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Comparative study of the effect of three antibiotics on renal function

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1. Therapeutic doses of colistin sulphomethate sodium B.P. (Colomycin), pento-N sulphomethylpolymyxin B sodium (Thiosporin) and ampicillin B.P. (Penbritin) as well as normal saline have been given to volunteers with normal kidney function.

2. A blind crossover technique was used and the effects of the compounds on blood levels and clearances of urea and creatinine studied.

3. All four treatments resulted in some changes in the parameters measured but were reversible. The most marked changes were found with pento-N sulphomethylpolymyxin B sodium and were also associated with severe side-effects.

4. In contrast to penicillins it is established that the process of tubular secretion sensitive to probenecid block plays no part in the renal excretion of polymyxins.

The effects of certain antibiotics on renal function have been the subject of numerous reports, especially when these compounds have a specific value in the treatment of urinary tract infections. The polypeptide antibiotics are mainly powerful bactericidal agents and among this group the polymyxins, such as colistin and polymyxin B are commonly used in the treatment of urinary tract infections due to certain Gram negative organisms such as *Pseudomonas pyocyanea* and *E. coli*. In the synthetic penicillin group, ampicillin has a broad spectrum, is bactericidal and is useful in the treatment of urinary infections due to *E. coli* and some strains of *Proteus mirabilis*.

Various authors have reported on the possible nephrotoxic effect of the polymyxins (Brumfitt, Black & Williams, 1966 ; Katz, 1963 ; Moyer, Mills & Yow, 1953) and it is important to assess the extent of this as these antibiotics are often used in patients who already have some degree of renal damage. The penicillins are claimed to have no effect on renal function, although some workers (Brauninger & Remington, 1968 ; London, 1967) have described the occurrence of severe haematuria and proteinuria following administration of methicillin and penicillin G. In a recent study using ampicillin in renal disease (Lee & Hill, 1968) three cases of permanent reduction in renal function following a sensitivity reaction were recorded and reduction in dosage recommended in severe kidney impairment.

Renal tract infections often have a depressing effect on renal function which improves as the condition clears during antibiotic therapy. Such changes serve to mask the actual effect of antibiotics on the kidney and as no previous work has been carried out on the effect of either colistin or polymyxin B in therapeutic doses on the normal kidney compared with a control, it was decided to investigate this and in addition to include ampicillin in the series. Some controversy has surrounded various methods for estimating renal function (Annotation, 1967) and the ones mainly used, in decreasing order of sensitivity are, inulin clearance, endogenous creatinine clearance, serum creatinine content and blood urea. In this work it was decided to determine blood creatinine and urea, and creatinine and standard urea clearances. In the event of any marked effect on renal function by any of the compounds employed it would thus have been possible to obtain an accurate measurement of glomerular filtration rate by taking a mean of the combined creatinine and urea clearances (Lubowitz, Slatopolsky, Shackel, Rieselbach & Bricker, 1967).

It has been established that penicillins are excreted by both glomerular filtration and tubular excretion (Robinson, 1964) but this has not been proved with polymyxins. Boger & Gavin (1961) suggested that there may be a tubular component involved in the excretion of colistin as serum levels of the antibiotic were increased following probenecid, but Baines & Rifkind (1964) found no such elevation. As a preliminary step these conflicting claims were re-investigated more extensively in order to establish the mechanism of colistin excretion.

Methods

Chemicals

Commercial samples of antibiotics used were obtained as follows: colistin sulphomethate sodium B.P. (C.S.M.S.) as Colomycin Lot E8733 (Pharmax Limited); pento-N sulphomethylpolymyxin sodium (S.P.B.M.S.) as Thiosporin Lot 62744 (Burroughs Wellcome & Co.); and ampicillin B.P. as Penbritin Lot F364/1 (Beecham Research Laboratories Ltd.). Probenecid for the preliminary study was supplied as Benemid (Merck, Sharp & Dohme Ltd.).

Urine and serum levels of C.S.M.S.

