

indexes are always below 1.0. On the other hand, the rate of emergence of resistance is not always reduced by the presence of trimethoprim (table 2).

**Discussion.** Our speculation, based on the mechanisms of action, that the combination of nalidixic or oxolinic acids and trimethoprim must lead to the strengthening of the antibacterial activity and delay the emergence of bacterial resistance, has been substantially confirmed by the experimental results. Bearing in mind the pharmacokinetics of these drugs<sup>21-24</sup>, it seems to us that such combinations deserve clinical evaluation of their potential usefulness in the treatment of urinary tract infections.

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## Renal polycystosis in the rat induced by prednisolone tertiary butyl acetate

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**Summary.** A single i.m. injection of 66 mg/kg prednisolone tertiary butyl acetate given on the 1st day of life produced glomerular degeneration and collecting duct and proximal tubule cysts in rat kidneys. There was evidence of delayed nephrogenesis leading to persistence of the neogenic zone.

Ignorance about the pathogenesis of human polycystic kidney disease has prompted a number of attempts to find suitable animal models<sup>2</sup>. Prednisolone tertiary butyl acetate (PTBA) has been shown to produce collecting duct cysts in rabbits<sup>3</sup> and collecting duct and distal tubular cysts in rats<sup>4</sup>. We report here histological studies of its effects on the development of the newborn rat kidney which extend the previous observations.

**Materials and methods.** 40 litters of Sprague-Dawley derived rats were used. Each litter was culled to 10 animals on the day of birth (= day 1) and 7 were given an i.m. injection of Codelcortone TBA (Merck, Sharp, and Dohme) equivalent to 66 mg PTBA/kg b.w. The 3 remaining animals in each litter received an equivalent volume of isotonic saline.

Animals were killed with chloroform on days 4, 8, 10, 12, 14 and 16 and the kidneys were either fixed in Bouin's fluid for paraffin processing, or in 10% phosphate buffered formalin for embedding in methyl methacrylate resin<sup>5</sup>. Paraffin sections were cut at 7 µm and stained with Masson's trichrome, Harris' haematoxylin and eosin, or PAS. Methacrylate sections were cut at 2 µm and stained with Delafield's haematoxylin and phloxine or PAS.

**Results.** The parts of the kidney most affected by the treatment were the glomeruli, the proximal tubules, and the collecting ducts. The terminal parts and ampullary regions of some collecting ducts were found to be slightly dilated at day 4 and dilation was more obvious at older ages (figure 1). From day 8 onwards variable numbers of medullary collecting ducts also had become cystic.

Signs of glomerular degeneration were observed on day 4 but this was more obvious from day 8 onwards. The glomerular tufts of some Malpighian bodies were degenerating and Bowman's space slightly or extensively dilated (figure 2). The glomeruli effected were at the periphery, the juxtamedullary ones were normal.

Occasional proximal tubules were mildly cystic in the outer cortex at days 4 and 8 but dilatation was more obvious at later stages (figure 2). From day 12 the cells in the walls of these cysts often became vacuolated and small spheres of cytoplasm or whole cells were seen being shed into the lumen. The PAS technique revealed sloughing-off the brush border and apices of the cells into the lumen of the larger cysts, all this material apparently contributing to PAS-positive casts found lower down in the collecting ducts. At days 14 and 16 there was often evidence of marked fibrosis around the cysts. None of the collecting duct or proximal tubule cysts exhibited obvious hyperplasia of their wall. The neogenic zone in control animals had disappeared by day 8 but it persisted in patches in many PTBA treated kidneys until day 16.

**Discussion.** An earlier study<sup>3</sup> of the effects of PTBA on newborn rats concluded that in the first 8 days post-partum renal cysts were distal tubular or collecting duct in origin and that the glomeruli were not affected. We have observed evidence of glomerular degeneration as early as day 4 and this was very marked at older ages. In addition proximal tubule dilatation was seen from day 8 onwards. It appears from our studies that the first parts of the kidney to be affected by PTBA are selected ampullary regions of the

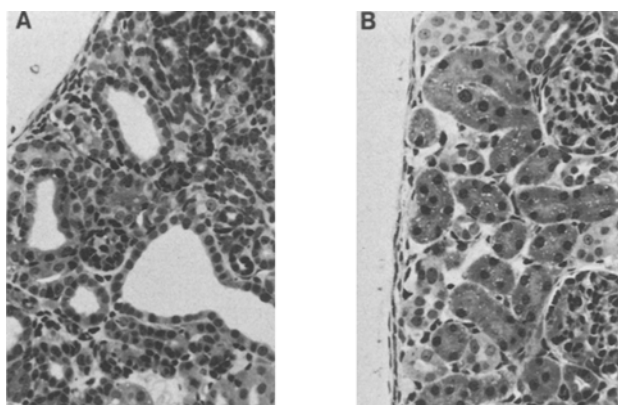


Fig. 1. *a* PTBA injected day 12, showing dilated ampulla in persistent neogenic zone. *b* Day 12 control.  $\times 200$ .

