

## Mitomycin C-induced organ toxicity in Wistar rats: a study with special focus on the kidney

J. Verweij<sup>1,3</sup>, S. Kerpel-Fronius<sup>1</sup>, M. Stuurman<sup>1</sup>, A. J. van Triet<sup>2</sup>, L. van Hattum<sup>3</sup>, J. de Vries<sup>4</sup>, and H. M. Pinedo<sup>1</sup>

<sup>1</sup> Department of Medical Oncology, Free University Hospital, Amsterdam

<sup>2</sup> Department of Clinical Chemistry, Free University Hospital, Amsterdam

<sup>3</sup> Department of Pathology, Free University Hospital, Amsterdam

<sup>4</sup> Department of Molecular Toxicology, Free University Hospital, Amsterdam, The Netherlands

**Summary.** Two studies were performed to investigate acute and chronic organ toxicity after Mitomycin C (MMC) administration in Wistar rats. Six rats received 2.5 mg/kg MMC i.p. once and were followed for 5 consecutive days. The alanine aminopeptidase (AAP)/creatinine ratio increased significantly, compared to a control group receiving saline. Four groups of rats were injected i.p. weekly for 5 weeks; 6 control rats with saline, 7 rats with 1.7 mg/kg of MMC, 7 rats with 10 mg/kg 5-fluorouracil (5-FU) and 7 rats with MMC as well as 5-FU. The latter two groups were included to study possible toxicity synergism between the two drugs. A significant decrease in AAP excretion in the MMC group, as well as a nonsignificant decrease in the MMC/5-FU group were the most remarkable observations. Light microscopy did not show renal changes, but did show alveolar septal congestion after repeated MMC injections. It is concluded that MMC causes tubular damage in Wistar rats, with acute leakage of enzyme from the cells, followed by enzyme depletion during chronic treatment. Also MMC induces pulmonary changes in Wistar rats. To what extent these changes represent early stages of toxicity remains to be elucidated.

**Key words:** Mitomycin C – Renal toxicity – HUS – NAG – AAP

### Introduction

In man Mitomycin C (MMC) is known to induce glomerular and tubular renal damage as a part of the hemolytic uremic syndrome [27]. The drug can also cause interstitial pulmonary changes and cardiotoxic-

ity [2, 4, 18, 26]. In animals, pulmonary toxicity has not been observed while MMC has been found in high concentrations in the guinea pig heart [7] and is actually known to cause histological changes in the heart of Wistar rats [13] and dogs [20]. Little is known about renal damage induced by MMC in animals. A single dose of 0.5–2 mg/kg in rhesus monkeys [20] has been shown to cause hemorrhages in the renal cortex and necrotizing nephrosis, while repeated daily i.p. injections of 0.008–0.016 mg in SM and C3H/He mice caused epithelial changes of the renal pelvis, ureters and bladder, dilatation of renal tubules, and hydro-nephrosis [14]. Two i.p. injections of 4 mg MMC/kg caused cellular tubular changes in ICR mice [11].

The present study was performed mainly to look for possible MMC-induced acute or chronic, glomerular and/or tubular renal toxicity in rats. Additionally, histology of heart and lungs was studied for possible toxicity in these organs.

### Materials and methods

#### General information

Male Wistar rats (190–230 g), obtained from TNO-Zeist, the Netherlands, were kept individually in stainless steel metabolism cages. They were given a standard diet (Hope Farm) and had free access to food and drinking water throughout the study. All rats were decapitated immediately after ether anesthesia at the end of the study, after which 3–4 ml of blood was collected from the carotid arteries in heparinized tubes, for determination of serum creatinine and blood urea nitrogen (BUN).

The normal values (mean and range) of glomerular function, serum creatinine, and BUN were obtained in 15 control male rats. Pretreatment 24-h urine samples in the test rats served as normal urine values. Urinalysis included tests for pH, creatinine, total protein, *N*-acetyl- $\beta$ -glucuronidase (NAG) and alanine aminopeptidase (AAP). The latter two were used as parameters of tubular damage [15, 16].

