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Enhancement by potent diuretics of renal tubular necrosis induced by cephaloridine

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Summary

1. Administration of frusemide or ethacrynic acid to cephaloridine-treated mice increased both the incidence and extent of proximal renal tubular necrosis compared with that obtained in cephaloridine-treated control mice.

2. Administration of frusemide to cephaloridine-treated rats produced significant changes in urine output, electrolyte excretion and proteinuria, and plasma urea nitrogen and creatinine values were significantly increased compared with controls or rats that received frusemide or cephaloridine alone.

3. Histological examination of the kidneys showed a higher incidence and greater extent of tubular necrosis in rats that received both frusemide and cephaloridine.

4. It is suggested that this adverse drug interaction may have contributed to the deterioration in renal function observed in some patients treated with diuretics and cephaloridine concurrently.

Introduction

Cephaloridine has been successfully used in the treatment of a wide variety of bacterial infections since its introduction in 1964 (Lewis, 1967). By the end of 1968 there were published reports of its use in 7,000 cases, of which 1,257 had been treated for kidney and urinary tract infections. At least 2% of these 7,000 patients were known to have had some degree of renal impairment before treatment with cephaloridine and in the great majority of cases renal function either remained unchanged or improved following treatment. In a very small number of patients renal function deteriorated following cephaloridine treatment, and Foord (1969) has collected and analysed the clinical reports of a total of thirty-six patients in whom cephaloridine treatment was associated with acute renal failure. Shock and infection were probably implicated in the development of acute renal failure in some cases, but a number of other factors may have contributed to the deterioration in renal function. These included the use of excessive dosage of cephaloridine, the concomitant administration of other potentially nephrotoxic drugs and the occurrence of hypersensitivity reactions. It was noted that diuretics, particularly frusemide, had been used simultaneously in several patients and this finding prompted the present investigation in animals. Large doses of cephaloridine produce a proximal renal tubular necrosis in a number of laboratory animal species (Atkinson,

Currie, Davis, Pratt, Sharpe & Tomich, 1966 ; Atkinson, Caisey, Currie, Middleton, Pratt, Sharpe & Tomich, 1966 ; Welles, Gibson, Harris, Small & Anderson, 1966), including the mouse and rat. Experiments were performed in these species to find out if the renal response to cephaloridine was exacerbated by administration of potent diuretics.

Methods

Mice

Female mice (A2G strain, body weight 15–20 g) were injected intraperitoneally with saline or diuretic, 0·5 h before subcutaneous treatment with cephaloridine or saline. Solutions of frusemide (Lasix), sodium ethacrynate (Lyovac Edecrin) or chlorothiazide sodium salt (Lyovac Saluric) were diluted in physiological saline for injection in a dose volume of 0·2 ml/20 g body weight. Cephaloridine (Ceporin) was administered to mice as a 5 or 8% w/v solution in physiological saline in a dose volume of 0·2 ml/20 g. After cephaloridine treatment the mice were kept under standard conditions for 48 h with free access to food and water. They were then killed by cervical dislocation and their kidneys removed and fixed in buffered formol saline. Each kidney was given an individual code number and the kidneys were selected at random for histological examination. The results of the histological examinations were expressed as "necrosis scores": the score for any particular kidney was designated 0, 5, 10, 15, etc., corresponding to the percentage of proximal tubules affected by necrosis.

Rats

In the first experiment four groups of five male rats (CFE strain, body weight 200–250 g) were dosed orally with vehicle (0·5% Tween 80) or frusemide in a dose volume of 0·5 ml/100 g 1 h before subcutaneous injection of saline or cephaloridine in a dose volume of 0·5 ml/100 g. The diuretic was administered as a fine suspension in 0·5% Tween 80, prepared by crushing 40 mg tablets of Lasix and grinding the material in a tissue grinder. Cephaloridine was injected as a 15% w/v solution in physiological saline. Immediately after subcutaneous treatment the rats were placed in individual metabolism cages and urine was collected for 5 h. The rats were then maintained under standard conditions with free access to food and water until a further 5 h urine collection was obtained from 48–53 h after treatment. At the end of the second urine collection period blood samples were taken by cardiac puncture under ether anaesthesia, collected in heparinized tubes and centrifuged. The rats were then killed and their kidneys examined in a manner similar to that described for mice. The urine samples were analysed for sodium, potassium and protein, and the plasma samples were analysed for plasma urea nitrogen (PUN), creatinine, sodium and potassium by standard Technicon AutoAnalyzer assay methods.

