus (indole-positive)	90					2%	7%	12%	37%	60%	40%
Esch. coli	155					5%	34%	70%	89%	93%	6%
Klebsiella-Aerobacter	60					3%	22%	57%	60%	65%	35%
Klebsiella	68			1%	4%	20%	41%	69%	84%	90%	10%
Aerobacter	36						3%	8%	30%	39%	61%
Enterococcus	78				2%	5%	9%	13%	14%	23%	77%
Pseudomonas	85			1%	1%					1%	97%
Paracolon	8							12%			88%
N. meningitidis	21			10%	57%	86%	100%				
Others	12					8%	33%	67%	75%		25%
TOTAL	1,119										
CUMULATIVE %		0.5%	4%	10%	15%	31%	49%	65%	73%	77%	23%

Note that strains of group A streptococci, pneumococci and H. influenzae are susceptible in vitro. For Neisseria meningitidis, the sensitivity values are higher than those anticipated with penicillin G or ampicillin (30).

Pharmacology: Concentrations of cephalexin in serum in subjects with normal renal function are shown in Figure 7.

FIGURE 7



Clark and Turck studied two patients with creatinine clearances of less than 5.0 ml/min. In these patients peak serum values were 24 μg/ml in contrast to 10 μg/ml in normals after a single 250 mg dose (29). In normals, 80% of the administered dose was recovered in the urine 6 hours after a single 250 mg dose, with the mean peak concentration in the urine being 830 μg/ml. In the two azotemic patients peak urine concentrations were only 78 and 90 μg/ml (29).

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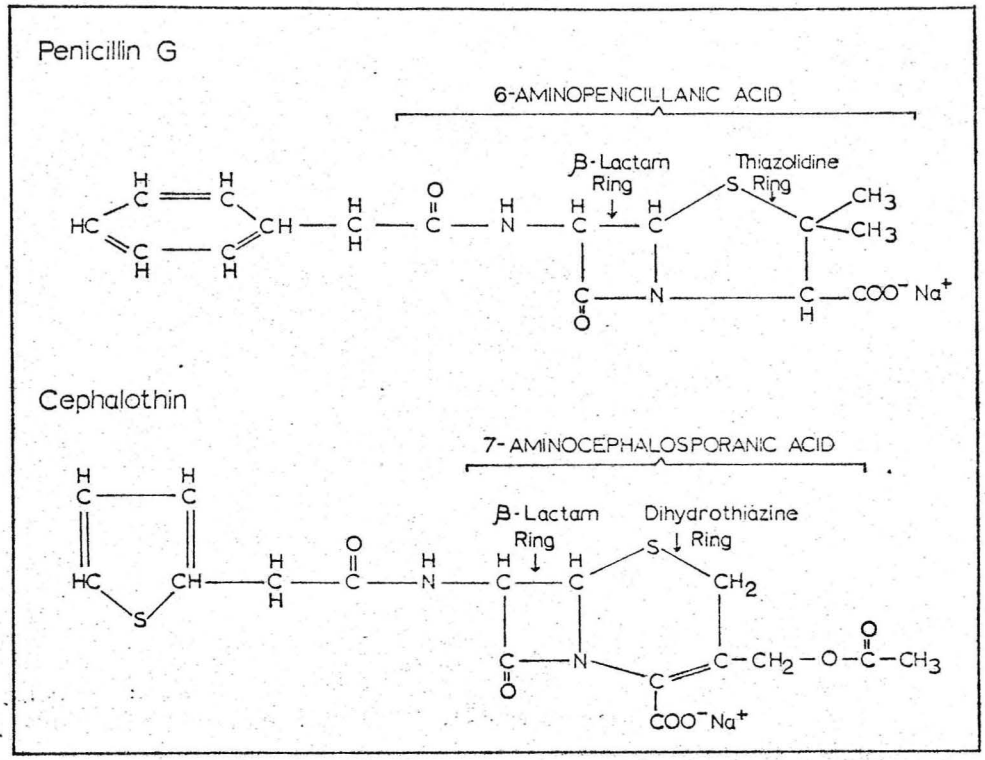
FIGURE 8



Brandriss, Smith and Steinman clearly demonstrated in 1964 that sensitization of rabbits with penicillin G, 6-amino penicillanic acid and cephalothin resulted in cross-reacting antibodies as measured by agar gel diffusion, passive cutaneous anaphylaxis and hemagglutination (36). Batchelor and associates reported similar data (37). Thoburn, Johnson and Cluff have analyzed their clinical experiences with cross-allergenicity (38). Of 54 patients treated with cephalothin, 7 had an allergic reaction. Three of these occurred before a program of surveillance of adverse drug reactions Excluding these 3 patients, they calculate a rate of allergic reactions of 8% (4 of 51). The reactions included 2 patients with anaphylaxis, 2 with urticaria and 3 with maculopapular rashes (one of the anaphylactoid reactions was immediate, and one was delayed). Five of the seven patients with reactions to cephalothin had a history of penicillin allergy. Allergic reactions to cephalothin occurred at a higher rate in Negro women than in Negro men or Caucasians. They concluded that "cephalothin must be used with caution in the treatment of patients with a history of allergic reactions to penicillin...and cannot be considered as a uniformly safe substitute for penicillin in such patients." Similarly, Greico demonstrated reagenic antibody (skin sensitizing) to cephalothin, cephaloridine and 7-aminocephalosporanic acid in a patient studied 5 months after an anaphylactoid reaction to penicillin G given orally (39,40). Our patient illustrates this need for caution, although we still recommend cephalothin as the alternative drug in many life-threatening infections. In contrast, sensitization to cephalosporins per se may occur without cross-sensitization, just as with the penicillins (41). Because of this potential for sensitization against the cephalosporins, we have decried its widescale usage and have favored holding it in reserve for "rainy days".

Other Toxic Reactions Encountered With the Cephalosporins: Other unusual reactions which may be encountered with the cephalosporins include positive Coombs tests, neutropenia and thrombocytopenia (42,43). With cephaloridine, the risk of nephrotoxicity is greater than with cephalothin, especially if the dosage of cephaloridine exceeds 4.0 gm daily or if the patient has underlying renal insufficiency (44,45). It is important to recall that many elderly patients may have endogenous creatinine clearances of < 40 ml/min with serum creatinine levels of < 1.5 mg.% because of less production of endogenous creatinine (46). It is such patients that potentially nephrotoxic drugs such as kanamycin, gentamicin or colistimethate but even cephaloridine must be administered with extreme caution.

While not a "toxic reaction", it has been reported that cephalothin may interfere with testing for glycosuria by use of "Clinitest" tablets (47). An unusual brown or brown-black color which can be misinterpreted occurs. Glucose oxidase methods (Testape or Combistix) work satisfactorily in the presence of cephalothin.

Gentamicin ("Garamycin"):

Gentamicin C is a broad-spectrum aminoglycoside antibiotic isolated from submerged fermentations of Micromonospora purpurea (48). Recently, the complex has been shown to consist of 3 components, C_1, C_2, and C_{1a}. The aminoglycoside antibiotics consist of 2 subclasses of agents (Table 8).