The enzyme assays were performed according to minor modifications of the methods reported by Roth [23] for AAP and Dance et al. [5] for NAG. Biopsies for light microscopic study were stained with H&E and elastic-van Gieson (EVG), the latter being performed to reveal possible changes consistent with fibrosis.

*Offprint requests to:* J. Verweij, at his present address: Department of Medical Oncology, Rotterdam Cancer Institute, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands

### Drug administration

The MMC was dissolved as a 1 mg/ml solution and administered i.p. at the LD<sub>50</sub> dose of 2.5 mg/kg [20] in the single dose experiment. In the repeated dose experiment, the dose of MMC was 1.7 mg/kg and based on recalculation of the drug dose commonly used in humans. In the repeated dose experiment a higher dose was not used in order to avoid early death of the animals caused by drug toxicity. 5-Fluorouracil (5-FU) was dissolved as a 25 mg/ml solution, and administered i.p. at a dose of 10 mg/kg. Doses of 25–100 mg/kg were found to be too toxic in combination with the 1.7 mg/kg MMC dose (unpublished data).

Control rats received i.p. injections of 0.9% saline. All rats received a similar volume (2.1 ml/kg) of injected fluid. The number of injections in the repeated dose experiment was chosen arbitrarily.

### Single dose experiment

Starting 48 h before treatment and continuing up to 5 days afterwards 24-h urine samples were obtained daily. Six rats received MMC, six rats served as controls. At each of the following time points two MMC-treated rats were killed: 48 h, 72 h, 96 h, and 120 h, in order to obtain blood and kidney samples for histology. The control rats remained in the cage for a maximum of 120 h.

### Repeated dose experiment

A total of 27 rats were studied: 6 control rats received i.p. injections of 0.9% saline, 7 rats i.p. MMC, 7 rats 5-FU i.p., and 7 rats received a combination of MMC plus 5-FU. The injections were given on days 1, 8, 15, 22, and 29. On days -2, -1, 15, and 29 24-h urine samples were collected. All rats were killed on day 30, for blood tests and histology. At autopsy kidneys, lungs, and heart were removed.

### Statistics

Changes for separate groups were statistically analyzed using the Wilcoxon-Wilcox test. For the repeated dose experiment differences between the groups were tested by means of a Kruskal-Wallis one-way analysis of variance, plus the Mann-Whitney *U* test.

## Results

### Normal values

The normal values of the different parameters in Wistar rats are given in Table 1. Urinalysis additionally showed variations in the pH range from 6.9 to 8.5 and protein excretion never exceeded 2500 mg/l or 11 mg/day.

Table 1. Normal values in Wistar rats

Parameter		Mean	(Range)
Serum creatinine	( $\mu$ mol/l)	48.8	38–62
BUN	(mmol/l)	6.6	6.0–8.7
Urine volume	(ml/day)	6.8	1.0–15
AAP	(mU/day)	236	75–400
	(mU/l)	37	10–70
AAP/Creatinine ratio	(U/mol)	4029	1400–7000
NAG	(mU/day)	46	10–150
	(mU/l)	8.6	3–17
NAG/Creatinine ratio	(U/mol)	916	400–1800

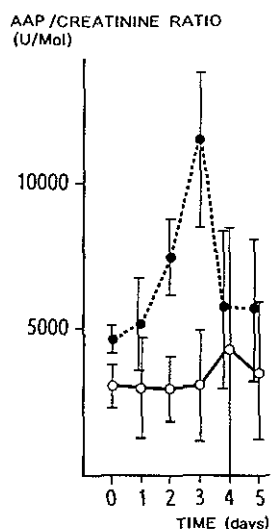


Fig. 1. Alaninoaminopeptidase (AAP)/creatinine ratio (median  $\pm$  2SEM, or range) in Wistar rats after administration of Mitomycin C (MMC) (●—●) or saline (○—○). The increase in the MMC group on day 3 was significant ( $P < 0.05$ )

### Single dose experiment

Serum creatinine, BUN, urinary volume, creatinine excretion, AAP excretion, and NAG excretion did not change in control animals over sequential days. For the MMC-treated animals the AAP/creatinine ratio was increased at 48–72 h (Fig. 1), partly due to a decrease in creatinine excretion, but mainly caused by an increase in AAP excretion. The increase was significant at 72 h ( $P < 0.05$ ). There were no significant changes in the other parameters studied, and renal histology was normal in all rats.

