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# *Preliminary Communications*

## Papillary Necrosis in Experimental Analgesic Nephropathy

*Brit. med. J.*, 1969, **1**, 161–162

**Summary: A proprietary aspirin, phenacetin, and caffeine preparation given to rats in a dose equivalent to that taken by patients with analgesic nephropathy produced papillary necrosis in 55%. There was a higher incidence in rats deprived of fluids overnight.**

**Papillary necrosis was not noted in rats receiving twice as much phenacetin.**

**These findings support the argument that phenacetin should not be singled out as the substance responsible for analgesic nephropathy in man.**

### INTRODUCTION

Animal experiments have usually failed to show any convincing lesions in the kidney after prolonged administration of analgesics (Shelley, 1967). Papillary necrosis is the characteristic lesion in analgesic nephropathy in man (Kincaid-Smith, 1967a), and this paper reports the occurrence of papillary necrosis in a high percentage of rats receiving a proprietary aspirin, phenacetin, and caffeine (A.P.C.) mixture. Papillary necrosis was not noted in rats receiving twice as much phenacetin as that contained in the A.P.C. mixture.

### MATERIALS AND METHODS

Wistar rats weighing 150 g. received by gavage on five days each week phenacetin 500 mg./kg/day or a proprietary analgesic mixture containing phenacetin 210 mg./kg./day, aspirin 210 mg./kg./day, and caffeine 80 mg./kg./day. A control group did not receive drugs. Half the animals in each group were deprived of fluids overnight (see Table). This produced a striking rise in urine osmolarity and may thus have potentiated any toxic effect of the analgesics on the medulla.

Findings in animals killed at three months and five months have been reported (Kincaid-Smith *et al.*, 1968). It had been planned to continue the experiment for a year, but very heavy losses in the A.P.C. group after six months made it necessary to kill all the remaining animals between six and nine months.

### RESULTS

Ten out of 18 rats receiving the proprietary A.P.C. mixture for more than six months developed papillary necrosis (see Table). A further 10 rats receiving A.P.C. died over this period but were not satisfactory for examination owing to cannibalism and autolysis. Papillary necrosis was noted in six (75%) out of eight rats deprived of fluids overnight and in 4 (40%) out of 10 rats with continuous access to water. Papillary necrosis was not noted in the control group (28) or in rats receiving phenacetin alone (27) in a dose of 500 mg./kg./day.

*Table of Results*

| | Control | | Phenacetin | | A.P.C. | |
|---|---|---|---|---|---|---|
| | Free Fluid | Restricted Fluid | Free Fluid | Restricted Fluid | Free Fluid | Restricted Fluid |
| No. of rats killed 6–9 months .. | 14 | 14 | 14 | 13* | 10† | 8‡ |
| Papillary necrosis | 0 | 0 | 0 | 0 | 4 | 6 |

* 1 not included owing to cannibalism and/or autolysis.
† 4 not included owing to cannibalism and/or autolysis.
‡ 6 not included owing to cannibalism and/or autolysis.

Four animals with papillary necrosis showed cortical scars, and these could be seen in serial sections to result from atrophy of tubules which were obstructed in the papillary lesion. The cortex was normal in animals with normal papillae except for a patchy increase in lipofuchsin pigment in tubular cells in some animals receiving phenacetin and A.P.C.

Some abnormalities were noted in the papillae in rats receiving phenacetin alone; these included patchy cast formation and an unidentified pigmented substance in the interstitium of the medulla in a few animals. The lesions in the vasa recta noted in the early months of this experiment (Kincaid-Smith *et al.*, 1968) did not seem to advance between six and nine months and no definite thrombosis or occlusion of these vessels was noted. Platelets were, however, noted adhering to the endothelium in some abnormal vessels.

### DISCUSSION

We have confirmed the high incidence of papillary necrosis in rats given a proprietary A.P.C. mixture for between six and nine months and also the pathogenesis of the renal lesions in these animals. Papillary necrosis precedes cortical scars, and these result from atrophy of tubules obstructed by the necrosis in the papilla (Abrahams and Levin, 1967; Kincaid-Smith *et al.*, 1968). This is a similar pathogenesis to that described in analgesic nephropathy in man (Kincaid-Smith, 1967a).

The dose of A.P.C. mixture given to the rats was equivalent in mg./kg./day to that taken by some patients with analgesic nephropathy.