TABLE 8

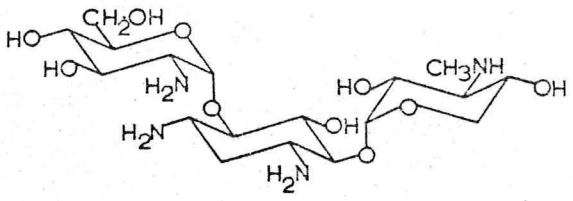
Classes of Aminoglycosidic Aminocyclitol Antibiotics

A. Streptidine Group:	B. Deoxystreptamine Group:
1. Streptomycin	1. Neomycins (2)*
2. Dihydrostreptomycin	2. Paromomycins (2)
3. Mannosidostreptomycin	3. Kanamycins (3)
4. Hydroxystreptomycin	4. Gentamicins (4)
5. Bluensomycin	5. Nebramycins

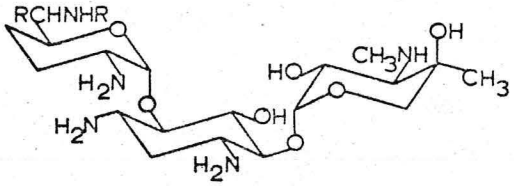
* Number in parentheses refers to number of components

The structure of gentamicin is illustrated in Figure 9. The gentamicins differ from the kanamycins in that they contain N-methyl and C-methyl groups, while the kanamycins do not (49).

FIGURE 9



GENTAMICIN A



GENTAMICINS C_1:R,R'=CH_3
C_2:R=CH_3,R'=H
C_{1a}:R,R'=H

In Vitro Antimicrobial Activity: Representative in vitro tube dilution sensitivities are presented in Table 9. In interpretation of this table, average peak blood levels of gentamicin are 5 μg/ml. Thus, gentamicin is not effective in vitro against pneumococci or N. gonorrheae.

TABLE 9

Organism	Strains (N)	No. (%) Inhibited By	
		5 μg/ml	10 μg/ml
Streptococcus sp.	37	37 (100%)	
Streptococcus beta hemolytic	6	5 (83%)	6 (100%)
Streptococcus group A	12	12 (100%)	
Diplococcus pneumoniae	50	9 (18%)	48 (96%)
Bacillus spp.	16	16 (100%)	
Corynebacterium spp.	2	2 (100%)	
Clostridium spp.	12	9 (75%)	11 (92%)
Listeria sp.	2	2 (100%)	
Neisseria gonorrheae	49	10 (20%)	49 (100%)
Brucella sp.	14	14 (100%)	
Paracolobactrium sp.	56	56 (100%)	
Providence sp.	13	13 (100%)	
Salmonella spp.	112	110 (99%)	112 (100%)
Serratia sp.	2	2 (100%)	
Shigella spp.	85	74 (87%)	85 (100%)
Mycobacterium tuberculosis	15	14 (93%)	15 (100%)
Mycoplasma pneumoniae	5	5 (100%)	

Limited studies with other, less common organisms are presented in Table 10.

TABLE 10

Organism	Strains (N)	MIC (μg/ml) Range	Medium*
Staphylococcus (mastitis)	5	0.01	1
Streptococcus agalactiae	3	0.3-3.0	1
Lactobacillus sp.	2	0.08	1
Clostridium spp.	3	64.0	2
Corynebacterium spp.	3	3.0-37.5	2
Sphaerophorus necrophorus	1	64.0	2
Bacteroides melaningogenicus	1	0.3	2
Pasteurella multocida	1	3.0-7.5	3
Aeromonas liquefaciens	1	37.5	3
Hemophilus influenzae	3	7.5	4
Corynebacterium minutissimum	4	37.5	2
Vibrio coli	1	0.7	3
Mycoplasma gallisepticum	1	1.4	5
Mycoplasma (rats)	2	0.75	5
Pseudomonas pseudomallei	9	17.5-75.0	1

* 1 = yeast beef broth, pH 6.8; 2 = brain heart infusion + 10% horse serum, pH 7.3; 3 = fluid thioglycollate broth, pH 7.1; 4 = brain heart infusion + 20% horse serum, pH 7.3; and 5 = PPLO broth

From such data it is difficult to generalize, but effectiveness against many anaerobes, H. influenzae and Pseudomonas pseudomallei would be questionable. Kirby and Standiford have compared gentamicin in vitro with other antibiotics against some of the more "resistant" gram-negative bacilli (Table 11) (50).

TABLE 11

Comparative In Vitro Activity

Antibiotic	Av. Peak Blood Level (μg/ml)	Organism (% Strains Inhibited)		
		Klebsiella	Enterobacter	Indole + Proteus
Gentamicin	5	95	95	90
Cephalothin	20	90	5	-
Carbenicillin	75	5	90	90
Kanamycin	20	95	95	90

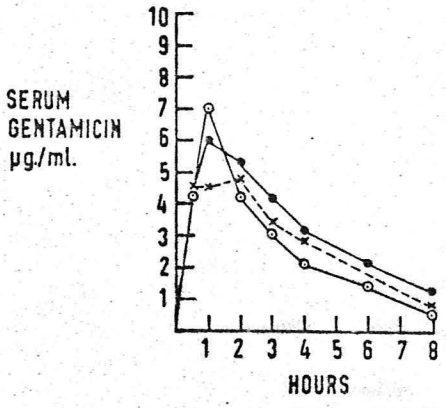
Of 87 strains of Staph. aureus, all were sensitive in vitro. Hoeprich studied 20 strains of "methicillin-cephalosporin" resistant staphylococci and found all were susceptible at 0.4 μg/ml of gentamicin (51). These observations are of particular importance with the emergence of methicillin resistant staphylococci in many areas

(52). Such strains constitute 2 to 4% of staphylococci studied in 2 British hospitals. In the U.S., occasional strains have been encountered in Seattle, Chapel Hill, Columbia, S.C., Houston, New York City and Portland. In a survey specifically looking for such strains at PMH in May-June 1969, Dr. John Berland found none in 150 cultures. Vancomycin has been considered the "last line of defense" against such staph, but as many as 2% are vancomycin-resistant, hence these data on gentamicin may be of considerable importance (53). Of 27 strains of enterococci, zone diameters were in an intermediate range and sensitivity is questionable (50).

Thus, gentamicin is effective in vitro against staphylococci and most aerobic gram-negative bacilli, but not particularly effective against pneumococci, neisseria, H. influenzae, enterococci, many anaerobes and Ps. pseudomallei.

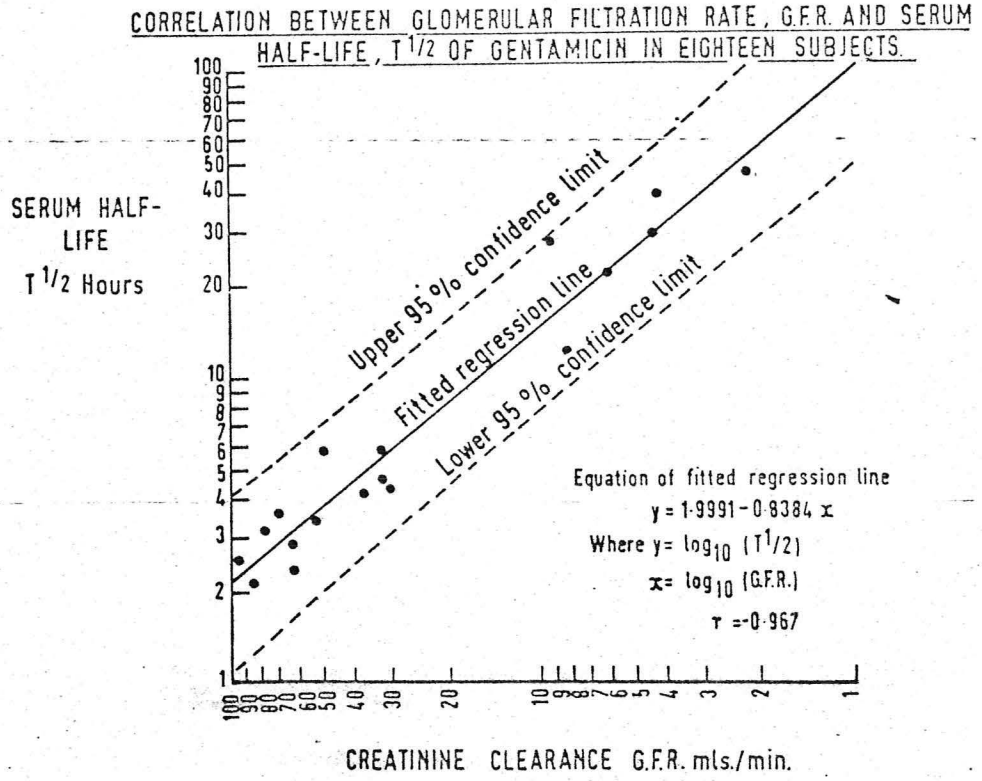
Pharmacology: The usual dose of gentamicin currently utilized is 2.5 to 3.0 mg/kg/day for systemic infections and 1.2 to 1.5 mg/kg/day for urinary tract infections, i.e., 80 mg q 8 hours for systemic infections. With such dosage, serum levels of $\geq$ 5 µg/ml will be obtained (Figure 10).