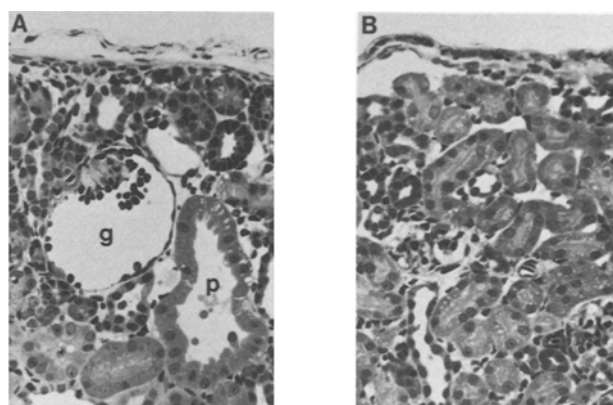


Fig. 2. *a* PTBA injected day 14, showing glomerular degeneration (g) and dilated proximal tubule (p). *b* Day 14 control.  $\times 200$ .

collecting ducts which enlarge. At a later stage the medullary collecting ducts dilate. The peripheral glomeruli and proximal tubules become involved from day 8 onwards although mild dilatation was seen on day 4. The ampullary regions of collecting ducts have a unique role because they induce new nephrons<sup>6</sup> before and after birth in the rat. Whether dilatation of an ampulla affects its inductive properties is not known although persistence of the neogenic zone in our older animals suggests that nephrogenesis has been delayed for some reason. The fact that glomerular degeneration and proximal tubule dilatation is limited to the outer cortex suggests that the steroid must affect the newly forming nephrons more than the older more deeply situated ones.

The action that PTBA and related steroids have at the cell level to produce the cysts is not known. Hypokalaemia has been implicated<sup>3,7</sup> as also have, hyperplasia of the tubule

wall<sup>4</sup>, and changes in elastic properties of the basement membrane<sup>4,7</sup>. We are at present studying the earliest ultrastructural changes that take place in the cells of the renal tubules in an attempt to determine the cause of the cysts.

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## Cytopathological effects of aristolochic acid on male houseflies causing sterility

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**Summary.** Feeding male houseflies with 0.075% aristolochic acid obtained from the plants of the genus *Aristolochia* induced sterility. High condensation of chromosomes, and breakages, have been shown to be responsible for sterility. When onion roots were exposed to aristolochic acid similar chromosomal aberrations were noticed in cells at the tip.

The autocidal method of insect control has added a new dimension to the concept of pest management, in which the environmental quality is saved from deterioration due to the dumping of toxicants. Cytotoxic compounds have a potential as chemosterilants, and aristolochic acid, obtainable from the roots of plants belonging to the genus *Aristolochia*<sup>2,3</sup> and reported to be cytotoxic<sup>4</sup>, was screened as part of our programme on the study of antgonadial agents on insects<sup>5</sup>. Already it has been proved that the root extract of *Aristolochia indica* L. induces sterility in male houseflies<sup>6</sup>, and has an antispermatic effect in male mice<sup>7</sup>.

**Material and methods.** Houseflies (*Musca domestica* L.) immediately after eclosion were fed on 0.075% aristolochic acid in the manner already described<sup>6</sup>. Testes of treated males siring sterile eggs (sterility 95%) were fixed in aceto-alcohol, stained in aceto-orcin and squashed in aceto-carmine for microscopic examination.

**Results.** When treated males have sired the eggs, hatchability of the latter has been severely reduced; however, in a few eggs one could see the development of a larva which failed to hatch and died shortly before hatching<sup>6</sup>. Possible clues to the reason for the sterile eggs and larval death have been provided by the behaviour of the chromosomes of the spermatogenic cells during meiosis under the influence of aristolochic acid. The pathological manifestations in the nucleus are: a) A smaller number of dividing cells as compared with the control; b) high condensation of chromosomes in meiotic prophase and metaphase as compared with the control; c) prolonged prophase; d) breakage of chromosomes (figures 1 and 2).

The sperms produced from the cells with cytological aberrations do not lead to embryogenesis, or embryonic development stops at a certain stage.

The male germ cells in the housefly are very small and, since light microscopy unravels little because of inherent