Biological assays were carried out using the plate method with nutrient agar seeded with *Bordetella bronchiseptica* (ATCC 4617). This procedure is sensitive to at least 1 μg/ml.

Blood urea levels

Both this and the urine urea level estimation were based on the method of Natelson, Scott, & Beffa (1951). Two ml. of heparinized whole blood plus 14 ml. of water was treated with 2 ml. of 10% w/v sodium tungstate solution and mixed. Two ml. of 2/3 N sulphuric acid was added and mixed again. The precipitated protein was filtered off through Whatman No. 4 paper after 20 min. Five ml. of the filtrate plus 5·0 ml. of water was heated in a boiling water bath for 20 min with 8·5 ml. of butanedione monoxime reagent (butanedione monoxime 0·5% w/v, sodium chloride 15% w/v) and 9·5 ml. ferric alum-acid reagent (ammonium ferric sulphate 0·33% w/v, orthophosphoric acid (wt./ml. 1·75) 33% v/v, sulphuric acid

33% v/v). The resulting yellow solution was cooled in the dark and made up to 50·0 ml. with water. Standards were prepared by appropriate dilution from a stock standard containing 2·14% w/v urea in 0·01 N sulphuric acid. The colours were measured against a reagent blank in 1 cm glass cells at 475 mμ, using a Unicam S.P.500 Series 1 spectrophotometer.

Urine urea levels

One ml. of urine was diluted to 100 ml. with water. To 1·0 ml. of the dilute solution, 9·0 ml. of water, 8·5 ml. of butanedione monoxime reagent and 8·5 ml. of ferric alum-acid reagent were added, and heated in a boiling water bath for 20 min. The resulting yellow solution was cooled in the dark, made up to 50·0 ml. with water and the optical density measured against a reagent blank in 1 cm glass cells at 475 mμ.

Blood and urine creatinine levels

These were determined according to the procedure of Owen, Iggo, Scandrett & Stewart (1954). Where Lloyds reagent is described for absorption, Fullers earth was substituted.

Clearances

Standard urea and creatinine clearance values were calculated from the blood level, the urine level and the rate of urine production, according to accepted formulae.

Microscopic examination of urine

This was performed on each sample of urine taken immediately after voiding. Examination was carried out for protein, blood, casts and cells.

Subjects and treatment protocols

(1) *Effect of probenecid on renal excretion of colistin*

Six healthy volunteers, four males (age range 18–33 yr; weight range 117–224 lb.) and two females (aged 20 and 23; weight 119 and 126 lb.) took part, each acting as his or her own control. On day 1 of the experiment probenecid 0·5 g 6 hourly was given to three volunteers followed by the same dose on day 2 together with 1,000,000 u. of C.S.M.S. at 0930 hours. The remaining three volunteers were given 1,000,000 u. of C.S.M.S. on day 2, the probenecid on both day 1 and day 2 being omitted.

Blood and urine samples were taken from all volunteers on day 2 at 1130, 1330, 1530 and 1730 hours plus overnight urine and 24 hr urine and blood samples on day 3. The reverse procedure was carried out 2 weeks later to complete crossover. C.S.M.S. levels in blood and urine were determined on all samples.

(2) *Effects of three antibiotics and saline on renal function*

Six healthy volunteers, four males (age range 18–40; weight range 117–154 lb.) and two females (aged 18 and 25; weight 110 lb. and 147 lb.) were used and each

received all four compounds randomized and at intervals according to the schedule shown in Table 1. The key to the compounds used was only known by the practitioner making up and administering the preparations, who did not take part in observing or assessing results. The materials and dosages were as follows:

Compound A: normal saline (2 ml./injection);

Compound B: ampicillin (500 mg/injection in 2 ml. normal saline);

Compound C: colistin sulphomethate sodium (C.S.M.S.) (1·5 M-u./injection in 2 ml. normal saline);

Compound D: Pento-N sulphomethyl-polymyxin B sodium (S.P.B.M.S.) (0·5 M-u./injection in 2 ml. normal saline).