FIGURE 10



The correlation between glomerular filtration and serum half-life is illustrated in Figure 11 (54). It is excreted almost entirely in active form by glomerular filtration with little or no reabsorption.

FIGURE 11



Based upon these data, the following recommendations have been made (Table 12).

TABLE 12

Approximate Scheme of Dosage for Gentamicin

Blood urea mg/100 ml	Creatinine Clearance (GFR) ml/min	Dose and Frequency of Administration
< 35	> 70	80* mg 8-hourly
50-100	30-50	80 mg 12-hourly
> 200	5-10	80 mg every 48 hr
Twice weekly intermittent hemodialysis	< 5	80 mg after dialysis

* Use 60 mg if body weight is less than 60 kg

Therapeutic Results: Attempts to collate clinical data prove to be difficult but the following tabulation will provide a sense of the effectiveness of gentamicin.

TABLE 13

Results in Various Infections (55)

Type Infection	No. Cases	Successfully Treated	Improved	Failed	Indeterminant
Systemic infection	3	0	1	1	1
Complicated UTI*	7	5	1	1	0
UTI in paraplegics	6	3	0	3	0
Acute UTI	10	10	0	0	0

* UTI = urinary tract infection

TABLE 14

Pulmonary Infections in Patients With Underlying Pulmonary Disease (56)

Organism	No. Cases	Recovered or Improved	Failed
Pseudomonas	29	14	15
Klebsiella pneumoniae	7	1	6
Hemophilus influenzae	4	1	3
Staphylococcus aureus	2	0	2
Escherichia coli	1	1	0
Proteus sp.	3	0	3
Corynebacterium parvum	1	1	0
Proteus and Pseudomonas	1	1	0

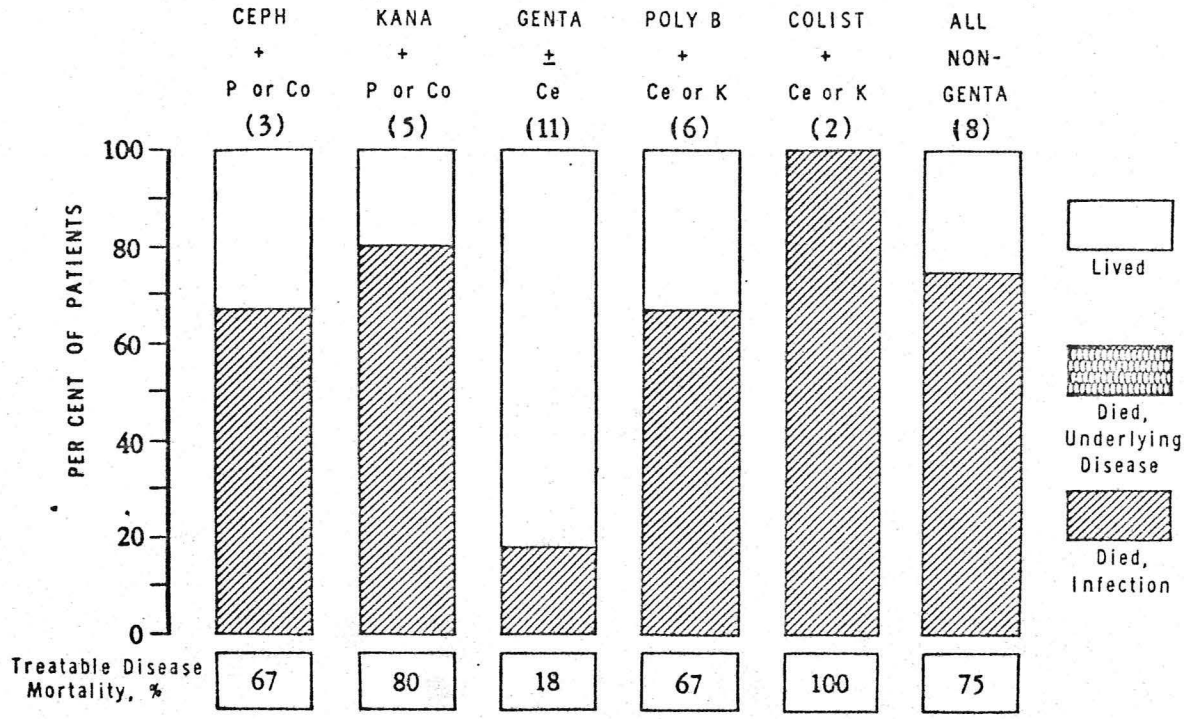
TABLE 15

Gentamicin Therapy in Urinary Tract Infection (57)

Diagnosis	No. Patients	Cure	Improvement	Unsatisfactory
Acute pyelonephritis	9	7	2	0
Chronic pyelonephritis	30	20	9	1
Chronic cystitis	12	8	3	1
Chronic prostatitis	7	6	0	1
Total	58	41	14	3

The studies of Martin, et al. provide the most striking evidence of efficacy (58). The study involved the assignment of all patients with a clinical diagnosis of gram-negative rod bacteremia to one of six randomized treatment regimens: cephalothin + polymyxin B (11 cs), cephalothin + colistimethate (9 cs), gentamicin (54 cs), gentamicin + cephalothin (14 cs), kanamycin + polymyxin B (31 cs) and kanamycin + colistimethate (31 cs). In 150 trial patients, final diagnoses of gram-negative rod bacteremia were made in 90 patients and were bacteriologically verified in 85 patients. The results of therapy in 19 patients with gram-negative rod bacteremia and shock are illustrated (Figure 12).

FIGURE 12



Dr. Riff of the University of Illinois has reported similar data (Table 16) (59).

TABLE 16

Recovery from Gram-Negative Bacteremia

Bacterial Etiology	Total Cases	Recovery No.	Recovery %
Escherichia coli and coliforms	10	8	80
Klebsiella-Enterobacter	5	4	80
Proteus species	3	1	33
Pseudomonas	7	0	0

From these observations, gentamicin appears to be the agent of choice in the treatment of serious aerobic gram-negative bacillary infections of the usual type encountered in hospitals, except perhaps for pseudomonas infections where it should be combined with carbenicillin (when available).