### Repeated dose experiment

All rats survived and were decapitated on day 30. The data obtained are shown in Figs. 2–4. The changes in urine volume were not significant, either sequentially or between the groups (Fig. 2).

The creatinine excretion changed significantly in the MMC group ( $P < 0.01$ ), while changes in the other groups were nonsignificant (Fig. 2). The differences in creatinine excretion between the groups were nonsignificant on days 0 and 15. On day 29 the value for the MMC group was significantly different compared to the control group ( $P < 0.02$ ) and the 5-FU and MMC/5-FU groups ( $P < 0.006$ ).

In the MMC-treated animals protein excretion decreased significantly ( $P < 0.001$ , Fig. 3), but not in the other groups. The differences between groups were not significant on days 0 and 15. The decrease in the MMC group on day 29 was significant as compared to the other groups ( $P < 0.001$ ). A significant decrease ( $P < 0.01$ ) in AAP excretion was only noted in the

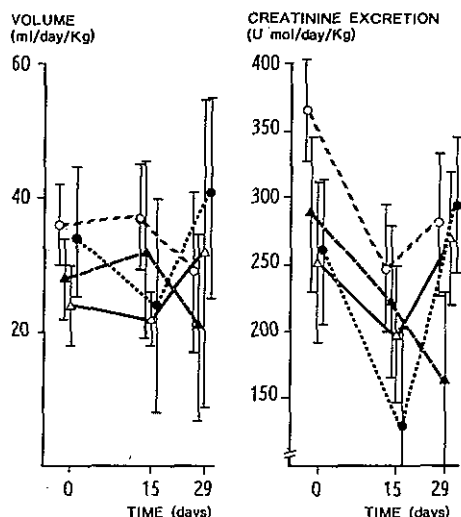


Fig. 2. Urine volume, and urinary creatinine excretion (median  $\pm$  2 SEM) during weekly administration of either saline ( $\Delta$ — $\Delta$ ), MMC ( $\blacktriangle$ — $\blacktriangle$ ), 5-fluorouracil (5-FU) ( $\circ$ — $\circ$ ) or MMC/5-FU ( $\bullet$ — $\bullet$ )

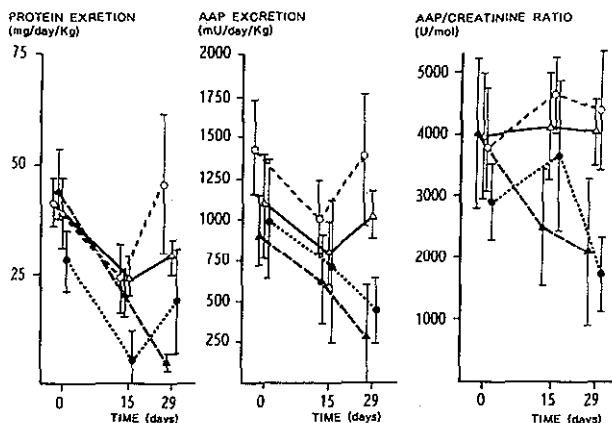


Fig. 3. Urinary protein excretion, AAP excretion and AAP/creatinine ratio (median  $\pm$  2SEM) during weekly administration of either saline ( $\Delta$ — $\Delta$ ), MMC ( $\blacktriangle$ — $\blacktriangle$ ), 5-FU ( $\circ$ — $\circ$ ) or MMC/5-FU ( $\bullet$ — $\bullet$ )

MMC group (Fig. 3). The differences between the groups were not significant on days 0 and 15, but the values on day 29 of the MMC and MMC/5-FU groups on one hand, and control and 5-FU groups on the other, differed significantly ( $P < 0.01$ ). The AAP/creatinine ratio changed significantly in the MMC and MMC/5-FU groups ( $P < 0.05$ ). The differences between groups were not significant on day 0 and 15, but the values on day 29 of the MMC and MMC/5-FU groups on one hand and the control and the 5-FU groups on the other, were significantly lower ( $P < 0.05$ ).