During each week of treatment the appropriate compound was given on day 2 (three injections) and day 3 (one injection). This total of four injections over a 24 hr period represented the usual daily therapeutic dose for each antibiotic. Blood and urine creatinine and ureas were determined and examination of urine was carried out on days 1, 2, 3, and 4 of each treatment week and day 1 of the following week (day 8 in Results).

TABLE 1. *Treatment protocol—renal function study*

	Compound given on week							
Subject	1	2	3	4	5	6	7	8
1	B		D		C		A	
2	D		B		A		C	
3	C		A		D		B	
4	A		C		B		D	
5	B		D		C		A	
6	A		C		B		D	

TABLE 2. *Serum concentrations (μg/ml.) following 1 M-u. intramuscular C.S.M.S. with and without administration of probenecid*

	No probenecid					Probenecid				
Subject	2 hr	4 hr	6 hr	8 hr	24 hr	2 hr	4 hr	6 hr	8 hr	24 hr
1	5·9	6·0	4·6	4·1	0	5·6	5·4	3·3	3·0	0
2	6·8	4·9	3·8	9·5	0	9·1	8·4	4·0	3·6	0
3	8·0	6·8	4·4	2·8	0	10·3	8·8	6·1	5·2	0
4	8·2	8·0	5·6	4·6	0	6·0	3·6	3·2	2·2	0
5	7·3	5·4	3·6	2·8	0	8·9	8·1	7·5	4·3	0
6	6·1	4·8	3·6	2·9	0	6·3	3·9	3·1	2·8	0
Total	42·3	35·9	25·6	26·7	0	46·2	38·2	27·2	21·1	0
Mean	7·05	5·98	4·26	4·45	0	7·7	6·36	4·53	3·51	0

TABLE 3. *Urine concentrations (μg/ml.) following 1 M-u. intramuscular C.S.M.S. with and without administration of probenecid*

	No probenecid					Probenecid				
Subject	2 hr	4 hr	6 hr	8 hr	24 hr	2 rh	4 hr	6 hr	8 hr	24 hr
1	67	113	33·6	16	2·5	186	73	74	37	4·2
2	410	350	124	38·5	0·8	320	320	124	29	4·2
3	162	156	80	8·2	2·4	264	276	104	23·5	3·2
4	190	184	128	N.S.	6·0	240	224	112	15·8	1·5
5	194	94	54	34·5	1·8	84	472	188	39	3·7
6	204	192	80	18	0	104	94	108	19·8	0·7
Total	1227	1089	499·6	115·2	13·5	1198	1459	710	164·1	17·6
Mean	204·5	181·5	83·3	23·0	2·3	199·7	243·1	118·3	27·4	2·9

N.S., No sample taken.

Results

(1) *Effect of probenecid on renal excretion of colistin*

The individual serum concentrations of C.S.M.S. and their means, both with and without probenecid, are shown in Table 2 and the individual urine concentrations and their means with and without probenecid in Table 3. Figures 1 and 2 show

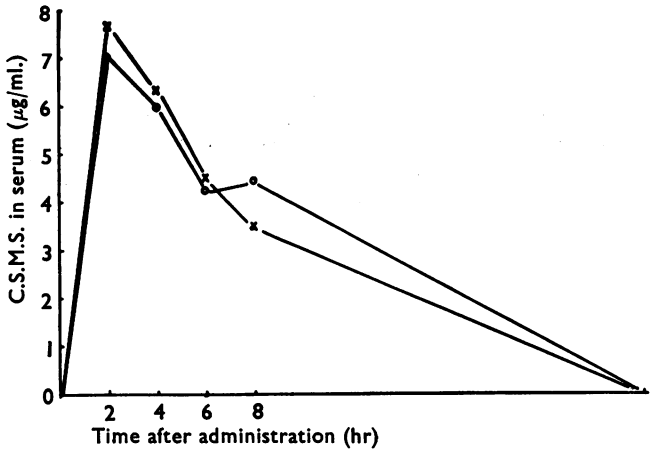

FIG. 1. Serum concentrations of C.S.M.S. following intramuscular injection of 1 M-u. ○, No probenecid ; ×, probenecid.