Similar protocols were observed in experiments on rats in which ethacrynic acid or hydrochlorothiazide were examined instead of frusemide. Ethacrynic acid and hydrochlorothiazide were administered as fine suspensions of the pure compounds (supplied by Merck, Sharpe & Dohme Ltd.) in 0·5% Tween 80.

In two other experiments with frusemide (Fig. 1 and Table 5) male Charles River CD rats (body weight 180–210 g) were used. Similar methods to those in the first

experiment were employed; only the numbers of animals and dose levels were different.

Results

Experiments on mice

Enhancement of cephaloridine-induced renal tubular necrosis by frusemide or ethacrynic acid

The kidneys of mice that received cephaloridine alone in a dose of 500 mg/kg exhibited necrosis of the proximal renal tubules in 16% of kidneys examined with an average score of 1 (Table 1*a*). Kidneys from mice given frusemide (10 and 100 mg/kg) or ethacrynic acid (1, 10 and 50 mg/kg) 0·5 h before cephaloridine treatment showed an increased incidence and extent of tubular necrosis. No renal damage was observed in the kidneys of mice that received diuretic alone.

The kidneys of mice injected with cephaloridine alone in a dose of 800 mg/kg showed a necrosis incidence of 84% and an average score of 12 (Table 1*b*). Treatment with 1 mg/kg of either frusemide or ethacrynic acid increased the incidence and extent of tubular necrosis. Increase in the dose of diuretic was not always accompanied by a corresponding increase in the incidence or degree of renal damage; the top doses of frusemide and ethacrynic acid produced less than the maximum effect.

TABLE 1. *Enhancement of cephaloridine-induced renal tubular necrosis in mice by frusemide and ethacrynic acid*

Diuretic dose (mg/kg i.p.) at −0·5 h	Cephaloridine dose (mg/kg s.c.) at time 0	Number of kidneys examined	Incidence of necrosis: Number of kidneys with necrosis	Incidence of necrosis: Incidence %	Extent of necrosis. *Average necrosis score
(*a*) Saline	500	38	6	16	1
F 1	500	10	0	0	0
F 10	500	10	6	60	9
F 100	500	10	4	40	14
F 100	Saline	10	0	0	0
EA 1	500	10	7	70	30
EA 10	500	10	10	100	19
EA 50	500	10	6	60	9
EA 50	Saline	10	0	0	0
(*b*) Saline	800	50	42	84	12
F 1	800	20	20	100	27
F 10	800	20	20	100	50
F 100	800	20	20	100	29
EA 1	800	20	20	100	26
EA 10	800	20	20	100	48
EA 50	800	10	8	80	32
Chlor 10	800	20	17	85	16
Chlor 100	800	20	14	70	17
Chlor 1,000	800	20	0	0	0
Chlor 1,000	Saline	10	0	0	0

F, Frusemide; EA, ethacrynic acid; Chlor, chlorothiazide

* Average necrosis score $= \frac{\text{Total necrosis scores}}{\text{Number of kidneys examined}}$.

TABLE 2. *5 h urine volume and urinary electrolyte and protein excretion values in rats*

Treatment and group							
Oral dose at −1 h (mg/kg)	Subcutaneous dose at time 0 (mg/kg)		Rat weight (g)	Urine volume (ml)	Na^+ excretion (μequiv.)	K^+ excretion (μequiv.)	Protein excretion (mg)
Frusemide 160	Saline	*a*	229±2	1·5±0·3	281±37	155±20	0·51±0·04
Frusemide 160	Cephaloridine 1,500	*b*	212±5	6·3±0·6	157±49	240±23	4·35±0·68
Vehicle	Cephaloridine 1,500	*c*	231±4	1·8±0·5	170±53*	144±45*	0·80±0·30*
Vehicle	Saline	*d*	246±6	2·5±0·4	507±116	316±49	0·66±0·24
		a vs. *d*	<0·02	NS	NS	<0·02	NS
P value		*b* vs. *d*	<0·002	<0·01	<0·05	NS	<0·001
(Student's *t* test)		*c* vs. *d*	NS	NS	<0·05	<0·05	NS

5 h urine collections were obtained from groups of CFE male rats from 48 to 53 h after subcutaneous treatment. Values are group mean ± S.E. (n=5).
* Four observations.