Toxicity: Gentamicin has the potential for both nephrotoxicity and ototoxicity. Tabulations of clinical trial data demonstrated increases in BUN in 4 of 172 patients (2%), a figure similar to streptomycin. In contrast, Riff observed increases of $\geq$ 5 mg.% in BUN in 15 of 92 patients (17%). Martin observed nephrotoxicity in 6% of patients treated with gentamicin in comparison to 21% with kanamycin, 20% with polymyxin B and 13% with colistimethate. Ototoxic reactions are more frequently serious; at least 24 cases have been recorded, 8 with hearing disturbances, 10 with complete loss of vestibular function, and 7 questionable vestibular disturbance (60). Most occurred in patients with impaired renal function. Meyers reported studies for ototoxicity in 40 patients, 10 of whom evidenced toxicity (61). 5 of the 10 had normal pre-treatment tests and in the other 5, pre-treatment status was not determined because the patients were too ill (these were classed as "presumptive") (Table 17).

TABLE 17

Patient No.	Ototoxic Effect		BUN (mg/100 ml)	Dose of Gentamicin (mg/ml)		Level of Gentamicin in (μg/ml)	
	Cochlear	Vestibular		Daily	Total	Serum	Urine
I	Normal	Complete right loss with dizziness	30	141	1,887	4	64
II	Normal	Complete right loss with dizziness	140	75	740	8	256
III	Normal	Transient right loss	16	51	600	4	-
IV	Normal	Transient right loss	15	150	900	8	125
V	40 db neural loss (flat curve)	Complete right loss	170	150/wk	1,050	16	32

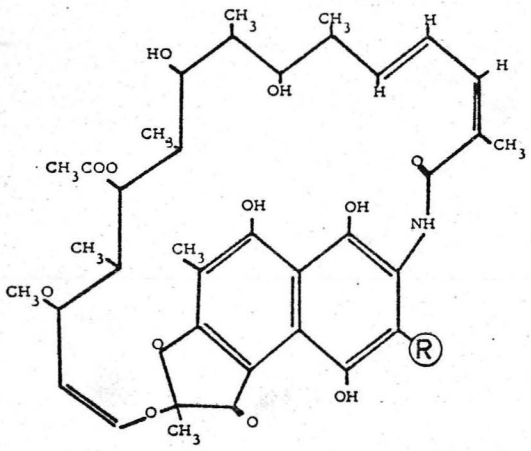
Of note, 5 of 10 disturbances were unilateral and 7 of 10 patients had increased BUNs.

Gentamicin appears to be a most important addition to the armamentarium of antimicrobials, but its use should not be indiscriminate both because of potential toxicity and because widescale usage may well be associated with the occurrence of a high proportion of resistant strains, as will be discussed briefly in the subsequent section of "transferable resistance".

Rifampicin or Rifampin (investigational in U.S.)

The rifamycins are a group of antibiotics which have been isolated from Streptomyces mediterranei. A parenteral form, rifamycin SV, has been employed clinically in Europe. Recently a semisynthetic derivative, rifampicin, which is well absorbed orally, has been introduced in the U.S. (Figure 13).

FIGURE 13



McCabe and Lorian studied in vitro activity and found rifampicin to be quite effective against pneumococci and group A streptococci, less active against enterococci and showed minimal activity against gram-negative bacilli (62). Mutants highly resistant to rifampicin were observed with 40% of strains of staphylococci. Atlas and Turck made similar observations against 250 clinical isolates, finding neisseria also to be sensitive in vitro (63). Resistant mutants were commonly seen. Deal and Sanders studied 30 strains of meningococci isolated from carriers and found all to be inhibited by 1.0 μg/ml of rifampicin or less, although only 2 of the 30 strains were "sulfadiazine-resistant" (63A). Treatment of 27 patients with bacteriuria resulted in clinical improvement in only 5 with most failures associated with the development of resistance (Table 18).

TABLE 18

Result of Therapy	No. of Patients
Elimination of bacteriuria	5 (18.5%)
Recurrence with different species or strain (reinfection)	2 (7%)
Recurrence with same species or strain (relapse)	5 (18.5%)
Persistence of bacteriuria during treatment	15 (56%)
Total	27 (100%)

These observations suggested little future for rifampicin.

However, strains of M. tuberculosis, either sensitive or resistant to other agents, are susceptible in the range of 0.01 to 2.0 μg/ml (64). M. kansasii strains are susceptible to ≤ 0.5 μg/ml, while some strains within Runyon's Group II also appear susceptible. In addition, the rate of development of resistance by mutation was slow. Thus, rifampicin may be an effective agent in retreatment programs involving M. tuberculosis strains and against M. kansasii.

Rifampin was highly effective in vitro against one strain of Pseudomonas pseudomallei (0.04 μg/ml) and effective in treating mice infected with Ps. pseudomallei (65).

Deal and Sanders treated 15 meningococcal carriers with rifampicin (600 mg daily for 4 days) and compared them with a group of 15 subjects who received placebo (63A). Cultures in only 2 of the placebo group became negative during study (13%), as compared to an overall success rate of 14 of 15 (93%) in the rifampicin group. These results are superior to any regimen other than sulfadiazine in carriers with "sulfa-sensitive" organisms (Table 19). Meningococci isolated after treatment did not show increased resistance.

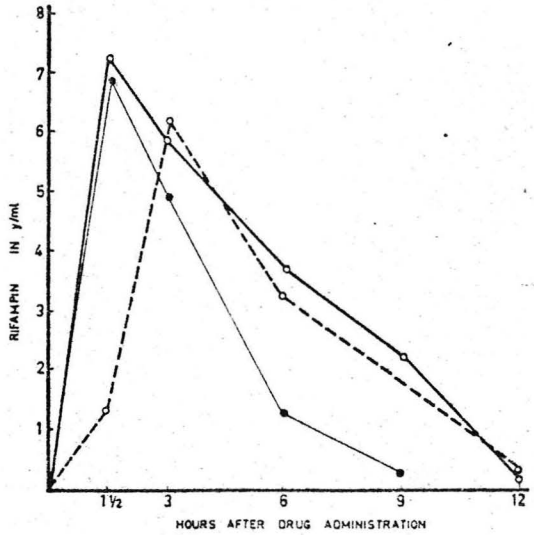
TABLE 19

Drug	Dose	Duration of Therapy (Days)	No. of Subjects Treated	Carrier Rate (% of Subjects with Positive Cultures)			
				Before Treatment	1 wk after Treatment	2 wk after Treatment	4 wk after Treatment
Sulfadiazine[17]	8 gm total	3	161	57.7	0.6	–	–
Sulfadiazine[15]	3 gm/day	3	100	30.0	0	2.0	–
	2 gm/day	2	100	36.0	3.0	2.0	–
Oxytetracycline[6]	0.5 gm twice/day	4	33	100.0	57.0	100.0	–
	0.5 gm twice/day	8	49	100.0	37.0	90.0	–
Erythromycin[8]	1 gm/day	4	12	100.0	63.6	100.0	–
	1 gm/day	10	28	100.0	53.8	60.0	–
Penicillin G[6]	1,000,000 units twice/day	4	23	100.0	61.5	75.0	–
	1,000,000 units twice/day	10	37	100.0	31.5	66.7	–
Penicillin G[6]	1,500,000 units	10	20	100.0	46.0	75.0	–
Ampicillin[8]	500 mg 3 times/day	10	26	100.0	32.0	38.0	–
Ethoxzolamide[7]	125-375 mg	3	8	100.0	100.0	100.0	–
Procaine penicillin[8]	1,200,000 units/day	2	118	100.0	–	51.0	–
Erythromycin[8]	500 mg 3 times/day	2	7	100.0	–	100.0	–
Rifampin	600 mg/day	4	15	100.0	6.7*	6.7	6.7

Meningococci isolated after treatment did not show increased resistance.