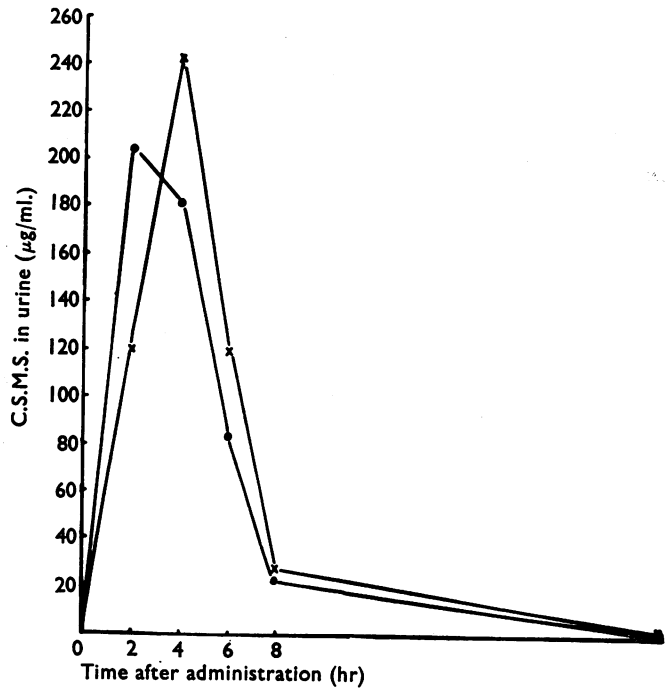

FIG. 2. Urine concentrations of C.S.M.S. following intramuscular injection of 1 M-u. ○, No probenecid ; ×, probenecid.

these results graphically. From these it can be seen that probenecid has no appreciable effect on the serum and urine levels of C.S.M.S., indicating that the process of tubular secretion sensitive to probenecid block is negligible as far as elimination of this antibiotic is concerned.

(2) *Effects of three antibiotics and saline on renal function*

The " base line " value for blood creatinine and urea and creatinine and urea clearance was taken as the day 1 estimation of each subject for each treatment week (before any compound had been given). These are shown in Table 4 together with the values for days 2, 3, 4, and 8 of each treatment week for each individual, with each of the four substances employed. The means of the individual values are also shown in Table 4 and these figures are represented graphically in Figs. 3 and 4.