TABLE 3. *Terminal plasma urea nitrogen (PUN), creatinine and electrolyte concentrations in rats*

Treatment and group						
Oral dose at −1 h (mg/kg)	Subcutaneous dose at time 0 (mg/kg)		PUN (mg/100 ml)	Plasma creatinine (mg/100 ml)	Plasma Na^+ (mequiv/l.)	Plasma K^+ (mequiv/l.)
Frusemide 160	Saline	*a*	13·3±1·4	0·40±0·02	143·2±0·3	3·2±0·4
Frusemide 160	Cephaloridine 1,500	*b*	45·7±5·1	1·14±0·11	131·8±1·0	2·9±0·2
Vehicle	Cephaloridine 1,500	*c*	18·7±2·8	0·53±0·09	140·6±0·2	4·8±0·9
Vehicle	Saline	*d*	14·7±0·5	0·43±0·02	139·4±0·9	4·9±0·1
		a vs. *b*	NS	NS	<0·01	<0·01
P value		b vs. *d*	<0·001	<0·001	<0·001	<0·002
(Student's *t* test)		*c* vs. *d*	NS	NS	NS	NS

Blood samples were obtained from CFE male rats by cardiac puncture under ether anaesthesia 53 to 54 h after subcutaneous treatment. Values are group mean ± S.E. (n=5).

Protective effect of chlorothiazide

Chlorothiazide did not increase renal damage in cephaloridine-treated mice; paradoxically, at the highest dose level used it completely prevented damage to the kidneys. The property of a number of drugs to protect mice and other laboratory animal species against cephaloridine-induced renal tubular necrosis has been reported previously by Child & Dodds (1966, 1967).

Experiments on rats

Enhancement of cephaloridine-induced renal dysfunction by frusemide

Frusemide produces a very intense diuresis and natriuresis in the rat, although it is much less potent in this species on a weight for weight basis when compared with its potency in the dog or man: the oral dose of 160 mg/kg used in these experiments is approximately the maximal diuretic dose in male rats of the CFE strain.

In the first rat experiment, four groups of rats were dosed orally with vehicle or frusemide 1 h before saline or cephaloridine injection. During the initial 5 h urine collection period, begun immediately after subcutaneous treatment, the group mean urine volume of the control group of rats that received vehicle and saline was $1{\cdot}6 \pm 0{\cdot}2$ (S.E.) ml and the group mean urinary Na^+, K^+ and protein excretion values were 323 ± 63 μequiv., 314 ± 44 μequiv. and $0{\cdot}63 \pm 0{\cdot}10$ mg respectively. Urine volume was significantly increased in the group of rats that received cephaloridine alone to $3{\cdot}9 \pm 0{\cdot}6$ ml ($P<0{\cdot}01$, Student's t test) and Na^+ excretion (583 ± 91 μequiv.), K^+ excretion (531 ± 50 μequiv.) and protein excretion ($1{\cdot}60 \pm 0{\cdot}23$ mg) were also increased significantly compared with the control group values ($P<0{\cdot}02$, $P<0{\cdot}02$ and $P<0{\cdot}01$ respectively). The group mean urine volume and urinary electrolyte and protein excretion values of the groups that received frusemide alone or frusemide and cephaloridine were similar. Diuretic treatment significantly increased urine output and Na^+ excretion in both groups compared with the control group of rats. The values for the group given frusemide alone were $6{\cdot}6 \pm 0{\cdot}5$ ml ($P<0{\cdot}001$) and 665 ± 63 μequiv. ($P<0{\cdot}01$) and for the group that received both frusemide and cephaloridine they were $6{\cdot}8 \pm 0{\cdot}9$ ml ($P<0{\cdot}001$) and 715 ± 97 μequiv. ($P<0{\cdot}02$).

The results of the second urine collection period of 5 h, obtained 48 to 53 h after treatment, are given in Table 2. Rats in group *b* (the combined treatment group) had lost weight and excreted large volumes of urine; Na^+ excretion was reduced and K^+ was now the preponderant cation in the urine, accompanied by marked proteinuria. Less marked changes were observed in the other treatment groups. Rats in group *a* (frusemide alone) lost weight and K^+ excretion was reduced, and in group *c* (cephaloridine alone) urinary electrolyte excretion was lower than in the control group *d*.

Analysis of the plasma samples obtained 53–54 h after treatment clearly distinguished group *b*, which had received both frusemide and cephaloridine, from the other treatment groups (Table 3). The group mean PUN and plasma creatinine values were significantly elevated and plasma Na^+ and K^+ concentrations were significantly reduced below control levels. Values in group *c* (cephaloridine alone) were not significantly different from control. The elevated plasma Na^+ and low plasma K^+ concentrations in group *a* (frusemide alone) probably resulted from increased aldosterone activity following the initial acute Na^+ depletion.