Pharmacology: Following 450 mg by mouth, blood levels of 7 μg/ml are attained at 2 hours. In some studies they have still been 2 μg/ml at 12 hours, with a therapeutic blood level still present at 24 hours (66,66A) (Fig. 14). Active concentrations appear in most tissues and body fluids.

FIGURE 14



Rifampin concentration in human blood after a single dose. o—o, 600 mg on an empty stomach; ●—●, 450 mg on an empty stomach; o---o, 600 mg in addition to breakfast.

Therapeutic Results: Results in the treatment of INH resistant tuberculosis are promising (Table 20) (67,68).

TABLE 20

Treatment Regimen	No. of Patients	Sputum "Conversion" to Negative for Mycobacteria		Bacteriologic Relapses	
		Yes	No	Under Treatment	After Cessation of Treatment
Group I: One active drug					
A. Ethambutol	14	9	5	3	1
B. Rifampin	7	7	-	2	-
Group II: Two active drugs					
A. Ethambutol plus viomycin, capreomycin, ethionamide, or thiocarlide	19	16	3	5	1
B. Ethambutol plus rifampin	12	11	1	1	-

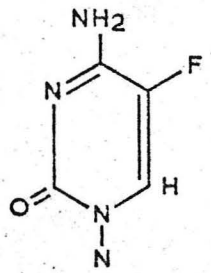
In 7 patients previously treated unsuccessfully with ethambutol, rifampicin resulted in reversal of positive cultures in all, although 2 had bacteriologic relapse during continued treatment. Of 12 patients treated with a combined regimen of rifampicin and ethambutol, sputum conversion was obtained in 11 and bacteriologic relapse occurred in only one (67). The 3 bacteriologic relapses were associated with the appearance of resistant strains (> 80 μg/ml).

Rifampicin and Viruses: It is of considerable interest that an antibiotic of proved pharmacologic acceptability in man inhibits the growth of some viruses (69-70). Rifampicin inhibits various poxviruses and an adenovirus. Of note, this observation was not the result of an empirical observation but was based on sound reasoning. It had been shown that the drug inhibits the DNA dependent RNA polymerase of E. coli (71). Mammalian cells also contain an RNA polymerase but the enzyme is less sensitive to the drug than the bacterial one. Recently it has been shown that vaccinia virus also has an RNA polymerase in the virus particle (72). Hence, it was logical to see if rifampicin inhibited the growth of vaccinia and of other DNA viruses (cowpox and alastrim). Rifampicin was effective against massive doses of virus, but 75 to 150 μg/ml was required. This concentration has not been possible in man, but the approach is most encouraging. Inhibition of the trachoma agent also has been reported and may be of clinical value (73).

5-Fluorocytosine (Ancobon®)

5-fluorocytosine is an antifungal agent with potential clinical usefulness (Figure 15).

FIGURE 15



In vitro studies reveal fungistatic concentrations in the range of 0.46 to 3.9 μg/ml against Cryptococcus neoformans, and 0.46 to 3.9 μg/ml for Candida albicans (74). A strain of Aspergillus flavus and A. niger were also susceptible in vitro (75). Studies in mice infected with Aspergillus fumigatus have been equivocal (75).

Pharmacology: Specimens obtained from patients on 100 mg/kg/day ranged from 11 to 32 μg/ml in serum and 8.5 to 26.0 μg/ml in spinal fluid (74). The serum half-life of 5-FC is 4 to 8 hours. Two-thirds is excreted unchanged in the urine.

Therapeutic Results: Patients with infections due to candida, cryptococci and aspergillus have been treated (76-78). Tassel and Madoff reported a patient with candida sepsis and a patient with cryptococcal meningitis recurring after amphotericin B who were favorably affected. The patient with candidemia did well. The patient with cryptococcal meningitis had lymphosarcoma and initially responded with clearing of her CSF only to deteriorate 8 months later (76). Utz and associates treated 15 patients with cryptococcosis with doses of 1.0 to 6.0 gm daily in divided doses, orally, for 14 to 42 days (77). Three patients with pulmonary disease improved, one patient with synovial involvement did not appear to improve but died of other causes,and the cultures were negative. In 9 of 11 patients with meningitis, improvement and inability to culture the microorganism was noted. During the follow-up period, relapse occurred in 4 of these 9 patients. We have treated two patients with disseminated candidiasis with apparent success.

Toxicity: 5-FC is relatively non-toxic, in comparison to amphotericin B, and has the virtue of being suited to oral use.

In the patients treated by Utz, et al., there was a fall in hemoglobin and increase in SGOT in one patient. In the patients treated by Tassel, one had no side-effects, while the second on 3.0 gm/day decreased her WBC to 3,000/mm^3 but it didnot progress despite further treatment. In the patient reported by Watkins, an increase in serum enzymes occurred. Other side-effects reported in 83 patients include alopecia (1 cs), optic atrophy (1 cs), malaise and diarrhea (1 cs), dermatitis (1 cs), leucopenia (9 cs), decreased hemoglobin (2 cs), thrombocytopenia (1 cs), elevated SGOT (7 cs) (75). Of these patients, 8 were also on amphotericin B and 5 were on antineoplastic drugs. The patient with eye damage had papilledema for one month before 5-FC was started.

The optimal dosage has not been established but current recommendations are 50 to 150 mg/kg/day, divided into 4 doses, not to exceed a course of 90 days.

5-fluorocytosine shows considerable promise as an agent which can be used in the treatment of candidiasis and cryptococcosis and possibly aspergillosis in patients with renal insufficiency. Data on the pharmacology of 5-FC in patients with renal insufficiency is not available as yet, but dosage modification would seem reasonable.

II. General Considerations Regarding Older Antimicrobials

A. Causes of Failure of Chemotherapy

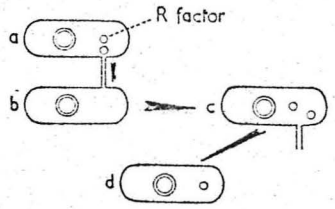
As outlined by Crafton, the following have to be considered: (1) bacterial drug resistance, (2) bacterial "persistence", (3) poor host defenses, (4) poor drug absorption, (5) drug inactivation by the host's protein or flora, and (6) poor penetration of the drug into tissues or cells (ref. B). Discussion of all aspects of failure of therapy is beyond this presentation, but review of recent knowledge may be of interest.

Bacterial Drug Resistance: The basis by which a bacterium becomes resistant to a given antibiotic has been assumed to result from a mutation in the chromosome of that cell. Such an event occurs spontaneously at frequencies of approximately 1 in 10^8 cell divisions. The mutation is perpetuated at the replication of the involved cell, the drug resistant phenotype being expressed by the daughter bacteria. Since different (families of) antibiotics, e.g., tetracyclines, chloramphenicol, affect bacteria by different mechanisms, a mutation to resistance to a given antibiotic does not affect the susceptibility of that cell to other antibiotics; resistance to multiple antibiotics develops as a consequence of separate mutations and is a rare event. Thus, resistance to two antibiotics occurs at a frequency of 1 in approximately 10^{16} cell divisions; that to three antibiotics, at a frequency of 1 in approximately 10^{24} cell divisions.

Observations made during epidemic shigellosis in Japan (1959) that not only were the responsible microorganisms resistant to four major antibiotics but also that the resistance was transferable to bacteria of different species were the stimuli for most of the research of the past decade on this problem. The early work in this area was summarized in 1963 by Watanabe (79). The mechanism of transfer of resistance involved conjugation which consists of the transfer of genetic material by physical

contact between individual bacterial cells by means of a cytoplasmic bridge or "pilus" (Figure 16).

