24-hour period. The tubular reabsorption of solute-free water for the six days of therapy was calculated to be 315 ml./24 hours. Sodium excretion decreased from an average control value of 78 mEq/24 hours to 11 mEq on the sixth day of diazoxide therapy. The administration of diazoxide had no apparent effect on endogenous creatinine clearance, indicating that the fluid retention was not due to a reduced glomerular filtration rate. Blood-pressure fell from an average of 156/105 mm. Hg during the control period to 128/88 mm. Hg by the third day of therapy.

Discussion

Previous observations have indicated that the antihypertensive property of chlorothiazide and its congeners was not directly due to a natriuretic effect. Hollander *et al.* (1959), for example, reported that chlorothiazide maintained a lowered blood-pressure in hypertensive patients treated with fludrocortisone (9α-fluorohydrocortisone) during a period of positive sodium balance. In the present study the fall in blood-pressure produced by diazoxide was also accompanied by sodium retention. These results therefore support the view that a depletion of total body sodium is not the primary mechanism responsible for the decrease in blood-pressure caused by the benzothiadiazine drugs. The decrease in vascular reactivity which has been observed during the administration of chlorothiazide (Mendlowitz *et al.*, 1960) may therefore be due to a direct inhibitory effect on the peripheral arteriole.

In addition to causing sodium retention, diazoxide was observed to decrease solute-free water clearance. Taylor *et al.* (1961) also noted a reduction in free water clearance after diazoxide in dogs during water diuresis. This decrease in free water clearance is of interest in relation to the mechanism of the sodium and water retention during diazoxide therapy. The benzothiadiazine diuretics have been shown to decrease free water clearance in dogs (Earley *et al.*, 1961), control subjects (Heinemann *et al.*, 1959; Takasu and Hutcheon, 1960), and cases of nephrogenic diabetes insipidus (Wenzl *et al.*, 1961). Calesnick and Brenner (1961) reported that chlorothiazide reduced free water clearance in diabetes insipidus by an action similar to that of the antidiuretic hormone. It is therefore possible that diazoxide shares this effect on the tubular reabsorption of solute-free water with the chlorothiazide diuretics, while it lacks their natriuretic action. Increased tubular reabsorption of sodium would then be expected during this state of water retention to maintain the osmolal concentration of the plasma within normal limits.

The fluid-retaining property of diazoxide was antagonized by thiazide diuretics. This observation indicated that the occurrence of salt and water retention should not prevent the clinical development of diazoxide as a useful antihypertensive agent.

Summary

Metabolic balance studies were undertaken in seven hypertensive patients to evaluate the action of diazoxide, a new chlorothiazide congener. Three additional cases were treated under general ward conditions.

In contrast to chlorothiazide, diazoxide reduced urine volume and renal sodium excretion. An increase in total blood-volume was observed during diazoxide therapy and the patients gained weight. Some patients developed ankle oedema when dietary sodium was not restricted.

In spite of its antidiuretic effects, diazoxide caused a significant drop in systolic and diastolic blood-pressure. Chlorothiazide diuretics such as trichlormethiazide antagonized the sodium-retaining properties of diazoxide without interfering with its antihypertensive action.

The results of this study support the view that the antihypertensive action of chlorothiazide and its congeners is independent of their effects on renal sodium excretion.

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"COLOMYCIN"—LABORATORY AND CLINICAL INVESTIGATIONS

BY

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"Colomycin" ("colistin") is an antibiotic originally isolated by Koyama *et al.* (1950) from the micro-organism *Bacillus colistinus*. Although only recently introduced into this country, colomycin has been available elsewhere for some time, and reports from Japan, Italy, France, and the United States have claimed it to be an effective antibiotic against a wide range of Gram-negative organisms. The antimicrobial spectrum of colomycin closely resembles that of polymyxin B, but the methane sulphonate of colomycin is reported to have fewer toxic effects than unsubstituted polymyxin B.

We report the results of laboratory and clinical studies with colomycin methane sulphonate.