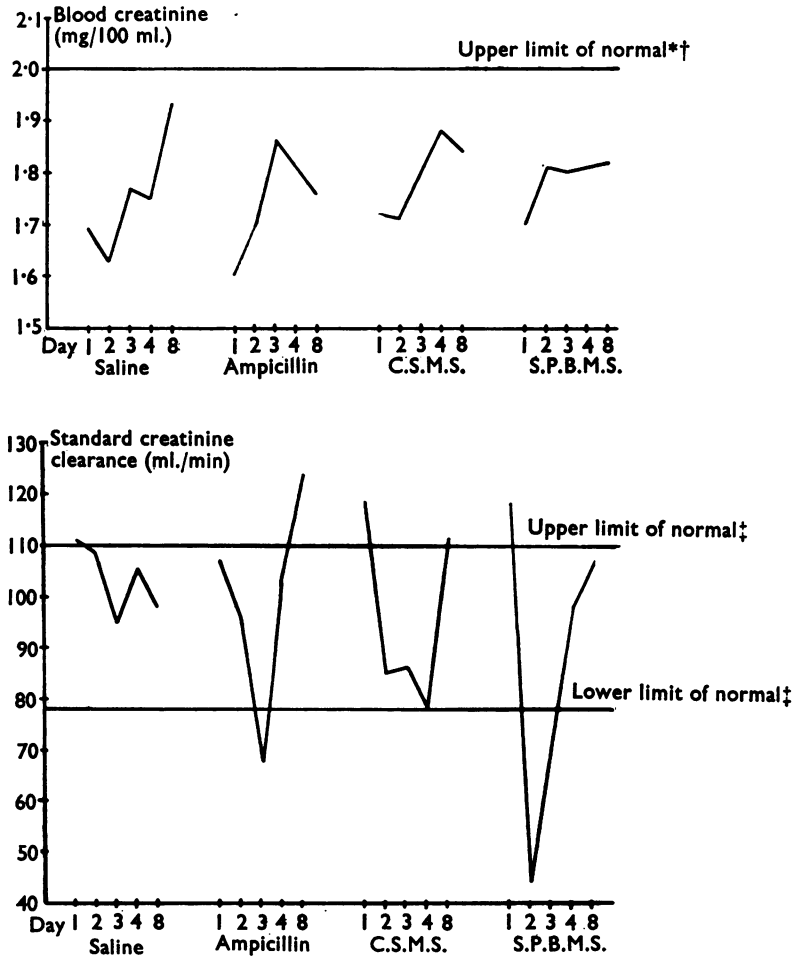

FIG. 3. Blood creatinine and creatinine clearance, four compounds. *Cantarow & Trumper (1962); †Martindale (1955); ‡*Documenta Geigy* (1962).

From these results there is an apparent effect on the kidneys when each of the substances under consideration is given to healthy human volunteers.

Microscopic examination of the urine detected no abnormalities ; tests for protein and blood, and observations for cells and casts all proving negative.

Side-effects

Side-effects were pronounced and severe following the injection of S.P.B.M.S. to such an extent as to exclude three volunteers receiving this compound. The three

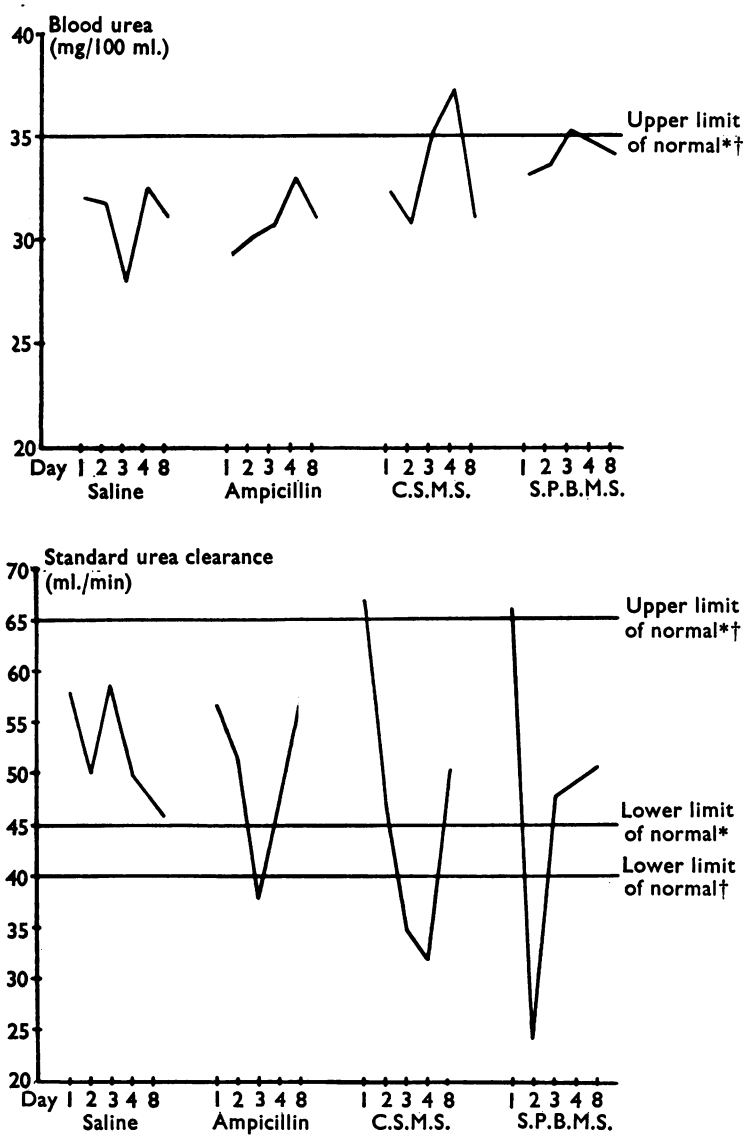

FIG. 4. Blood urea and urea clearance, four compounds. * Cantarow & Trumper (1962); † Martindale (1955).

