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MECHANISM OF METHIONINE PROTECTION AGAINST THE NEPHROTOXICITY OF POLYMYXIN A

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Of the five polypeptide antibiotics derived from various strains of *Bacillus polymyxa*, one of them, polymyxin A, has a nephrotoxic action in both animals and man (Brownlee, Bushby, and Short, 1949). The kidney damage, as indicated by proteinuria, occurs within twenty-four hours of administration, the peak protein excretion lasting between three and four days, the proteinuria then gradually decreasing in spite of continued dosage with the antibiotic. Cellular and granular casts continue to be excreted occasionally for several weeks after the cessation of treatment.

Histological examination of the kidneys at various stages of treatment in rats, guinea-pigs, and dogs shows that the damage is mainly confined to the convoluted tubules. The progressive effects were followed in dogs given a five-day course of treatment consisting of four three-hourly injections of 1 mg./kg. each day. Three days after the commencement of treatment, the picture was one of acute severe nephrosis with the secretory epithelium in all stages of disintegration and large numbers of cellular, granular, and hyaline casts. Seven days later there was still histological evidence of active damage with generalized cloudy swelling, stripping of the tubular epithelium, some granular and hyaline casts, and patches of severe oedema resulting in blockage of the damaged nephrons. After twenty-one days, there was no evidence of progressive damage ; although many of the nephrons were blocked and apparently non-functional, many others appeared normal.

The similarity of the nephrotoxic action of polymyxin A to that of DL-serine, as described by Artom, Fishman, and Morehead (1945), has already been pointed out (Brownlee and Short, 1949) and confirmation obtained that a similar protection in rats and dogs is obtained with certain amino-acids (Brownlee, Bushby, and Short, 1948) to that reported for serine by Wachstein (1947).

An investigation of the possible mechanisms of the nephrotoxicity and this protection is described here.

METHODS

Assessment of the renal toxicity

In rats.—The resultant proteinuria in rats has been used to evolve a semi-quantitative method for measuring the effect of antagonists on the renal toxicity of polymyxin A. Groups of four rats given graded doses of polymyxin A showed increased amounts of

protein in the urine during 72 hours. The animals were kept in metabolism cages at a constant temperature of 20–22° C. to minimize variations in renal flow due to temperature and were hydrated with 10 ml. water/100 g. rat before dosage. The dose selected as giving the best response was 1 mg./100 g. rat, since higher doses approached too near to the LD50 and lower doses gave uncertain responses. The total weight of protein excreted in 72 hours, expressed as mg. protein/100 g. rat, was compared with that excreted by a group of animals given a standard batch of polymyxin A and a control group given saline. The reproducibility of the test is low, largely because of the variations in the normals.

In dogs.—The effect of continuous treatment with the antibiotic was studied in the dog, since it proved more sensitive in detecting small amounts of damage. The maximum protein concentration in the urine (g./litre) attained in 96 hours was used as a measure of the extent of the renal damage, since it was observed that after this time the degree of proteinuria decreased even when treatment was continued.

Estimation of protein

The protein was precipitated with trichloroacetic acid and then estimated absorptiometrically by means of the biuret reaction in the presence of ethylene glycol. This modification (Mehl, 1945) has the advantage that a clear solution is obtained and centrifuging to remove copper hydroxide is avoided.

Estimation of methionine

The methionine concentration in the urine was determined by a modification of the method described by Albanese, Frankston, and Irby (1944). It depends on the selective oxidation of the sulphydryl group with hydrogen peroxide in the presence of perchloric acid. Samples of urine (15 ml.) were treated with a measured excess of hydrogen peroxide-perchloric acid mixture, and after exactly one hour the excess oxidizing reagent was determined by the addition of potassium iodide and titration of the liberated iodine with 0.1 N-sodium thiosulphate The method is accurate to within ± 2.5 per cent.

Estimation of polymyxin

The concentration of polymyxin in serum and urine was determined microbiologically by the serial dilution method in a glucose medium containing 1 per cent phenol red broth and 50 per cent (v/v) horse serum inoculated with *Bact. coli* (Brownlee and Bushby, 1948).

