

Effect of the anti-oestrogen Tamoxifen on the development of renal cortical necrosis induced by oestrone + vasopressin administration in rats

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Summary. Bilateral renal cortical necrosis was observed after vasopressin administration in rats pretreated with oestrone acetate. Histochemical (succinic dehydrogenase, trichrome, periodic acid Schiff) and electronmicroscopic methods were used to examine how the anti-oestrogen, Tamoxifen, influences the development of this renal cortical necrosis. The experiments revealed that in most rats vasopressin did not induce renal tubular necrosis if the anti-oestrogen was administered simultaneously, even during oestrogen pretreatment. The results suggest that oestrogen receptors in the kidney are involved in the induction of renal cortical necrosis by vasopressin.

Keywords: oestrone, vasopressin, renal cortical necrosis, anti-oestrogen

The administration of vasopressin preparations to rats following prolonged oestrone treatment induces bilateral renal cortical necrosis (Byrom 1938; Byrom & Pratt 1959; Kovács *et al.* 1964). Without oestrogen pretreatment, the renal cortical necrosis does not occur after the given vasopressin injection alone. Study of the pathomechanism revealed that oestrogen sensitizes the renal vessels to the vasoconstrictive effect of vasopressin (Lloyd 1959*a,b*; Lloyd & Pickford 1962; Deis *et al.* 1963; Kovács *et al.* 1965; Kocsis *et al.* 1979; 1987; László 1981). To decide the question of whether the oestrogens exert their sensitizing effects via their specific receptors, experiments were carried out with the anti-oestrogen Tamoxifen (4β -dimethylaminoethoxyphenyl-1,2-diphenyl-1-butane citrate). The anti-oestrogenic acti-

vity of Tamoxifen can be attributed to competitive oestrogen antagonism acting at the periphery: it acts by blocking the oestrogen receptors (Skidmore *et al.* 1972; Lippman *et al.* 1976; Jordan *et al.* 1977; Capony & Rochefort 1978; Katzenellenbogen *et al.* 1978; Borgna & Rochefort 1980). The problem was also investigated of whether the renal cortical necrosis can be prevented by the simultaneous administration of the anti-oestrogen. The work reported here was carried out in the hope of answering these questions.

Materials and methods

Examinations were performed on 75 male white R-Amsterdam rats weighing 170–210 g, maintained on a standard diet. The ani-

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mals were divided into five groups, each of 15 rats.

Group 1. For 10 days a daily dose of 1.0 mg oestrone acetate (Hogival, Chinoim) was administered s.c.

Group 2. A dose of 10 iu synthetic lysine-vasopressin (Sandoz) was administered s.c.

Group 3. For 10 days a daily dose of 2.0 mg Tamoxifen (Zitazonium, EGYT) was administered via a feeding tube.

Group 4. For 10 days a daily dose of 1.0 mg oestrone acetate was administered s.c., with a single dose of 10 iu synthetic lysine-vasopressin s.c. on day 10.

Group 5. For 10 days the rats were treated with a daily dose of 1.0 mg oestrone acetate s.c. and a daily dose of 2.0 mg Tamoxifen via a feeding tube. On day 10, 10 iu lysine-vasopressin was administered s.c.

The animals treated with oestrone acetate or with Tamoxifen only, were killed by decapitation on day 10 of treatment, and the other animals 24 h after vasopressin administration (10 animals in each group) and the kidneys were examined histologically. For light microscopic investigations two pieces of tissue were prepared. One half of the kidney was sectioned with a cryostat. Succinic dehydrogenase (SDH) activity was detected according to the method of Pearse (1972). The other half of the kidney was fixed in buffered formaldehyde and embedded in paraffin. Slides 4–6 μm thick were stained with the periodic acid Schiff (PAS) technique, and by Masson's trichrome method (Putt 1972).

For electron-microscopy, the five animals remaining in each group were anaesthetized with ether and perfused through the left ventricle with a fixative containing 4% formaldehyde (freshly prepared from paraformaldehyde) and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer ($\text{pH}=7.4$). After

perfusion for 30 min, pieces of tissue excised from the kidney were postfixed in the same solution for 6 h, washed overnight in 0.1 M sodium cacodylate buffer ($\text{pH}=7.0$) and postfixed in 1% buffered OsO_4 for 2 h. Subsequently, the blocks were dehydrated in graded alcohols and were finally embedded in Araldite. Ultrathin sections were cut on a Reichert Ultratome, contrasted with uranyl acetate and lead citrate, and examined under a Tesla BS 500 electron-microscope.