Phenacetin has been generally assumed to be the cause of renal damage in analgesic nephropathy in man, though evidence against phenacetin depends mainly on the fact that it is present in most of the mixtures said to cause renal damage. Recently attention has been drawn to the lack of direct evidence against

phenacetin and increasing evidence that other analgesic substances may damage the kidney (Creasy, 1964 ; Prescott, 1965 ; Kincaid-Smith, 1967b ; Shelley, 1967 ; Brown and Hardy, 1968 ; Bulger *et al.*, 1968 ; *Lancet*, 1968).

This study adds further weight to the argument that phenacetin should not be singled out as the substance responsible for papillary necrosis in patients who take large amounts of various analgesic mixtures.

Papillary necrosis did not develop in rats receiving phenacetin alone even though those rats received more than twice the dose of phenacetin given to those on the A.P.C. mixture. The papillary necrosis noted in animals rceiving A.P.C. must therefore have been due to some ingredient of the A.P.C. mixture other than phenacetin, perhaps a contaminant, or to a combined effect of more than one ingredient.

In view of our findings, and because of increasing evidence that salicylates, antipyrine, amidopyrine, and other analgesic substances may cause renal damage, including papillary necrosis (Lotze, 1934 ; Axelsson, 1958 ; Harvald, 1963 ; Eknoyan and Matson, 1964 ; Prescott, 1965 ; Lawson and Maclean, 1966 ; Olafsson *et al.*, 1966 ; Brown and Hardy, 1968 ; Bulger *et al.*, 1968), it seems unreasonable to attempt to control analgesic nephropathy by warning labels attached only to preparations containing phenacetin.

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# Kidney Lesions Induced in Rats by P-aminophenol

*Brit. med. J.*, 1969, **1**, 162–164

**Summary: Necrosis of the terminal third of the proximal convulated tubule develops in rats after a single intravenous injection of p-aminophenol hydrochloride. As the tubules regenerate a chronic inflammatory reaction occurs in the interstitial tissue, and this reaction extends beyond the original zone of injury. These findings are additional evidence that some aromatic compounds are selectively nephrotoxic and may be particularly relevant to the problem of renal damage associated with heavy and prolonged doses of analgesics.**

## INTRODUCTION

The association of prolonged and heavy doses of analgesic compounds with both papillary necrosis and chronic renal lesions was first described by Spühler and Zollinger (1953). Its exact pathogenesis is still unknown. Three factors are believed to be implicated: ischaemia (Kincaid-Smith, Saker, McKenzie, and Muriden, 1968), infection (Lauler, Schreiner, and David, 1960), and direct toxicity of phenacetin metabolites (Zollinger, 1960). In man 80–90% of 1–2-g. doses of phenacetin is excreted within 24 hours as *p*-acetamidophenol (paracetamol) or its conjugates (Brodie and Axelrod, 1949). A small amount is deacetylated to *p*-phenetidine, and this metabolite and its breakdown products have been considered to be responsible for the methaemoglobinaemia sometimes seen in patients taking phenacetin (Brodie and Axelrod, 1949 ; Gilman, 1964). We are currently investigating the nephrotoxicity to rats of compounds structurally related to phenacetin, using a single sublethal intravenous dose. This procedure avoids many of the difficulties associated with prolonged oral administration of relatively large doses of analgesic compounds. This communication describes the changes following injection of *p*-aminophenol hydrochloride.

## METHODS

Thirty female hooded rats, each weighing 150–200 g., were divided into three groups. The animals in each group received a single intravenous injection of 20, 40, or 60 mg. of *p*-aminophenol hydrochloride (B.D.H.) in freshly prepared aqueous solution. Pairs of animals from each group were killed at 24, 48, and 96 hours and at one and two weeks after injection. Five similar animals served as controls. Tissues were fixed in 10% neutral formalin and embedded in paraffin ; sections were stained with haematoxylin and eosin and some with P.A.S. and Mallory's trichrome.

## RESULTS

Almost immediately after injection the animals became cyanosed and remained so for one to two hours. The animals continued to excrete urine and appeared well until the end of the experiment.

The renal capsular surfaces were generally normal ; those of three animals killed at two weeks were finely granular. On hemisection a pale zone, 1–2 mm. in width, was situated on the cortical side of the corticomedullary junction. This was easily seen in animals killed at one week or earlier, but not in those killed at two weeks. Renal papillae were normal.