FIGURE 16



At present it is uncertain whether genetic material actually passes through the pilus or whether the pilus merely acts as a "grappling hook" allowing the formation of a cytoplasmic bridge elsewhere in the cell. In R factor type of conjugation, a cytoplasmic episome (resistance determinant) which is responsible for multiple drug resistance replicates itself within the cytoplasm and the resistance transfer factor (RTF) also replicates and induces the formation of a pilus which allows transfer of an R factor to a previously sensitive female type cell. This makes the recipient cell resistant and also induces the formation of a pilus in this cell, rendering it a male. The interbacterial transfer and expression of the R factor is complete within minutes in in vitro cultures, and 90% of sensitive bacteria are "infected" by R factors within 2 to 4 hours after the introduction of a few bacteria bearing the R factor (R^+ bacteria). There has been some confusion in the literature concerning terminology, but it seems to be resolved that the episome, which is responsible for the transmission of drug resistance between bacteria, is termed an R factor. It is composed of DNA autonomous of chromosomal DNA. The R factor seems to be composed of two entities, the resistance determinants and the resistance transfer factor (RTF) such that the R factor without the RTF can produce resistance but it cannot be transferred to other cells; the R factor without the resistance determinants can be transferred but exhibits no ability to cause resistance (80). There is evidence that the resistance determinants for the various antibiotics can themselves be transferred independently (79).

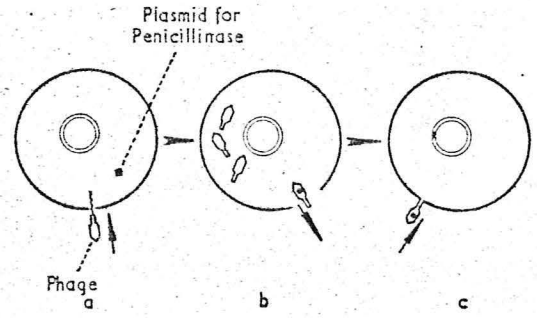
At the time of Watanabe's review there had accumulated a number of suggestions in the literature that a defect in permeability to the antibiotics was a prime mechanism of expression of resistance. The reasoning was as follows: chloramphenicol, for instance, inhibited protein synthesis in a sensitive strain but not a resistant strain of E. coli. However, chloramphenicol was equally effective in inhibiting protein synthesis in lysates of both strains. It was thus suggested (81) that in the resistant strain there was a permeability barrier which did not permit the chloramphenicol to reach the protein synthesizing apparatus. However, the demonstration by Okamoto and Suzuki (82) that cell-free systems could inactivate antibiotics if suitable cofactors were added obviated the need for a permeability defect explanation in R factor-mediated resistance since inactivation of the chloramphenicol adequately explained the above observations. Chloramphenicol, streptomycin, kanamycin, neomycin, ampicillin and dihydrostreptomycin have all been found to be inactivated by enzymes from R factor-bearing microorganisms and the products have been characterized.

R factors mediate resistance to streptomycin by one of two mechanisms, adenylation or phosphorylation of the 3'-OH of the N-methyl-glucosamine moiety (82,83). The adenylated or phosphorylated streptomycin is biologically inactive. Smith has found two R factor-mediated phosphorylases, one specific for streptomycin, the other

specific for kanamycin and neomycin. The streptomycin phosphorylase has *in vitro* and *in vivo* activity against gentamicin; however, the *in vivo* gentamicin resistance thus far is lower than concentrations clinically attainable. Unfortunately, such R factors can mutate at high frequencies to high levels of streptomycin and presumably gentamicin resistance. Chloramphenicol resistance appears to be due to enzymatic acetylation (84). *E. coli* and klebsiella-enterobacter which are kanamycin-resistant are usually resistant to 4 to 7 other drugs, and in particular chloramphenicol, due to the linkage of the loci mediating kanamycin and chloramphenicol resistances. Resistance to ampicillin, through the enzyme penicillinase, is also R factor-mediated (85). Smith demonstrated that resistance to ampicillin was often transferable whereas that to cephalothin was not.

In addition to transfer of resistance by conjugation which appears limited to bacilli, genetic material may be transferred by transduction. This consists of the transfer of genetic material conferring resistance from a resistant to a sensitive strain by means of bacteriophage (Figure 17).

FIGURE 17



This has been shown particularly with staphylococci. The phage may infect a coccus containing a cytoplasmic plasmid capable of inducing, for instance, penicillinase formation (86).

Whereas R factor-mediated resistance to the antibiotics mentioned above has been ascribed to inactivation of the antibiotic, such has not been demonstrated for tetracycline (87). Arima and Izaki demonstrated that an R factor-bearing strain of *E. coli* had a greatly reduced uptake capacity for tetracycline and postulated a permeability defect (88).

This mechanism of antibiotic resistance differs notably from that of the classic model in that (1) acquisition of drug resistance is not random or spontaneous but occurs only through contact of sensitive and R^+ bacteria; (2) multiple resistance is acquired at a single, rapid event; (3) multiple resistance to antibiotics can be "infectious"; (4) the transmission of multiple antibiotic resistance can occur between all genera of enteric bacteria; (5) the genetic apparatus responsible for the multiple antibiotic resistance is self-regulated and can be eliminated from the host bacterium without affecting bacterial survival.

The potential spread of R factors among enteric bacteria was immediately recognized as a threat. Concern increased during the ensuing years as R factors were associated with the rapid emergence of antibiotic-resistant shigella in Japan (89), salmonella and *E. coli* in Great Britain (90), and were found to play the major role in the mediation of antibiotic resistance among enteric bacteria (associated

with nosocomial infections) in the United States (91). Recent studies indicate that R factors now infect approximately 85% of all shigella in Japan (89), 60% of the salmonella isolated in England (92), and 20% of those in the United States (93,94), 25% of all enteric bacteria and 60 to 70% of all resistant enteric bacteria isolated at medical centers in the United States (91,95).

Less is known about the epidemiology and clinical role of R factors, however. The prevalence of R factors has been correlated generally with the commercial usage of antibiotics in Japan (89), and it was originally presumed that R factors arose solely because of the selective force of antibiotics used by man. This thesis was found to be an overstatement since R factors have been found in bacteria isolated before the antibiotic era (96). That antibiotics play a major role in the selection of R^+ bacteria, however, has been demonstrated by preliminary experiments (89,97).

Despite the rapidity of interbacterial transfer in in vitro cultures, R factor transfer in nature may be much less frequent than originally anticipated. Smith demonstrated antibiotic resistance could be transferred to the resident E. coli in the alimentary tract of a volunteer when cultures of E. coli of animal and human origin were taken in large doses (98). The amount of transfer was small and the resistant resident organisms did not persist. Similarly, Jarolmen and Kemp induced experimental infections in weanling pigs with both nalidixic acid sensitive and resistant strains of S. cholera suis (99). Transfer of drug resistance in vivo was rare.

B. Combinations of Antibiotics and Antibiotic Combinations

Within the past months there has been a resurgence of interest as to the role which fixed combinations of antimicrobial agents should have in medical practice. As background, the 1962 amendments to the Federal Food, Drug and Cosmetic Act of 1938 fixed requirements as to efficacy as well as to safety of drugs. To implement these amendments, the FDA sought the assistance of the National Academy of Sciences-National Research Council in reviewing the evidence for drug efficacy (11).

In a consideration of fixed combinations of antimicrobials, it is advantageous to review the potential advantages and disadvantages for combined antimicrobial therapy. There are five potential advantages to treatment with more than a single antimicrobial agent.