Results and Discussion

Complete protection in rats against the nephrotoxicity of a dose of 1 mg./100 g. rat of polymyxin A was obtained by treatment with methionine or S-methyl-cysteine. When the antibiotic was given alone, about five times as much protein was excreted by the test group of animals as by the controls, while simultaneous administration of 200 mg. methionine or S-methyl-cysteine per 100 g. rat reduced the weight of protein excreted to values similar to those obtained with the controls. No protective action was shown by the substances containing an unmethylated thiol group, such as L-cysteine and 2 : 3-dithiopropanol.

A similar protective action was obtained with methionine and S-methyl-cysteine in dogs. Table I shows the effect of the two substances on a course of treatment with polymyxin A of 1.0 mg./kg. given by subcutaneous injection, three times daily for three days. The proteinuria was completely prevented by simultaneous treatment with ten times the amount of antagonist in each case. With the lower doses of

TABLE I

PROTECTIVE EFFECT OF METHIONINE AND S-METHYL-CYSTEINE AGAINST NEPHROTOXICITY OF POLYMYXIN A IN DOGS

1 mg./kg. polymyxin A, three times daily for three days

Antagonist	Dose mg./kg.	Max. conc. protein in urine in 96 hours (g./l.)
Methionine	10	0
	5	0, 0.1
	2.5	7.0
S-Methyl-cysteine	10	0
	5	0.3
Nil		5.0, 2.0

antagonist, some proteinuria developed, but urinary deposits were free from renal epithelial cells and casts, and histological examination of the kidneys showed no evidence of stripping but only a cloudy swelling of the epithelial cells.

Wachstein (1947) suggested that the protective effect of methionine and other substances against the renal damage produced by serine in the rat was due to com-

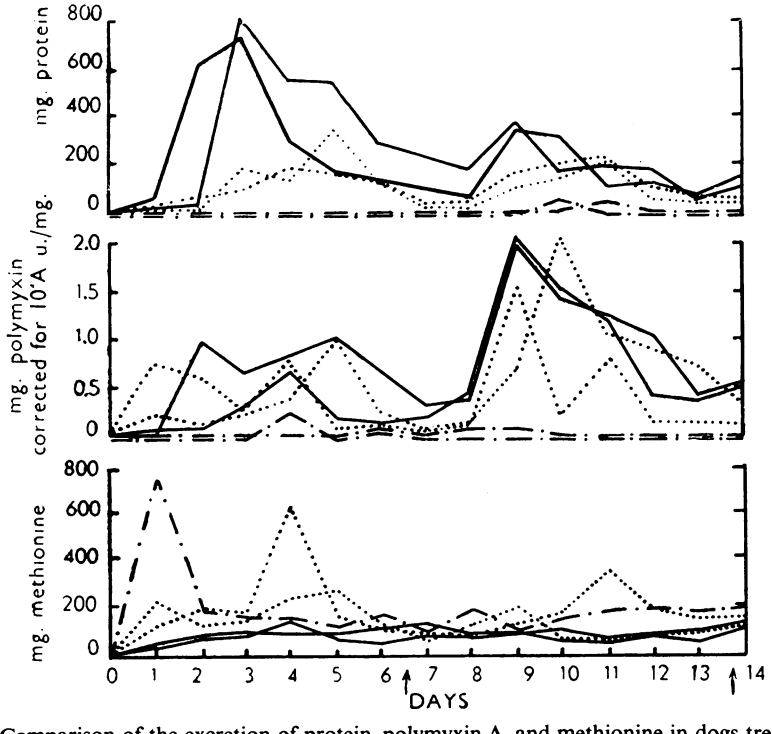

FIG. 1.—Comparison of the excretion of protein, polymyxin A, and methionine in dogs treated three times daily, except at the arrows, with 1 mg./kg. of antibiotic. Continuous lines: polymyxin A alone; dotted lines: with methionine (20 × dose); and dot-dash lines: with methionine (40 × dose).

petitive tubular resorption, the preferential reabsorption of the antagonist preventing the reabsorption of the damaging substance. A study of the comparative excretions of antibiotic, methionine, and the extent of proteinuria does not support this mechanism in the case of polymyxin A. Fig. 1 compares the excretion of protein, polymyxin A, and methionine in dogs treated three times daily with 1 mg./kg. of antibiotic alone and with twenty and forty times the dose of methionine. Treatment was continuous except at the arrows. Contrary to what would be expected if methionine protected entirely by preferential tubular reabsorption, more antibiotic was excreted when proteinuria was present and the excretion of methionine did not run concurrently with the protein excretion. Confirmation is given by the results obtained in a similar experiment with polymyxin B (Fig. 2), which is