For NAG excretion (Fig. 4) there was a significant increase in the MMC/5-FU group ( $P < 0.05$ ). The differences between groups were not significant on days

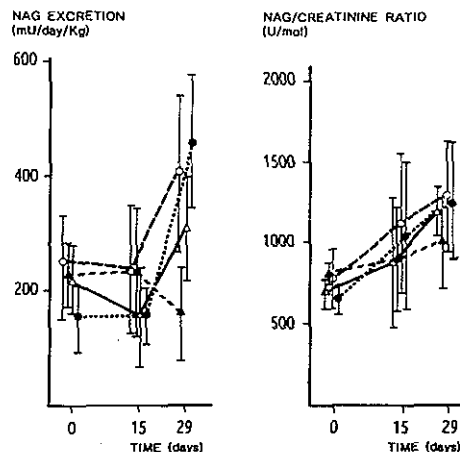


Fig. 4. Urinary *N*-acetyl- $\beta$ -glucuronidase (NAG) excretion and NAG/creatinine ratio (median 2SEM) during weekly administration of either saline ( $\Delta$ — $\Delta$ ), MMC ( $\blacktriangle$ — $\blacktriangle$ ), 5-FU ( $\circ$ — $\circ$ ) or MMC/5-FU ( $\bullet$ — $\bullet$ )

0 and 15. The value of the MMC/5-FU group on day 29 differed significantly from the MMC group ( $P < 0.001$ ) but not from the other groups. In fact, for the MMC group there was a slight decrease in NAG excretion, while for the other groups an increase was noted. The NAG/creatinine ratio (Fig. 4) changed significantly for the MMC group ( $P < 0.05$ ) and the MMC/5-FU group ( $P < 0.01$ ), but the differences between groups were not significant on all study days.

BUN and serum creatinine values were within normal ranges for all animals. Renal and cardiac histology was normal in all animals; the lungs showed changes in the MMC and MMC/5-FU groups (Fig. 5). In MMC-treated animals there was a varying degree of congestion in the alveolar septa, with normal EVG staining. In the MMC/5-FU group similar congestion was noted coinciding with marked lymphoid infiltration of the alveolar septa, again with normal EVG staining.

### Discussion

In the present study we investigated the occurrence of MMC-induced renal, pulmonary, and cardiac toxicity in Wistar rats as well as possible synergism with 5-FU concerning this toxicity.

A significant initial increase and late decrease in AAP excretion following i.v. MMC administration was found. AAP is a brush border enzyme [17] with a molecular weight estimated to be between 157,000 and 230,000 daltons [19, 24]. It is normally not filtered by the glomerulus and the main part of urinary AAP originates from the renal tubular cell membrane bound fraction [16]. The serum fraction is only excreted in the urine in cases of severe glomerular damage [1], which always coincides with proteinuria. Our single dose experiment on MMC indicated an increase