Under specific circumstances which depend upon the type of drug, its mode of action, specific dosage and phase of bacterial growth, synergistic or enhanced antimicrobial activity can be demonstrated. The classic example of this is the usefulness of penicillin and streptomycin in the treatment of bacterial endocarditis due to enterococci (group D streptococci) (100). Another example of enhanced although not synergistic efficacy is the combined chloroquine-primaquine prophylaxis employed particularly against strains of Plasmodium vivax. In this situation the chloroquine is effective against the erythrocytic schizont, while the primaquine is active against the secondary tissue schizont (101). Thus, the combination is effective against both phases of the life cycle of the plasmodia in man.

Delay in the development of resistance is a second potential advantage to combined therapy. When antimicrobial resistance is dependent upon spontaneous mutation, the simultaneous spontaneous mutation at two genetic loci is infinitesimally smaller than at one. Unfortunately, recent evidence suggests that resistance, particularly among gram-negative bacteria, against many antimicrobials can be transferred en masse

through episomal resistance factors (102). The classic example of combined therapy resulting in delay in the appearance of resistant organisms lies in the treatment of infections due to Mycobacterium tuberculosis.

Combined antimicrobial therapy may permit reduction in dose of a potentially toxic drug. Another circumstance where combined antimicrobial therapy has major potential advantages is the circumstance where there are mixed infections in which all of the organisms are not susceptible to a single antimicrobial agent. For example, in peritonitis following fecal contamination of the peritoneal cavity, multiple organisms including coliform organisms such as Escherichia coli, anaerobic non-sporulating gram-negative rods such as bacteroides, and fecal streptococci may be involved. While the aminoglycoside antibiotics, e.g., kanamycin, are effective against coliform organisms, they are ineffective against bacteroides. Agents which are effective against bacteroides include tetracycline, penicillin in high dosage, cephalothin in high dosage, and possibly some of the newer agents (103,104).

Finally, more than a single antimicrobial agent is often employed in the patient with a life-threatening infection where it is not possible on clinical grounds to reasonably predict the specific bacterial etiology or where even if predicted, the antimicrobial sensitivities are variable. In the patient with gram-negative bacillary bacteremia related to an indwelling urethral catheter, a number of gram-negative organisms may be involved and combinations of agents effective against gram-negative bacilli such as kanamycin and colistimethate or colistimethate and cephalothin are often employed pending the results of cultural studies (105).

There are a series of potential disadvantages which can accompany the use of more than a single agent. These include the possibility of antagonism between the agents, enhanced toxicity and a false sense of security. Clinical evidence of antagonism has been best illustrated in the observations on the treatment of pneumococcal meningitis with penicillin and parenteral tetracycline (106,107). The lessened mortality in children with meningitis treated with ampicillin in contrast to "triple therapy" with penicillin, chloramphenicol and sulfonamide is consistent with the earlier observations (108). Additional reports include antagonism between tetracycline and penicillin in scarletina and in combined therapy in urinary tract infections (109, 110). However, antagonism of clinical significance has been difficult to demonstrate and must be relatively infrequent. The most important potential disadvantage is that of enhanced toxicity, due to the inherent toxicity of each agent in the combination. In addition, "covering the possibilities" has the potential serious disadvantage of resulting in a false sense of security.

Despite these potential disadvantages, in certain of the situations outlined earlier, the potential advantages appear to outweigh the disadvantage and combined antimicrobial therapy is utilized.

It is against this body of information that the fixed combinations of antimicrobial agents should be evaluated. The common preparations which are in relatively wide usage include large numbers of combinations of penicillin with streptomycin, penicillin with sulfonamides, tetracycline with novobiocin, novobiocin with a sulfonamide, and tetracycline with antifungal agents such as mycostatin or amphotericin B.

The potential advantages for fixed dosage antibiotic combinations are listed as those for combined antimicrobial treatment with variable dosage of independent agents. Thus, the justifications usually stated include treatment of mixed bacterial infec-

tions, enhancement of antibacterial activity and treatment of infections before the etiology is known or in instances where it is impossible to determine etiology. The data in support of these claims are limited (111,112). Weinstein, Samet and Chew have reported that under some circumstances a "broad-spectrum" effect is produced when sulfonamide-penicillin mixtures are given (113). However, the degree of antibacterial activity is generally unpredictable and antimicrobial effectiveness is often decreased by this type of therapy. In the treatment of life-threatening infections before the etiology is known, certainly oral sulfonamide-penicillin mixtures would not be employed inasmuch as parenteral therapy would be indicated. A possible exception is the use of penicillin-sulfonamide combinations in the treatment of acute otitis media. Group A streptococci, pneumococci and Hemophilus influenzae are the common bacterial causes of acute otitis media. However, Hemophilus influenzae only rarely causes otitis media after the age of 4 years. Because H. influenzae, group A streptococci and pneumococci cause otitis media during the first years of life, it has been common practice to use sulfonamide-penicillin combinations in this age group. Data to support this regimen are minimal and, indeed, recent in vitro studies indicate that sulfonamides in the commonly used triple formulation inhibited only 20% of the typable and 40% of the non-typable strains of H. influenzae at concentrations within the usual therapeutic range (114). Also, the results of treatment of otitis media by the intramuscular injection of a mixture of benzathine, procaine and crystalline penicillin G were comparable to those with the same penicillin mixture in combination with an oral triple sulfonamide mixture (115).

Recently a report has appeared in which the frequency of gastrointestinal distress following the administration of tetracycline alone was compared with that of a tetracycline-antifungal (mycostatin) combination. While the tetracycline-antifungal combination was associated with less frequency of candida in the stool, it was not associated with a decreased frequency of gastrointestinal disturbance (116). Thus, even this combination can be questioned.

In the face of these limited potential advantages, there are serious potential disadvantages. These potential disadvantages are essentially the same as noted with combination of antimicrobial agents. In addition, there is the major disadvantage that in fixed combination, the patient usually receives an inadequate dosage of one of the components or an excessive dosage of the other, the latter situation being associated with increased toxicity. For example, penicillin and streptomycin are recommended for the management of enterococcal endocarditis; however, only 1 of 17 preparations of penicillin-streptomycin examined would provide 2.4 million units of penicillin per day at the same time 2 gm of streptomycin per day would have to be administered. This is far less than the recommended dose of penicillin for enterococcal endocarditis. In patients with peritonitis, penicillin and streptomycin has been employed and there is experimental evidence to support its efficacy (117). Again, the amount of penicillin required to be effective against bacteroides would require the administration of toxic amounts of streptomycin to achieve optimal effectiveness. The tetracycline-novobiocin mixture widely utilized incorporates 125 mg of novobiocin with 250 mg of tetracycline as a means of increasing the breadth of antimicrobial therapy. While novobiocin is an effective antistaphylococcal agent, the usual dosage administered in this combination, i.e., 125 mg four times daily or 500 mg per day, is suboptimal. Hirsch and Finland demonstrated that the administration of 500 mg of tetracycline with 250 mg of novobiocin resulted in lower antibacterial activity in the serum against a tetracycline-resistant staphylococcus as compared with the dose of 250 mg of novobiocin alone (118).

It is because of these potential disadvantages that fixed-dosage combinations of antibiotics have been decried by many (119). Despite skepticism regarding the need for fixed-dosage antibiotic combinations, there are possibilities on the horizon for additional indications of combined therapy and even antimicrobial combinations. There is some evidence that the polymyxins may enhance the antimicrobial action of sulfonamides (120). Also, the simultaneous administration of penicillinase-resistant penicillins which will tie up penicillinase may enhance susceptibility to the penicillinase-susceptible penicillins such as ampicillin. Thus, combinations of oxacillin and ampicillin may be effective against organisms such as Escherichia coli which would either be not affected by either agent alone or require very large doses (121).