TABLE 4. *Blood creatinine, urea, and creatinine and urea clearances*

	Subject	Normal saline (A)					Ampicillin (B)					C.S.M.S.(C)					S.P.B.M.S.(D)				
		Day 1 Base line	Day 2	Day 3	Day 4	Day 8	Day 1 Base line	Day 2	Day 3	Day 4	Day 8	Day 1 Base line	Day 2	Day 3	Day 4	Day 8	Day 1 Base line	Day 2	Day 3	Day 4	Day 8
Blood creatinine (mg/100 ml.)	1	1·464	1·476	1·860	1·782	2·640	1·720	2·328	2·400	2·652	2·292	1·932	2·010	1·992	2·100	2·112	2·028	2·146	2·058	—	2·004
	2	1·482	1·506	1·434	1·422	1·620	1·512	1·512	1·614	1·416	1·416	1·140	1·188	1·440	1·452	1·830	1·460	1·464	1·530	1·878*	1·644
	3	2·172	2·016	1·992	1·902	2·004	1·488	1·440	1·908	1·908	2·142	2·008	1·908	2·220	2·820	2·268	—	—	—	—	—
	4	1·808	1·632	2·082	2·058	1·794	1·788	1·680	1·650	1·680	1·896	1·866	1·908	1·974	1·686	1·662	—	—	—	—	—
	5	1·608	1·500	1·650	1·650	1·758	1·600	1·572	1·992	1·758	1·758	1·650	1·596	1·644	1·692	1·788	1·524	1·584	—	—	1·662*
	6	1·602	1·644	1·584	1·710	1·794	1·458	1·608	1·566	1·470	1·662	1·740	1·632	1·548	1·500	1·404	—	—	—	—	—
Mean		1·689	1·629	1·767	1·754	1·935	1·594	1·690	1·855	1·814	1·761	1·722	1·707	1·803	1·875	1·844	1·744	1·805	1·794	—	1·824
Blood urea (mg/100 ml.)	1	35·1	35·1	35·0	39·4	36·5	36·2	40·3	44·2	43·9	38·4	39·9	36·5	47·8	44·0	34·9	40·3	40·7	41·6	—	42·1
	2	29·4	28·9	15·0	31·6	31·3	27·2	32·1	32·7	29·2	33·0	29·7	32·1	34·1	35·4	33·9	26·0	26·7	28·6	30·0*	26·5
	3	36·3	40·8	36·3	36·7	36·8	31·5	35·0	38·1	40·1	29·1	29·8	30·0	32·3	44·1	29·6	—	—	—	—	—
	4	30·0	23·3	28·9	28·7	29·0	27·4	27·2	18·2	25·5	32·6	32·1	30·6	32·5	37·5	30·9	—	—	—	—	—
	5	30·4	30·3	26·1	28·7	22·1	26·0	19·1	25·5	29·5	24·8	22·9	24·4	31·0	30·3	25·0	31·6	27·1	—	—	30·0*
	6	31·6	33·2	27·2	30·0	31·5	28·1	26·9	25·7	30·3	28·7	38·7	32·2	33·5	32·8	32·8	—	—	—	—	—
Mean		32·1	31·9	28·0	32·5	31·2	29·4	30·1	30·7	33·1	31·1	32·2	30·9	35·2	37·3	31·1	33·2	33·7	35·1		34·3
Creatinine clearance (ml./min)	1	145·1	118·5	91·5	137·8	92·0	130·7	109·1	79·7	74·7	129·7	107·8	105·8	100·8	116·7	121·9	153·9	82·2	168·5	—	141·5
	2	80·3	73·0	55·3	108·1	71·3	74·1	50·5	32·2	119·6	140·3	122·3	110·0	44·3	59·4	90·1	82·1	6·7	28·6	54·0*	74·5
	3	108·7	106·4	170·4	136·2	57·3	101·3	140·4	104·0	131·8	134·8	107·7	22·0	81·6	72·4	98·4	—	—	—	—	—
	4	413·3	159·5	106·5	105·5	152·7	153·7	105·2	78·9	123·9	163·5	199·6	98·8	105·8	78·3	169·3	—	—	—	—	—
	5	93·4	107·2	84·0	88·6	121·3	96·0	92·6	58·4	106·1	86·2	40·4	86·7	97·6	113·8	93·9	82·6	28·9	—	—	137·0*
	6	99·8	92·1	64·5	62·1	90·6	89·8	80·4	59·6	65·5	92·5	134·1	86·9	87·6	29·5	94·6	—	—	—	—	—
Mean		111·7	109·4	95·2	106·3	97·5	107·6	96·3	68·8	103·6	124·5	118·6	85·0	86·2	78·4	111·3	118·0	44·4	98·5	—	107·5
Standard urea clearance (ml./min)	1	43·7	44·6	44·2	51·9	40·2	70·2	51·4	43·1	37·9	60·7	83·7	70·0	30·0	47·0	42·9	73·1	45·8	90·4	—	58·3
	2	59·3	60·2	72·4	40·3	33·4	48·2	26·7	18·6	67·6	60·7	48·6	38·7	20·6	26·4	46·0	59·3	3·6	5·9	31·3*	43·3
	3	63·3	39·1	79·8	55·9	41·2	40·5	52·4	35·3	63·3	81·3	70·9	12·4	40·3	28·4	59·3	—	—	—	—	—
	4	60·7	57·2	41·3	56·8	50·4	34·1	50·2	71·3	45·4	49·4	79·5	48·4	42·8	28·8	50·8	—	—	—	—	—
	5	41·7	46·5	70·6	39·8	65·7	59·0	48·4	23·8	40·9	49·3	36·2	70·3	26·8	49·9	60·5	36·7	12·8	—	—	45·8*
	6	79·8	53·4	44·7	53·8	43·3	89·8	82·1	34·9	36·1	43·7	84·2	46·7	51·2	14·1	44·1	—	—	—	—	—
Mean		58·4	50·1	58·8	49·7	45·7	56·9	51·8	37·8	48·5	57·5	67·1	47·7	35·2	32·4	50·6	66·2	24·7	48·2	—	50·8