The results of the histological examination of the kidneys from the four groups of rats are summarized in Table 4. All the kidneys from group *b* (frusemide and cephaloridine) exhibited necrosis of the proximal tubules, which correlates well with the elevated terminal PUN and plasma creatinine values observed in this group (Table 3). Only four of the ten kidneys from group *c* (cephaloridine alone) were affected. No renal pathology was observed in the kidneys of rats in group *a* that received frusemide alone or in group *d* that served as controls.

Lack of effect of ethacrynic acid and hydrochlorothiazide

In similar experiments an oral dose of 160 mg/kg ethacrynic acid failed to exert any effect in cephaloridine-treated rats ; however, this species is remarkable in being unresponsive to the diuretic action of this drug (Beyer, Baer, Michaelson & Russo,

TABLE 4. *Enhancement of cephaloridine-induced renal tubular necrosis in rats by frusemide*

Treatment and group			Number of kidneys examined	Number of kidneys with necrosis	*Average score
Oral dose at −1 h(mg/kg)	Subcutaneous dose at time 0 (mg/kg)				
Frusemide 160	Saline	*a*	10	0	0
Frusemide 160	Cephaloridine 1,500	*b*	10	10	41
Vehicle	Cephaloridine 1,500	*c*	10	4	10
Vehicle	Saline	*d*	10	0	0

* Average score $= \frac{\text{Total necrosis scores}}{\text{Number of kidneys examined}}$.

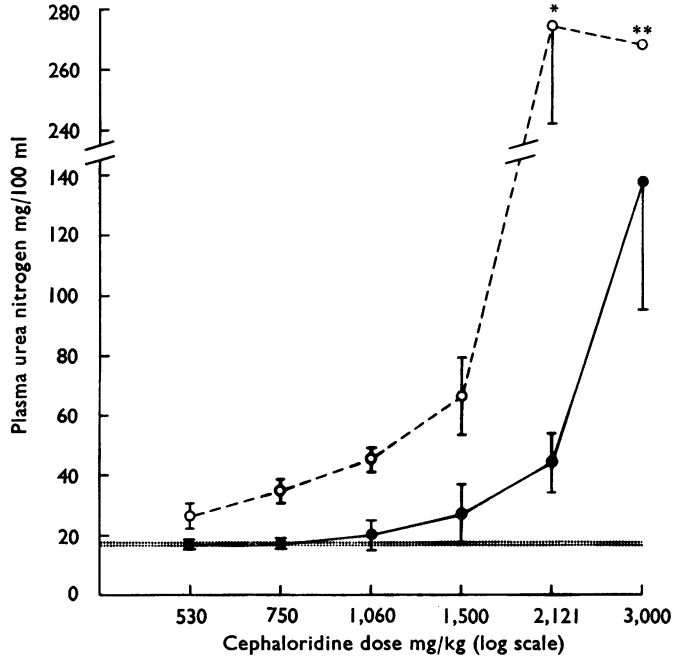

FIG. 1. Effect of treatment of rats with frusemide on the threshold for cephaloridine-induced renal damage. Values are group mean plasma urea nitrogen concentrations 48 h after treatment with cephaloridine. Vertical bars indicate ±standard error of the mean. ○- - -○, Groups given frusemide orally (160 mg/kg); ●——●, groups given vehicle; stippled bar, group mean PUN±2 S.E. of control rats given vehicle and saline. * Four observations, one rat died; ** two observations, three rats died.