Results

Vasopressin administered after oestrogen pretreatment gave rise to characteristic morphological changes. Both kidneys were slightly enlarged. On gross inspection the outer surface showed a reddish-yellow, irregular, patchy mottling. On the cut surface there were yellowish, infarct-like necrotic areas of various sizes and irregular outline, fairly sharply defined, and partially coalesced. This cortical necrosis was detected in every rat, but the extent varied from animal to animal.

Histologically, large areas of the cortex were necrotic, the proximal convoluted tubules had no nuclei and their cytoplasm was swollen, homogeneous and eosinophilic (Fig. 1a).

SDH activity was not detected in the necrotic tubular epithelia (Fig. 2a) and many casts developed from the desquamated tubular epithelial cells (Fig. 3a). Necrosis with complete disappearance of the brush border of the proximal tubules could be observed by means of the PAS technique. In small areas of the epithelial cells a hyaline-drop degeneration was observed (Fig. 4a).

In the rats treated simultaneously with Tamoxifen, vasopressin given after oestrone pretreatment did not cause a macroscopic change. Histologically, the structure of both glomeruli and tubules was preserved, and no necrotic tissue was seen in the renal cortex (Fig. 1b). However, two rats developed mild focal necrosis of the proximal convoluted tubules (Fig. 1c). In these animals (Group 5)