* Omitted from means due to inadequate data.

who received the antibiotic all complained of pain at the injection site, perioral paraesthesia, dizziness and ataxia. With C.S.M.S. three of the six volunteers complained of slight perioral paraesthesia passing off after the first injection but no local pain. Ampicillin caused pain at the injection site in two of the volunteers associated with swelling and tenderness in one and tingling of the left side and fingers in the other. Even saline was responsible for causing one volunteer to become hot and dizzy which was presumed to be a nervous reaction, although this could be explained on the basis of a " nocebo " effect, where a side-effect is produced in anticipation of receiving a powerful drug.

Discussion

A change in the values for creatinine and urea clearances follows the administration of each of the substances under consideration, although, in the case of saline, these stay within the limits considered normal as shown and referenced in Table 5 and Figs. 3 and 4. With the three antibiotics standard urea clearance was reduced to slightly below normal with ampicillin, more with C.S.M.S. and still more with S.P.B.M.S. With the latter two antibiotics small elevations in blood urea were also detected. Creatinine clearance was reduced to the lower limit of normal with C.S.M.S., just below normal with ampicillin and considerably more with S.P.B.M.S. Corresponding changes in blood creatinine were seen but all stayed within normal limits. A notable feature of these findings however was that in every case clearance had returned to within normal limits by day 8—5 days after the last dose of antibiotic had been given.

The findings with ampicillin in this study support the work of Lee & Hill (1968) and demonstrate that changes in clearance can take place in normal as well as diseased kidneys. With C.S.M.S. the effect on blood creatinine and clearance is similar to that reported by Brumfitt *et al.* (1966), with the exception that where the present results are outside normal limits a return to normal is shown in a considerably shorter time, despite the fact that three times the dosage of antibiotic was used.