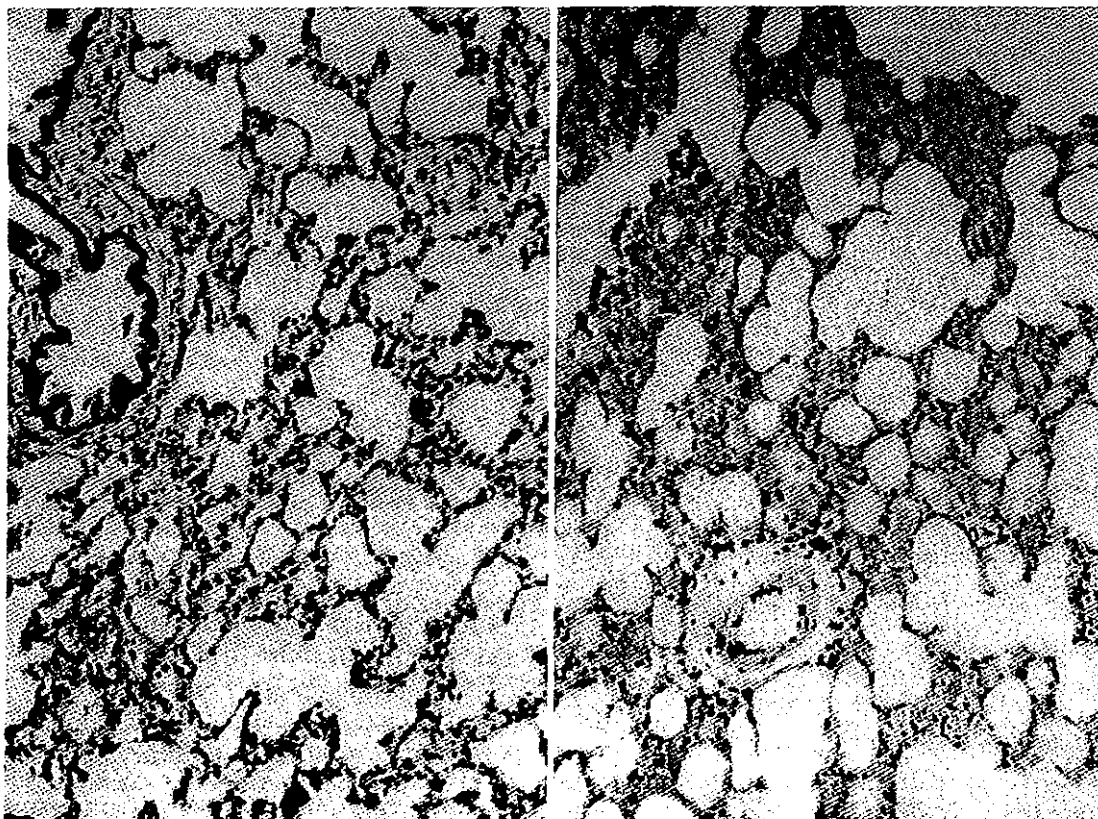


Fig. 5. Light microscopy of the lung (H&E staining) of a control rat (to the left) and MMC-treated rat (to the right), showing alveolar septal congestion in the MMC-treated rat

in the AAP/creatinine ratio, 48–72 h after administration of the drug. The repeated dose experiment showed a significant decrease in AAP excretion after repeated administrations of MMC, and a nonsignificant decrease after treatment with MMC/5-FU. The difference between those two groups on one hand and the control and 5-FU groups on the other was significant on day 29. The changes in AAP/creatinine ratio were in accordance with these data. These results indicate that MMC causes acute AAP leakage related to superficial tubular cell damage. This corresponds to electron microscopic observations in Sprague-Dawley and Lewis rats after renal perfusion with MMC [3]. Because of the repeated administrations of the drug in our study, repair from this damage may have been impossible, resulting in depletion of AAP in the tubular cells at the end of our repeated dose experiment, indicated by decreased levels of AAP in the urine. Such decreased levels of AAP have previously never been reported.

NAG is a hydrolytic enzyme, located mainly in the lysosomal fraction of the proximal renal tubular cell [12]. Since its molecular weight is 130,000–155,000 daltons [22] it does not normally pass through the glomerular membrane. For this reason the NAG in the urine has to originate from the renal tubular cells [10, 21]. The variation in NAG activity due to changing

rates of urine flow is almost eliminated by factoring enzyme activity by the urinary creatinine concentration [25, 28, 10]. In this way NAG has been shown to be a sensitive indicator of various types of renal damage such as nephrotic syndrome, allograft rejection, ischemia, glomerulonephritis, urinary tract infection, chronic renal failure, urinary obstruction, gentamycin nephrotoxicity, and hypertension [25, 28, 10, 6, 8, 9].