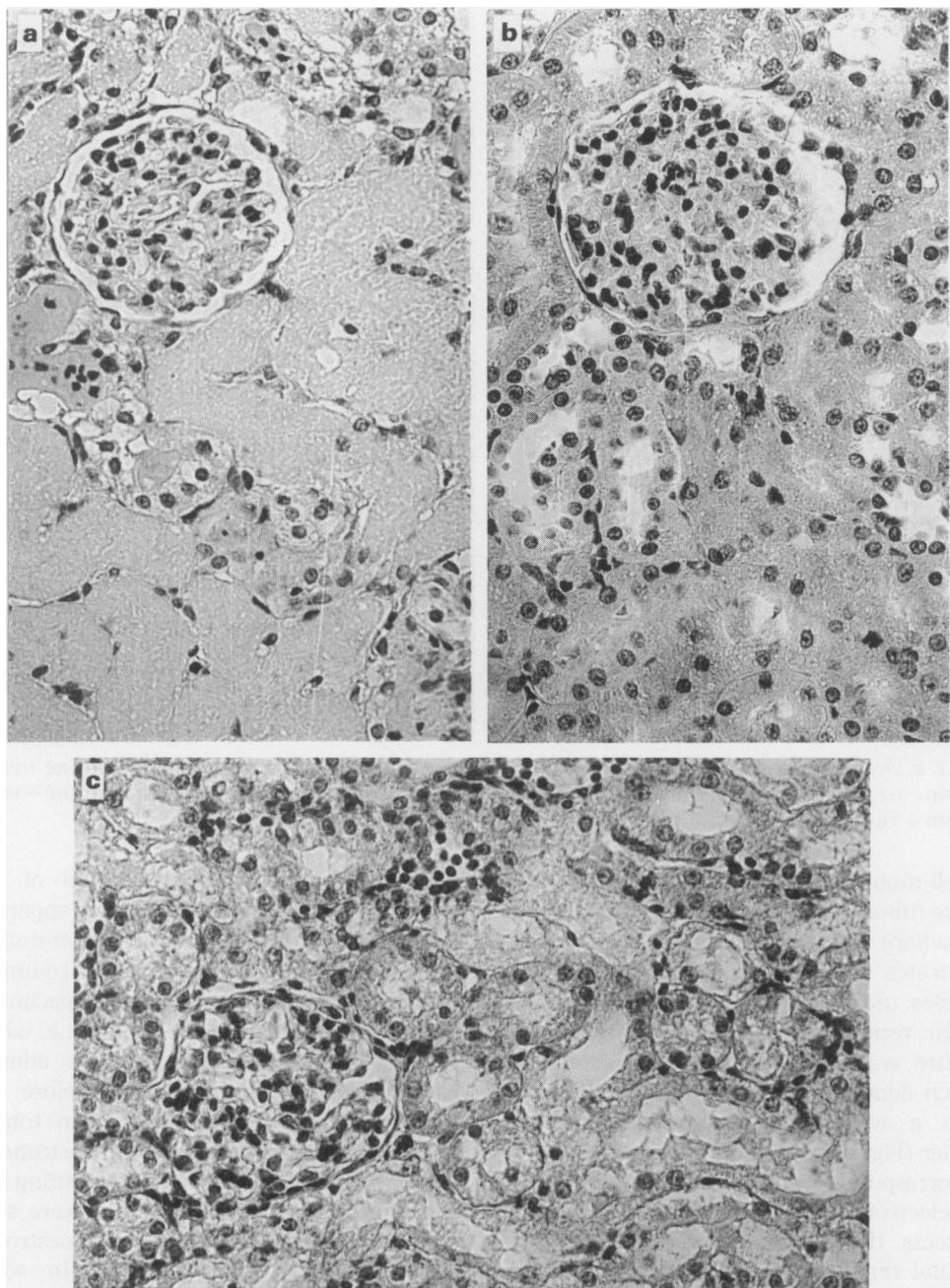


Fig. 1. *a*, Cortical necrosis with total damage of proximal convoluted tubular epithelia in rats treated with oestrone + vasopressin. H & E, $\times 300$. *b*, Preserved structure of renal cortex in animals treated with oestrone + vasopressin + Tamoxifen. H & E, $\times 300$. *c*, Partial prevention of damage to tubular epithelia in rats treated with oestrone + vasopressin + Tamoxifen. H & E, $\times 300$.

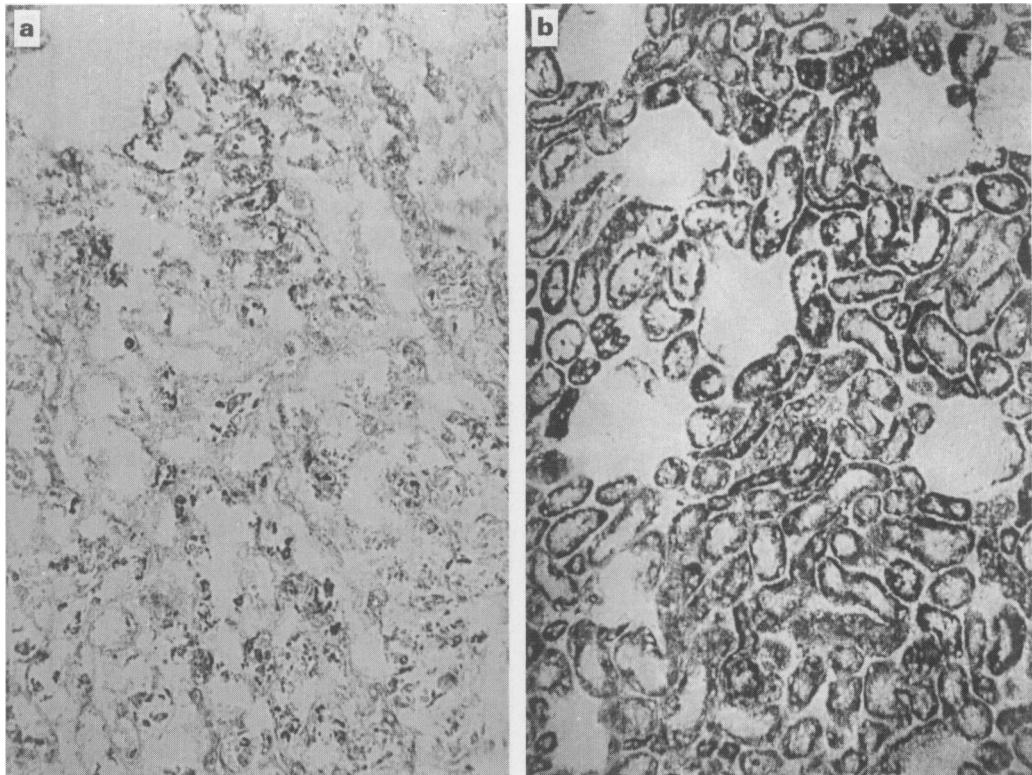


Fig. 2. *a*, Decreased or absent SDH activity in the tubular epithelia in rats treated with oestrone + vasopressin. $\times 125$. *b*, Well-maintained SDH activity of renal cortex in animals treated with oestrone + vasopressin + Tamoxifen. $\times 185$.

a well-maintained SDH activity could be seen in the tubular epithelial cells (Fig. 2*b*). Figure 3*b*, where the trichrome method was used, illustrates the preserved structure of the tubules; only a mild degeneration developed in the renal cortex. The same histological picture was observed after PAS staining, which demonstrated normal epithelial cells with a well-distinguished line of brush border (Fig. 4*b*).