It must be considered whether the changes in renal function demonstrated with the compounds used in this study represent nephrotoxicity. It is axiomatic in toxicology that any substance introduced into the animal body is toxic if given at a certain dose, by a particular route and in a selected vehicle. In the present study

TABLE 5. *Range of normals*

	Range
Blood creatinine	1·2–1·5 mg/100 ml.‡ 1·0–2·0 mg/100 ml.* 0·4–2·0 mg/100 ml.†
Blood urea	19–32·5 mg/100 ml.‡ 20–35 mg/100 ml.* 15–35 mg/100 ml.†
Creatinine clearance	78–110 ml./min‡ Ave–100 ml./min*
(Standard) Urea clearance	45–65 ml./min* 40–65 ml./min†

* Cantarow & Trumper (1962); † Martindale (1955); ‡ *Documenta Geigy* (1962).

recommended therapeutic doses were used, and all compounds given intramuscularly in the commercially available formulation. Ampicillin is excreted in the urine between 30% (Knudsen, Rollinson & Stevens, 1961) and up to 88% (Sutherland & Robinson, 1967) over a 6 hr period. M. McMillan, T. M. L. Price, D. M. Maclaren & G. W. Scott (personal communication 1961) have reported that excretion of C.S.M.S. is up to 80% in the urine over an 8–12 hr period (Colley & Frankel, 1963), but S.P.B.M.S. is reported as being excreted in the urine to a much lesser extent (Barnet, Bushby & Wilkinson, 1964). It now appears, however, that although levels of S.P.B.M.S. are comparatively low in the urine, this may be due to a slow excretion rate rather than excretion by another route (Beveridge & Martin, 1967). With all three antibiotics, therefore, an additional load is being placed on the kidney when the compound is given and slight changes in function may not be surprising. Where the change is most noticeable, however (with S.P.B.M.S.), this " loading effect " possibly contributes less, and here it may be that there is some direct action on renal tubular cells preventing re-absorption or excretion of certain compounds. It has been shown in this paper that polymyxins and penicillins are not excreted by the kidney in an identical manner, and it is possible that this factor may further influence the " loading effect " of the compounds on the kidney. The fact that probenecid can block the tubular excretion of penicillin is well known, but it is now thought that even this relatively safe uricosuric agent can cause renal damage (Scott & O'Brien, 1968) and its prolonged use in conjunction with penicillins as well as in the treatment of gout may have to be observed more carefully. A survey of the literature shows that the mechanism for effecting changes in urea and creatinine clearance by antibiotics is still largely unknown.

Brumfitt *et al.* (1966) considered that, although the renal effects of C.S.M.S. appeared to be reversible, the antibiotic should be considered as potentially nephrotoxic. This statement was based on changes in serum creatinine and creatinine clearance in volunteers, but it should be noted that the changes found after administration of C.S.M.S. virtually all remained within normal limits when the pre-treatment figure was also within normal limits. It is perhaps difficult for these authors to discuss the significance of their findings as their study was not comparative with any other antibiotic or a control. If their standards are applied to the present study it would appear that all three antibiotics and perhaps even normal saline would have to be regarded as nephrotoxic. The term " nephrotoxic " should be reserved for drugs causing irreversible damage to the kidneys which can be demonstrated histopathologically. Although no such changes were seen it is possible that any of the compounds used in this study at a higher dosage than that administered or over a prolonged period or in subjects with impaired renal function could bring about such changes. Use of these drugs in this manner should however be unacceptable in clinical practice.

One further observation has emerged from this study—the obvious difference in renal and systemic side-effects caused by C.S.M.S. and S.P.B.M.S. and previously noted (Beveridge & Martin, 1967). There has been much confusion between these two antibiotics and they have very often been reported as identical. C.S.M.S. has now, however, been positively identified as polymyxin E (Susuki, Hayashi, Fujikawa & Tsukamoto, 1965 ; Wilkinson & Lowe, 1964).

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