TABLE 5. *Enhancement of cephaloridine-induced renal damage by frusemide*

Group	Treatment at time 0: Oral dose (0·5 ml/100 g)	Treatment at time 0: Subcutaneous dose (1·0 ml/100 g)	Initial body weight (g)	5 h urine volume (ml)	PUN (mg/100 ml)	Plasma creatinine (mg/100 ml)
a	0·5% Tween 80	Saline	197±4	3·1±0·4	14·2±2·5	0·48±0·03
b	0·5% Tween 80	Cephaloridine 1,060 mg/kg	189±4	5·7±0·6	11·2±1·3	0·53±0·06
c	Frusemide 160 mg/kg	Saline	196±5	17·7±0·9	14·0±2·1	0·42±0·01
d	Frusemide 20 mg/kg	Cephaloridine 1,060 mg/kg	196±4	6·6±0·6	18·8±4·5	0·83±0·16
e	Frusemide 40 mg/kg	Cephaloridine 1,060 mg/kg	189±2	7·1±0·7	34·0±6·9	1·88±0·46
f	Frusemide 80 mg/kg	Cephaloridine 1,060 mg/kg	197±3	10·3±1·0	31·8±5·7	1·51±0·36
g	Frusemide 160 mg/kg	Cephaloridine 1,060 mg/kg	197±4	12·2±0·8	92·4±49·4*	3·61±1·22
	P value (Student's *t* test)	*a* vs. *b*	NS	<0·01	NS	NS
		b vs. *d*	NS	NS	NS	NS
		b vs. *e*	NS	NS	<0·01	<0·02
		b vs. *f*	NS	<0·01	<0·01	<0·05
		b vs. *g*	NS	<0·001	NS	<0·05

Values are group mean±S.E. (n=6). * Five observations.

1965), at least by the oral route. Intravenous injection of 100 mg/kg, the maximum dose tolerated without overt toxic symptoms, produced a modest diuresis and natriuresis but failed to increase cephaloridine-induced renal damage. Hydrochlorothiazide is a potent oral diuretic in the rat, although the intensity of diuresis is much less than that produced by frusemide. Oral administration of 25 mg/kg of hydrochlorothiazide, a dose which is about twice the maximal diuretic dose, 1 h before subcutaneous treatment with cephaloridine 1,500 mg/kg, did not significantly affect renal function in a group of rats compared with that of a group which received cephaloridine alone.

Further observations on the frusemide-cephaloridine interaction

The enhancement of cephaloridine-induced renal tubular necrosis by frusemide has been confirmed on numerous occasions in rats. The effect of frusemide treatment is always to increase the incidence and severity of renal dysfunction in cephaloridine-treated animals. By oral administration the diuretic is most active in increasing renal damage when given 1 h before or at the same time as the antibiotic. The threshold at which cephaloridine produced renal damage in rats was lowered by frusemide treatment. This effect is shown in Fig. 1, in which the group mean PUN concentrations 48 h after treatment with vehicle and cephaloridine or frusemide and cephaloridine have been plotted as a function of cephaloridine dose. PUN values of the frusemide-treated groups of rats were significantly increased ($P<0{\cdot}05$) in comparison with the corresponding vehicle-treated groups at each cephaloridine dose level except the lowest.

Although the maximum oral diuretic dose of frusemide has been used in these experiments enhancement of renal damage occurred with lower doses. Thus 40 and 80 as well as 160 mg/kg doses of frusemide significantly increased PUN and plasma creatinine concentrations measured 53 h after cephaloridine treatment (Table 5). Injection of this dose of cephaloridine increased urine volume during the 5 h period after administration in the vehicle-treated group of rats but reduced the diuretic response to frusemide (group mean urine volume of group *g* significantly less than that of group *c*, $P<0{\cdot}05$), an effect that has been observed in other experiments.

Discussion

These experiments have shown that treatment of mice or rats with frusemide, or mice with ethacrynic acid, can significantly increase the incidence and extent of renal tubular necrosis produced by large doses of cephaloridine. The results may be related to the clinical observation that in a total of thirty-six cases in which cephaloridine treatment was associated with acute renal failure, the clinical histories of nine patients mentioned the simultaneous administration of diuretic drugs, and in seven of them the diuretic used was frusemide (Foord, 1969). It is now reasonable to suspect that this combination of drugs may have adversely affected renal function through drug interaction of the kind described in these animal studies.

The effect of frusemide to increase renal damage has been observed also in rats treated with the nephrotoxic agents kanamycin, capreomycin, uranyl nitrate and glycerol (Dodds, unpublished). This enhancement of the nephrotoxic action of drugs is in contrast to the protective property that frusemide possesses in other forms of

experimental acute renal failure. Dodds (unpublished) has shown that frusemide treatment substantially protects rats against the pigment-induced nephropathy produced by intravenous injection of methaemoglobin and ferrocyanide, and similar protective effects have been described by Ruiz-Guinazu, Montoreano & Mouzet (1969) in dogs. Clinically both frusemide and ethacrynic acid have been employed in renal insufficiency to induce or sustain urine output (Muth, 1968 ; Auger, Dayton, Harrison, Tucker & Anderson, 1968).