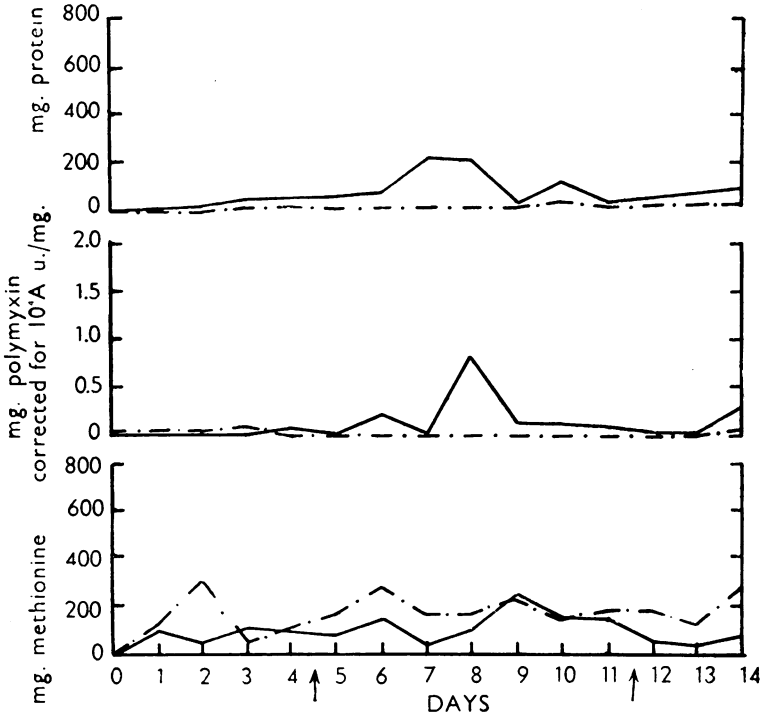

FIG. 2.—Comparison of the excretion of protein, polymyxin B, and methionine in dogs treated continuously, except at the arrows, with three daily doses of 1 mg./kg. Continuous lines: polymyxin B alone; dot-dash lines: with methionine (20 × dose).

practically free from nephrotoxicity. There was practically no proteinuria, and only a negligible excretion of antibiotic and no increase in methionine excretion occurred.

Thus it appears that competitive tubular reabsorption does not alone account for the protective action, and since it is unlikely that glomerular or tubular filtrates would damage specifically the distal tubules, the possibility of detoxication owing to concurrent chemical change accompanying the reabsorption of the toxic substance was investigated.

It is significant that L-cysteine and 2 :3-dithiopropanol gave no protection in the rat, so the reaction cannot be associated with the sulphydryl groups of methionine and S-methyl-cysteine.

Methionine contains a readily utilizable methyl group and is known to be capable of replacing choline as a transmethylating agent. Also S-methyl-cysteine, which gives as good protection as methionine, contains a labile methyl group. The possibility that polymyxin A produces its nephrotoxicity by an acquisition of labile methyl groups and that the protective mechanism is one of supplying such groups is thus indicated.

The effect of a number of compounds containing methyl groups was determined. The results given in Table II show that the two compounds which are known to

TABLE II

EFFECT OF METHYLATING COMPOUNDS AS PROTECTIVE AGENTS IN RATS

Dose of polymyxin A, 1.0 mg./100 g. rat

Substance	Dose mg./100 g. rat	Wt. protein excreted in 72 hours, mg./100 g. rat
Choline	50	24.7, 30.0
	200	9.2
Methyl isothiourea	50	18.2
	100	11.4
Caffeine sodium benzoate	10	48.0, 51.5
	20	32.5, 45.2
	40	22.5, 28.6
Theophylline sodium acetate	5	31.2
	10	38.3
	20	15.6
Nil		55.5, 56.3, 61.5
Controls		5.4, 10.7, 14.5

be active transmethylating agents, choline and S-methyl-thiourea, gave complete protection. The methyl-xanthine derivatives also gave some protection at the highest of the dose levels used.

Finally, it is significant that the nephrotoxicities of the acetyl derivative of polymyxin A and the product of the reaction of polymyxin A with diazomethane (probably methyl polymyxin) are less than that of the parent substance. The acetyl derivative had 12.3 per cent residual activity and the methyl compound 45.5 per cent. Given in doses equivalent to the activity of 1 mg. of the original antibiotic per 100 g. rat, complete protection was obtained in both cases (Table III).