NAG decreased significantly in the MMC/5-FU group, and slightly in the MMC group. Again we have to state that decreases have never been reported previously, but these data also are compatible with our postulation on MMC tubular toxicity. Light microscopy did not show marked tubular changes in any of the groups studied. However, using electron microscopy, Kuroda et al. have previously observed an edematous nuclear matrix, decreases in heterochromatin and euchromatin, dilation of the nuclear membrane and endoplasmic reticulum, and swelling of the mitochondria in renal tubular cells in mice after i.p. injections of MMC [11]. Moreover, Matsuyama et al. [14], using daily i.p. injections of the LD<sub>50</sub> in mice, observed amongst others a diffuse cystic dilation of the renal tubules.

After repeated MMC administrations we observed a significant decrease in creatinine excretion. This decrease was also noted after recalculations of the creati-

nine excretion per body weight. Serum creatinine did not change in any of the groups in both experiments. Combined with excretion data, this indicates that after repeated MMC administration, creatinine clearance is probably impaired. However, distinct histopathological glomerular abnormalities could not be found.

Another fact that remains to be elucidated is the observed significant decrease in protein excretion after repeated administrations of MMC. This decrease appears not to be related to a decrease in body weight, and cannot be explained by histopathological changes. A decreased supply of protein to the kidney, a decreased glomerular filtration, or increased protein reabsorption in the renal tubules, remain other possible reasons for the observed decrease in protein excretion.

While the light microscopy studies did not reveal renal or cardiac changes in any of the treatment groups, in the MMC group alveolar septal congestion was noted in the lungs without an increase in collagen in the EVG staining. Thus, fibrosis does not appear to be present. As the heart did not show histological changes in this group, the reason for this congestion remains unexplained. It may be that these changes represent early stages of pulmonary fibrosis.

In conclusion, our data suggest MMC-related damage of the brush border of renal tubular cells, as well as MMC-related alveolar septal congestion in the lungs. To what extent these changes are early stages of toxicity in both organs, remains to be elucidated. A synergism between MMC and 5-FU as far as toxicity is concerned, could not be found.