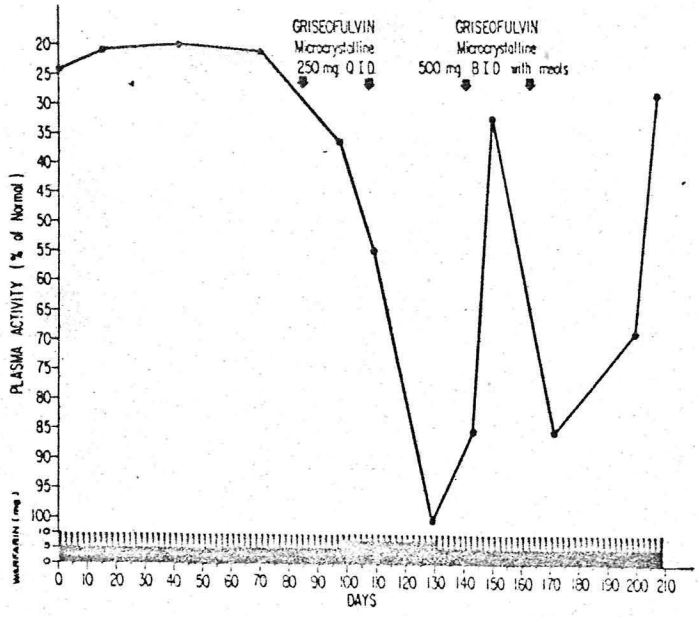
Physicians should look very critically at the need for fixed combinations of antimicrobial agents from the critical scientific evidence rather than emotionally reacting against what has been interpreted as bureaucratic intervention.

C. Drug Interaction-Enzyme Induction

The phenomenon of enzyme induction was accidentally discovered by Hart, et al. (122). They were studying the effects of starvation on hexabarbital metabolism in rats. In one group, microsomal enzyme activity was higher than anticipated with a consequent decrease in hexobarbital sleeping time. It was later discovered that this group of rats had been exposed to the insecticide chlordane, used to spray their cages. Many drugs are known to cause enzyme induction in animals. Awareness of the possibility of enzyme induction must be considered with many drug combinations, especially sedatives and anticoagulants (123). From the standpoint of antimicrobials, significant interactions may be seen.

Metabolism of both griseofulvin and coumarins is increased by treatment with phenobarbital. Hence, it seemed that one might act as an inducer to accelerate metabolism of the other. Cullen and Catatano demonstrated such to be the case (124) (Figure 18). This was observed in 3 of 4 subjects.

FIGURE 18



Diphenylhydantoin (DPH) is normally hydroxylated and conjugated with glucuronic acid in the liver (125). A daily dosage of 300 mg results in blood levels of 5 to 15 μg/ml. Blood levels of > 20 μg/ml are associated with nystagmus, ataxia and obtundation. Kutt, Winters and McDowell reported 8 epileptic patients who developed tuberculosis and then developed diphenylhydantoin intoxication 3 to 6 weeks after they were started on INH and PAS (125). Blood levels in these patients were > 20 μg/ml. The intoxication was associated with a low output of metabolites indicating depression of diphenylhydantoin metabolism. Folic acid (20 mg daily) stopped the accumulation, or the dose of DPH could be decreased since a lowered intake still protected against seizures.

III. Specific Comments About Older Antimicrobials--"Pearls or Graffiti?"

1. The question is often posed: at what *in vitro* sensitivity levels can one expect "sensitivity" or "resistance", assuming the drug is absorbed, etc.? Following are the values provided by Sherris at the University of Washington. These would hold reasonably for the agar dilution technique employed at PMH. Strains between "sensitive" and "resistant" are indeterminant (Table 21) (126).

TABLE 21

Approximate MIC Values for Breakpoints Using the Kirby-Bauer Diffusion Sensitivity Technique With Commonly Used Antibiotics

Antibiotic	Sensitive	Resistant
Penicillin	0.5 U/ml or less	2 U/ml or more
Ampicillin		
Gram-Negative	5-15* μg/ml or less	40 μg/ml or more
Staphylococci	0.5 μg/ml or less	
Cephalothin	10 μg/ml or less	32 μg/ml or more
Cephaloridine	10 μg/ml or less	40 μg/ml or more
Methicillin	2.5 μg/ml or less	
Tetracycline	5 μg/ml or less	12.5 μg/ml or more
Chloramphenicol	10 μg/ml or less	30 μg/ml or more
Erythromycin	1 μg/ml or less	25 μg/ml or more
Kanamycin	6.2 μg/ml or less	30 μg/ml or more
Lincomycin	2 μg/ml or less	
Neomycin	10 μg/ml or less	
Streptomycin	6 μg/ml or less	15 μg/ml or more
Polymyxin	Mean 12.5 U/ml or less	
Thiosulphil	10 mg.% or less	35 mg.% or more
Nitrofurantoin	50 μg/ml or less	100 μg/ml or more

* Depending on dilution method used

2. Penicillin G versus Penicillin V: It has usually been assumed that the action of penicillin V is comparable to that of penicillin G. Such is the case for group A streptococci and pneumococci (127). However, neisseria, both meningitidis and gonorrhoeae, are two to four dilutions less sensitive to penicillin V than penicillin G. While routine penicillin prophylaxis for meningococcal contacts is not usually recommended by us, if penicillin were to be given penicillin G would be preferable. Also, if oral penicillin were used in the treatment of gonorrhea (again not recommended), penicillin G would be preferable.

3. Chloramphenicol: Chloramphenicol probably gives rise to 2 forms of bone marrow toxicity. One is an acute change which affects most patients when blood levels rise above 25 μg/100 ml. WBC, platelets and RBC are depressed. These changes are readily and rapidly reversed when the drug is discontinued. Chloramphenicol may also give rise to aplastic anemia, which is usually fatal. Fatal aplastic anemia seemed 13 times more common after the use of chloramphenicol than in the untreated population (128). Wallerstein, et al. assessed the risk of dying as 1:36118 with an average dose of 4.5 gm and 1:21671 with an average dose of 7.5 gm. However, fatalities have been reported after a dose of 2.0 gm or even following the use of chloramphenicol eye drops on an average of two of three days over 23 months (129). Of note, this patient's niece had died of aplastic anemia after chloramphenicol. Nagao and Mauer recently reported the development of aplastic anemia in identical twins exposed to chloramphenicol (130). Studies suggested that the defect was a failure of delivery of differentiated forms from a damaged undifferentiated stem cell. Although the underlying biochemical changes are unknown, it seems probable that a genetically determined defect was responsible; this would also account for the observations after the prolonged use of the eye drops.

4. Skin rash with infectious mononucleosis and ampicillin: The incidence of skin rash in infectious mono is about 4%, yet in several series 95 to 100% of patients with infectious mono developed a skin rash 5 to 8 days after ampicillin (250 mg qid even for 1 to 2 days). None had histories of penicillin allergy! (131,132).

5. Kanamycin toxicity: The volume of distribution for kanamycin is similar to extracellular volume (19% of body weight). A dose of 7 mg/kg will give therapeutic blood levels. Kanamycin half-life could be estimated by multiplying the serum creatinine concentration (mg.%) by 3. Repetition of the loading dose every 3rd half-life will result in therapeutic, nontoxic serum levels in patients with renal failure (133). These data need to be confirmed, but look like most useful guidelines.

6. Increased intracranial pressure and papilledema after tetracycline or nalidixic acid (Negram): The complication is not related to dose or duration of therapy, being seen after 5 hours to 4 days of tetracycline. Cessation of tetracycline is followed by disappearance of signs in a few hours to 3 days (134,135). Awareness may avoid unnecessary neurosurgery.

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