The mechanism underlying the enhancement of the nephrotoxic action of drugs by frusemide or ethacrynic acid is not understood. It may be related to the acute contraction of the extracellular fluid space that is induced or the rapid rise in plasma renin activity that has been reported to occur in animals and man after their administration (Meyer, Menard, Papanicolau, Alexandre, Devaux & Milliez, 1968 ; Rosenthal, Boucher, Nowaczynski & Genest, 1968). The latter might be important if renin participates in the development of acute renal failure as discussed recently by Kokot & Kuska (1969) and Brown, Gleadle, Lawson, Lever, Linton, Macadam, Prentice, Robertson & Tree (1970).

As these animal studies have confirmed the clinical impression that renal function may be adversely affected by the combination of frusemide and cephaloridine, it is recommended that the drugs should not be given together. If it is essential to use a potent diuretic such as frusemide or ethacrynic acid together with cephaloridine, high dosage of the latter should be avoided and renal function monitored regularly.

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REFERENCES

ATKINSON, R. M., CAISEY, J. D., CURRIE, J. P., MIDDLETON, T. R., PRATT, D. A. H., SHARPE, HELEN M. & TOMICH, E. G. (1966). Subacute toxicity of cephaloridine to various species. *Tox. appl. Pharmac.*, **8**, 407–428.

ATKINSON, R. M., CURRIE, J. P., DAVIS, B., PRATT, D. A. H., SHARPE, HELEN M. & TOMICH, E. G. (1966). Acute toxicity of cephaloridine, an antibiotic derived from cephalosporin C. *Tox. appl. Pharmac.*, **8**, 398–406.

AUGER, A. G., DAYTON, D. A., HARRISON, C. E., TUCKER, R. M., & ANDERSON, C. F. (1968). Use of ethacrynic acid in mannitol-resistant oliguric renal failure. *J. Am. med. Ass.*, **206**, 891–893.

BEYER, K. H., BAER, J. E., MICHAELSON, JOAN K. & RUSSO, H. F. (1965). Renotropic characteristics of ethacrynic acid: a phenoxyacetic saluretic–diuretic agent. *J. Pharmac. exp. Ther.*, **147**, 1–22.

BROWN, J. J., GLEADLE, R. I., LAWSON, D. H., LEVER, A. F., LINTON, A. L., MACADAM, R. F., PRENTICE, E., ROBERTSON, J. I. S. & TREE, M. (1970). Renin and acute renal failure: studies in man. *Br. med. J.*, **1**, 253–258.

CHILD, K. J. & DODDS, M. G. (1966). Mechanism of urinary excretion of cephaloridine and its effects on renal function in animals. *Br. J. Pharmac. Chemother.*, **26**, 108–119.

CHILD, K. J. & DODDS, M. G. (1967). Nephron transport and renal tubular effects of cephaloridine in animals. *Br. J. Pharmac. Chemother.*, **30**, 354–370.

FOORD, R. D. (1969). Cephaloridine and the kidney. *VI International Congress of Chemotherapy, Tokyo, Proceedings*, **1**, 597–604.

KOKOT, F., & KUSKA, J. (1969). Plasma renin activity in acute renal insufficiency. *Nephron*, **6**, 115–127.

LEWIS, A. A. G. (1967). Cephaloridine. *Postgrad. med. J.*, **43**, Aug. Suppl.

MEYER, P., MENARD, J., PAPANICOLAU, N., ALEXANDRE, J. M., DEVAUX, CLAUDINE & MILLIEZ, P. (1968). Mechanism of renin release following furosemide diuresis in rabbit. *Am. J. Physiol.*, **215**, 908–915.

MUTH, R. G. (1968). Diuretic properties of furosemide in renal disease. *Ann. int. Med.*, **69**, 249–261.

ROSENTHAL, J., BOUCHER, R., NOWACZYNSKI, W. & GENEST, J. (1968). Acute changes in plasma volume, renin activity, and free aldosterone levels in healthy subjects following fursemide administration. *Can. J. Physiol. Pharmac.*, **46**, 85–91.

RUIZ-GUINAZU, R., MONTOREANO, B. & MOUZET, MARIA T. (1969). Prevention from the initial oliguria of acute renal insufficiency by the administration of furosemide. *IV International Congress of Nephrology, Stockholm*, Abstracts, pp. 216.

WELLES, J. S., GIBSON, W. R., HARRIS, P. N., SMALL, R. M. & ANDERSON, R. C. (1966). Toxicity, distribution, and excretion of cephaloridine in laboratory animals. *Antimicrob. Ag. Chemother.*, 1965, pp. 863–869.

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