Materials and Methods

The organisms examined in the laboratory study consisted of 227 recently isolated pathogens. In each case the minimum inhibitory concentration (M.I.C.) of colomycin was determined, using a serial dilution tube technique. In addition a disk sensitivity was carried out using 8-mm. blotting-paper disks impregnated with

40,000 units of colomycin methane sulphonate. The unit is a biological unit used by the manufacturers, and 12,500 units are equivalent to 1 mg. of colomycin methane sulphonate. In the M.I.C. determination serial doubling dilutions of colomycin were made in 0.5-ml. amounts of an assay broth consisting of nutrient broth No. 1 ("oxoid") with added 0.2% glucose and 0.5% Andrade's indicator. The tubes were each inoculated with 0.02 ml. of a 1 in 1,000 dilution of an overnight broth culture of the bacterium under test and were then incubated for 18 hours at 37° C. The M.I.C. was taken as the minimum concentration of colomycin which prevented visible growth.

The bactericidal activity of the antibiotic was determined by subculturing from the tubes used in the M.I.C. determinations containing ×1, ×2, ×4, and ×8 M.I.C. of colomycin. The subcultures were incubated for 48 hours and the bactericidal concentration was the least concentration from which no organisms could be isolated. In addition a more precise examination of the bactericidal activity was carried out. To four series of tubes containing ×½, ×1, ×2, and ×4 M.I.C. of colomycin in assay broth was added a suspension of organisms to give a final concentration of 10^4 to 10^5 bacteria per ml. At zero and at intervals of from half an hour to 24 hours a viable count was determined by the method of Miles *et al.* (1938).

Attempts to induce resistance were made by growing the organism in broth containing a sub-inhibitory concentration of colomycin and subculturing to a fresh series of colomycin-containing broth every one to two days. In all, six organisms—four strains of *Pseudomonas pyocyanea* and two of *Escherichia coli*—were treated in this way for up to 24 days.

Colomycin was used to treat 13 patients suffering from active infections due to *Ps. pyocyanea*. These comprised six patients with infections of the urinary tract and seven cases of wound or other infection due to the same organism. The dose used varied from 3,000,000 to 6,000,000 units a day by intramuscular injection. The course of antibiotic treatment ranged from 5 to 15 days, and appropriate specimens for culture were taken daily during this period. The M.I.C. of the organisms isolated from the specimens was determined in order to see if resistance had developed during treatment.

Results

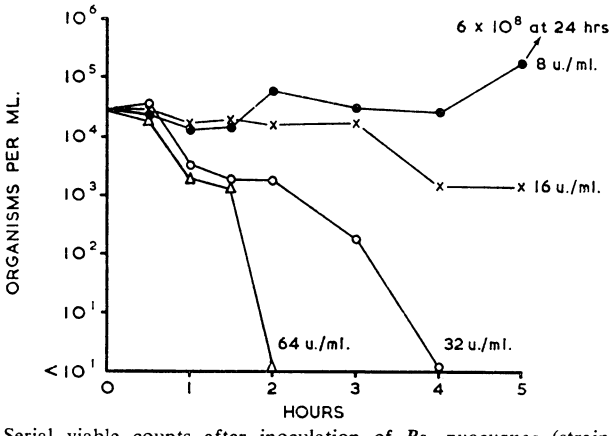
Antibacterial Activity

The results of the M.I.C. determinations are presented in Table I. The Gram-positive organisms tested proved to have very high M.I.C.s while with the exception of *Proteus* and *Providencia* most of the Gram-negative bacilli had M.I.C.s of 64 u./ml. or less. Fifteen strains of *Proteus* were tested against higher concentrations of colomycin than are shown in Table I. Most strains were not inhibited by a concentration of 2,500 u./ml.—indeed, several strains would tolerate 25,000 u./ml. The M.I.C.s of strains of *Ps. pyocyanea* varied from 2 to 128 u./ml. and fell into the range which was thought to indicate sensitivity. With the disk technique on 100 organisms, zones of greater than 15-mm. diameter were produced from organisms giving M.I.C.s of 128 u./ml. or less. By using disks containing 500 u. of polymyxin B in parallel it was found that the antibacterial spectra of the two antibiotics exactly corresponded. Correspondence between colomycin and other antibiotics was not found.