## References

- Burchardt U, Peters JE, Neef L, Thulin H, Grundig CA, Haschen RJ (1977) Der diagnostische Wert von Enzymbestimmungen im Harn. *Z Med Lab Diagn* 18:190-212
- Buzdar AU, Legha SW, Tashima ChK, Hortobagyi GN, Yap HY, Krutchik AM, Luna MA, Blumenschein GR (1978) Adriamycin and Mitomycin C: possible synergistic cardiotoxicity. *Cancer Treat Rep* 62:1005-1008
- Cattell V (1985) Mitomycin-induced hemolytic uremic kidney. An experimental model in the rat. *Am J Pathol* 121:88-95
- Creech RH, Catalano RB, Shah MK, Dayal H (1983) An effective low-dose mitomycin regimen for hormonal- and chemotherapy-refractory patients with metastatic breast cancer. *Cancer* 51:1034-1040
- Dance N, Price RG, Robinson D, Stirling JL (1969) Galactosidase,  $\beta$ -glucosidase and *N*-acetyl- $\beta$ -glucosaminidase in human kidney. *Clin Chim Acta* 24:189-197
- Diener U, Knoll E, Langer B, Rautenstrauch H, Ratge D, Wisser H (1981) Urinary excretion of *N*-acetyl- $\beta$ -D-glucosaminidase and alanine aminopeptidase in patients receiving amikacin or cis-platinum. *Clin Chim Acta* 112:149-157
- Fujita H (1971) Comparative studies on the blood level, tissue distribution, excretion and inactivation of anticancer drugs. *Jpn J Clin Oncol* 12:151-162
- Gibey R, Dupond JL, Alber D, Leconte des Floris R, Henry JC (1981) Predictive value of urinary *N*-acetyl- $\beta$ -D-glucosaminidase (NAG), alanine aminopeptidase (AAP) and B<sub>2</sub>-microglobulin (B<sub>2</sub>M) in evaluating nephrotoxicity of gentamycin. *Clin Chim Acta* 116:24-34
- Johnston IDA, Jones NF, Scoble JE, Yuen CT, Price RG (1983) The diagnostic value of urinary enzyme measurements in hypertension. *Clin Chim Acta* 133:317-325
- Kunin CM, Chesney RW, Craig WA (1978) Enzymuria as a marker of renal injury and disease: studies of *N*-acetyl- $\beta$ -glucosaminidase in the general population and in patients with renal disease. *Pediatrics* 62:751-760
- Kuroda K, Teranishi S, Akao M (1982) Toxicity of Mitomycin C and anti-intoxication by fumaric acid in liver and kidney cellular fine structure. *Gann* 73:656-660
- Le Hir M, Dubach UC, Schmidt U (1979) Quantitative distribution of lysosomal hydrolases in the rat nephron. *Histochemistry* 63:245-251
- Levillain R, Cluzan R (1973) Cardiac toxicity of antimetabolic drugs. *Proc 3rd Meeting EACR*, p 100
- Matsuyama M, Suzumori K, Nakamura T (1964) Biological studies of anti-cancer agents. I. Effects of prolonged intraperitoneal injections of mitomycin C on the urinary organs. *J Urol* 92:618-620
- Mattenheimer H (1977) Enzymes in renal disease. *Am Clin Lab Sci* 7:422-432
- Mondorf AW (1982) Enzymologie der Niere. In: Losse H, Renner E (eds) *Klinische Nephrologie*. Thieme, Stuttgart, S 123-129
- Mondorf AW, Kinne R, Scherberich JE, Falkenberg F (1972) Isolierung enzymatische und immunologische Charakterisierung einer Plasmamembranfraktion von proximalen Tubulus der menschlichen Niere. *Clin Chim Acta* 37:25-32
- Orwoll ES, Kiessling PJ, Patterson JR (1978) Interstitial pneumonia from mitomycin. *Ann Int Med* 89:352-355
- Peters JE, Schneider I, Haschen RJ (1972) Bestimmung der L-alanyl-peptidhydrolase (Alaninaminopeptidase, Aminosaurerearylaminidase) im menschlichen Harn. *Clin Chim Acta* 36:289-301
- Philips FS, Schwartz HS, Sternberg SS (1960) Pharmacology of mitomycin C. I. Toxicity and pathologic effects. *Cancer Res* 20:1354-1361
- Price RG, Dance N (1967) The cellular distribution of some rat and kidney glycosidases. *Biochem J* 105:877-883
- Robinson D, Price RG, Dance N (1967) Separation and properties of  $\beta$ -galactosidase,  $\beta$ -glucosidase,  $\beta$ -glucuronidase, and *N*-acetyl- $\beta$ -glucosaminidase from rat kidney. *Biochem J* 102:525-532
- Roth M (1964) Une ultramicro methode fluorometrique pour le dosage de la leucine aminopeptidase dans les liquides biologiques. *Clin Chim Acta* 9:448
- Scherberich JE, Falkenberg F, Mondorf AW, Muller H, Pfeleiderer G (1974) Biochemical and immunological studies on isolated brush border membranes of human kidney and their membrane surface proteins. *Clin Chim Acta* 45:179-197
- Sherman RL, Drayer DE, Leyland-Jones BR, Reidenberg MM (1983) *N*-acetyl- $\beta$ -glucosaminidase and  $\beta$ 2-microglobulin. *Arch Int Med* 143:1183-1185
- Suzuki Y, Nübe H (1975) Radiation-induced heart injury: radiopathological study. *Kita Kanto Igaku* 25:395-407
- Verweij J, Boven E, van der Meulen J, Pinedo HM (1984) Recovery from mitomycin C-induced hemolytic uremic syndrome. A case report. *Cancer* 54:2878-2881
- Wellwood JM, Ellis BG, Price RG, Hammond K, Thompson AE, Jones NF (1975) Urinary *N*-acetyl- $\beta$ -D-glucosaminidase activities in patients with renal disease. *Br Med J* 3:408-411

Received July 6, 1987/Accepted October 12, 1987