It has been observed that, during a five-day course of treatment with the antibiotic in man, the peak proteinuria developed at three and a half to four days and was resolving on the fifth day, even though dosage was continued until the fifth day (Brownlee, Bushby, and Short, 1949). This phenomenon was studied in dogs which were treated continuously with 1 mg. polymyxin A per kg., three times daily for three weeks. The development of proteinuria is shown in Fig. 3. A first peak, similar to that obtained in man, developed between the third and fourth day of treatment, and a second smaller peak between the ninth and tenth day, after which, in spite of continued treatment, only low concentrations of protein were found in

TABLE III

RENAL TOXICITY OF POLYMYXIN A DERIVATIVES

Doses equivalent to 1 mg. polymyxin A/100 g. rat

Substance	Wt. protein excreted in 72 hours mg./100 g. rat
Methylated polymyxin A	13.1, 6.1
Acetylated polymyxin A	5.1, 5.8
Polymyxin A	43.5, 41.7
Controls	5.7, 7.6, 14.5

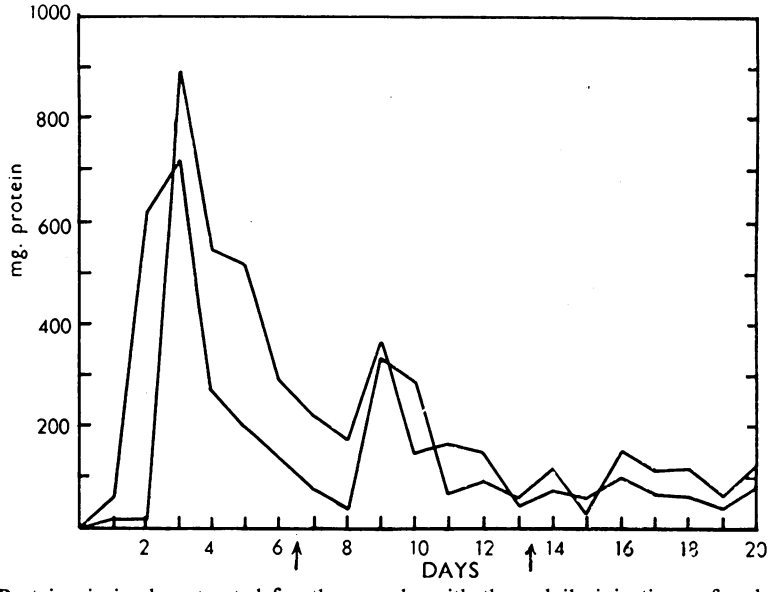

FIG. 3.—Proteinuria in dogs treated for three weeks with three daily injections of polymyxin A, 1 mg./kg.

the urine. These results, together with the histological evidence of the failure of the lesions to progress, suggest that the body is able to mobilize a defensive mechanism similar to that afforded by methionine and other methylating agents.

SUMMARY

1. Polymyxin A produces renal damage in both experimental animals and man.

2. The mechanism of the nephrotoxicity and of the action of certain antagonists has been studied by semi-quantitative methods in rats and dogs based on estimates of the resultant proteinuria.

3. DL-Methionine and S-methyl-L-cysteine in sufficient dosage give complete protection against the nephrotoxicity of polymyxin A in rats and dogs.

4. Comparison of the excretion of protein, antibiotic, and methionine after treatment with polymyxin A and polymyxin B, which has only negligible nephrotoxicity,

indicated that the protective action was not due simply to competitive tubular resorption.

5. Methionine and S-methyl-cysteine both contain labile methyl groups, and two other transmethylating agents, choline and S-methyl-thiourea, also gave good protection in rats.

6. The acetyl and methyl derivatives of polymyxin A did not show any nephrotoxicity.

7. It is concluded that the nephrotoxicity of polymyxin A is due to a drainage of methyl groups and that antagonists produce protection by supplying these groups.

8. There is some evidence that the body mobilizes a defence mechanism which may be analogous to the protection given by methylating compounds, since in both animals and man the peak proteinuria occurs between the third and fourth day of treatment, after which the amount of protein excreted diminishes in spite of continued dosage with the antibiotic.

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