By using the relatively crude test for bactericidal activity of subculturing from the tubes used in the determination of the M.I.C. it was found that 17 out of the 23 strains examined were killed by concentrations of four times the M.I.C. or less. The results of counting viable organisms after incubation for various times in different concentrations of colomycin are shown in the Chart. It will be seen that the test strain of *Ps. pyocyanea* was killed by twice the M.I.C. in four hours and by four times the M.I.C. in two hours.

TABLE I.—*Distribution of the Minimum Inhibitory Concentrations of Colomycin by Species*

Organism	No. of Strains Tested	No. of strains having M.I.C. (units/ml.) of:								
		>256	256	128	64	32	16	8	4	2	1
Staph. pyogenes	35	25	10								
Str. faecalis ..	20	20									
Proteus sp. ..	31	30	1								
Providencia ..	2	2									
Escherichia ..	54	1		4	14	23	8	3			1
Klebsiella ..	10		1		6	1	1	1			
Shigella sp. ..	9						2	1	2	2	2
Salmonella sp. ..	9		1		2	1	4	1			
Ps. pyocyanea ..	51			2	9	17	14	4	3	2	
Cloaca	1		1								
Serr. marcescens	1	1									
Achromobacter sp.	4			1	1	2					



Serial viable counts after inoculation of *Ps. pyocyanea* (strain T/402) into broths containing four different concentrations of colomycin. The M.I.C. of strain 402 under the same conditions was 16 u./ml.

Resistance to colomycin proved to be difficult to induce. Of the six organisms used only one strain of *Ps. pyocyanea* showed any significant change in M.I.C. After nine days' serial subcultures the M.I.C. increased more than 32-fold and an associated increase in the M.I.C. of polymyxin B occurred. None of the strains re-isolated during the course of treatment showed any significant change in sensitivity.

Clinical Trial

The results of using colomycin to treat cases of pseudomonas infection are shown in Table II. In 9 of the 13 cases *Ps. pyocyanea* was eliminated after four days' treatment or less. In two of the failures the infection was associated with considerable necrotic malignant tissue, and the other cases were respectively a terminal chest infection and an infection of a large skin defect following closure of an arteriovenous fistula.

Only one minor toxic effect was noted in this series of cases. In one patient a maculo-papular skin rash involving the lower limbs occurred five days after starting treatment with colomycin. This rash cleared three days after stopping the antibiotic. As the patient was receiving other drugs at the same time the rash may not have been due to colomycin. No other general toxic effects were noted. Repeated estimations of blood urea

TABLE II.—*Summaries of Clinical Results*

No.	Sex and Age	Diagnosis	Infecting Organism	Colomycin (units/day)	Bacteriological Results	Clinical Results
1	M 74	Post-prostatectomy urinary tract infection	*Ps. pyocyanea* *Staph. pyogenes*	3 million* for 6 days	Infecting organisms cleared in 3 days. Thereafter *Proteus* was cultured	Residual *Proteus* infection
2	F 65	Wound infection after biopsy secondary carcinoma in femur	*Ps. pyocyanea*	3 million for 5 days	*Ps. pyocyanea* persisted	Failure—died after 5 days with carcinoma bronchus
3	M 34	Chest infection, tracheostomy for emphysema	,,	3 million for 5 days	,, ,,	Failure—died after 5 days of cor pulmonale
4	F 9	Subphrenic abcess, ruptured liver	,,	3 million for 15 days	*Ps. pyocyanea* eliminated in 3 days	Cure
5	F 49	Post-operative wound infection	,,	3 million for 6 days	*Ps. pyocyanea* eliminated in 4 days	,,
6	M 40	Post-operative urinary tract infection	,,	3 million for 6 days	*Ps. pyocyanea* cleared in 24 hours	,,
7	M 71	Urinary tract infection, prostatic hypertrophy	,,	4·5 million for 5 days	,, ,,	,,
8	M 34	Varicose ulcer to be skin-grafted	,,	6 million for 8 days	*Ps. pyocyanea* eliminated in 2 days	Cure ; graft successful
9	M 55	Wound infection, biopsy carcinoma of mouth	,,	4·5 million for 9 days	*Ps. pyocyanea* persisted	Failure
10	M 19	Wound infection, closure of A-V fistula	,,	4·5 million for 8 days	,, ,,	,,
11	F 80	Urinary tract infection; fracture femur	,,	4·5 million for 8 days	*Ps. pyocyanea* cleared in 4 days. Thereafter *Proteus* cultured	Residual *Proteus* infection
12	F 36	Urinary tract infection vesico-lithotomy	,,	4·5 million for 5 days	*Ps. pyocyanea* eliminated in 24 hours	Cure
13	M 81	Urinary tract infection benign prostatic hypertrophy	,,	4·5 million for 6 days	*Ps. pyocyanea* eliminated in 24 hours. Thereafter *Proteus* cultured	Residual *Proteus* infection

*Plus erythromycin 1 g. day for six days.

and the absence of casts on frequent microscopy of the urine suggest that the antibiotic did not produce renal damage. Absence of significant change in the cellular elements of the blood as shown by frequent blood counts would indicate absence of toxic effect on the haemopoietic system, at least on short-term administration. On direct questioning there were no complaints of pain following the intramuscular injections.

Reinfection with *Proteus* sp. was found in three of the nine cases from which *Ps. pyocyanea* was cleared. All three were cases of long-standing infection of the urinary tract, and one patient had an indwelling catheter. In no case was *Proteus* isolated from the pre-treatment urine specimen.

Discussion

This study shows that colomycin is an antibiotic having *in vitro* activity again Gram-negative bacilli with the notable exceptions of *Proteus, Providencia,* and occasional strains of *Escherichia* and *Klebsiella.* It is bactericidal at concentrations only slightly higher than those required to inhibit growth, and resistance to this antibiotic does not develop rapidly either *in vitro* or *in vivo.* These findings are in agreement with the results of previous workers (Forni and Guidetti, 1956; Chabbert, 1958; Petersdorf and Hook, 1960; Courtieu *et al.,* 1961).

In the limited clinical trial reported here colomycin appears to be very effective in eliminating *Ps. pyocyanea* from various sites. In all four cases in which failure occurred other factors were present which militated against cure. In the presence of much necrotic tissue and in areas with a poor blood supply, any antibiotic would have been at a great disadvantage. It would seem that the main clinical indication for the use of this antibiotic will be to treat infections due to *Ps. pyocyanea* and to antibiotic-resistant strains of coliform organisms.

The main disadvantage of colomycin was the resistance of *Proteus* which resulted in reinfection with this organism during or after treatment. A similar high incidence of secondary *Proteus* infections has been reported by Petersdorf and Hook (1960), Carroll and Malette (1961), and Yow *et al.* (1961). The development of resistance to colomycin by previously sensitive organisms as a result of treatment was not noted in our series, but has been reported by Carroll and Malette (1961): this report gives insufficient detail to estimate the significance of the developed resistance.

Toxic effects so far reported appear to be infrequent and trivial. In this respect colomycin methane sulphonate has a considerable advantage over polymyxin B, which it closely resembles in antibacterial activity. This relative lack of toxicity may depend on the methane sulphonate substitution, as Clifford and Stewart (1961) have found polymyxin methane sulphonate to be much less toxic than the unsubstituted polymyxin. Colomycin does not appear to be nephrotoxic, but temporary paraesthesia of the face and mouth has been noted by Yow *et al.* (1961) and by Carroll and Malette (1961), who also record two cases of skin rash similar to the one reported here.

Conclusions and Summary

Colomycin is a bactericidal antibiotic with a range of activity against *Pseudomonas pyocyanea* and most coliform organisms. It is clinically effective in the treatment of infections due to *Ps. pyocyanea* and is thought to be the best antibiotic so far available for this purpose. The development of resistance to colomycin and toxic side-effects were insignificant.

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