Corresponding changes could be seen in the electron-microscopic pictures. Figure 5*a* presents the ultrastructural picture of a normal renal cortex. At 24h after the administration of vasopressin, extensive necrosis of the epithelial cells could be observed in the region of the proximal convoluted tubules of the oestrone-pretreated rats (Group 4). Rupture of the cell membrane

and the complete disorganization of the structure of the epithelial cells were apparent (Fig. 5*b*). In sharp contrast, electron-microscopy did not indicate any structural damage of the epithelial cells in tissue specimens obtained from the rats of Group 5, i.e. when the anti-oestrogen Tamoxifen was administered together with oestrone before the injection of vasopressin Tamoxifen totally inhibited the sensitizing effect of oestrone in most rats (Fig. 5*c*) while in the remaining few rats the ultrastructural changes were substantially milder than following oestrone+vasopressin treatment alone. In some places the brush border was disorganized, and intracellular organelles, e.g. mitochondria, were swollen, while the intercellular spaces and the basal invaginations were enlarged (Fig. 5*d*).

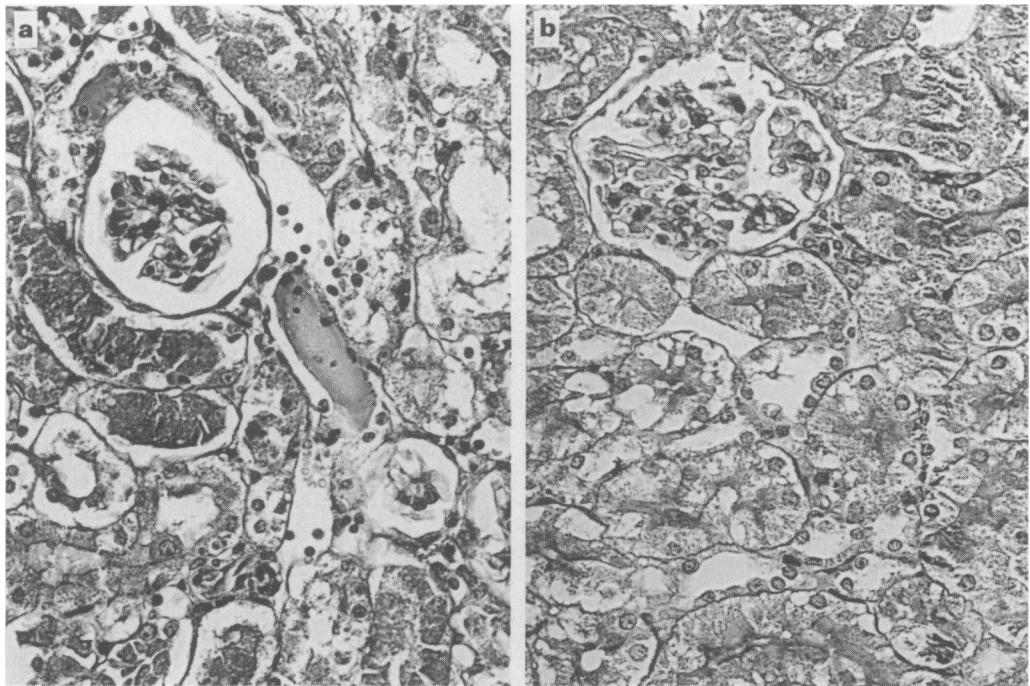


Fig. 3. *a*, Casts from desquamated tubular epithelial cells in rats treated with oestrone + vasopressin. Trichrome, $\times 300$. *b*, Mild degeneration of tubular epithelia in animals treated with oestrone + vasopressin + Tamoxifen. Trichrome, $\times 300$.

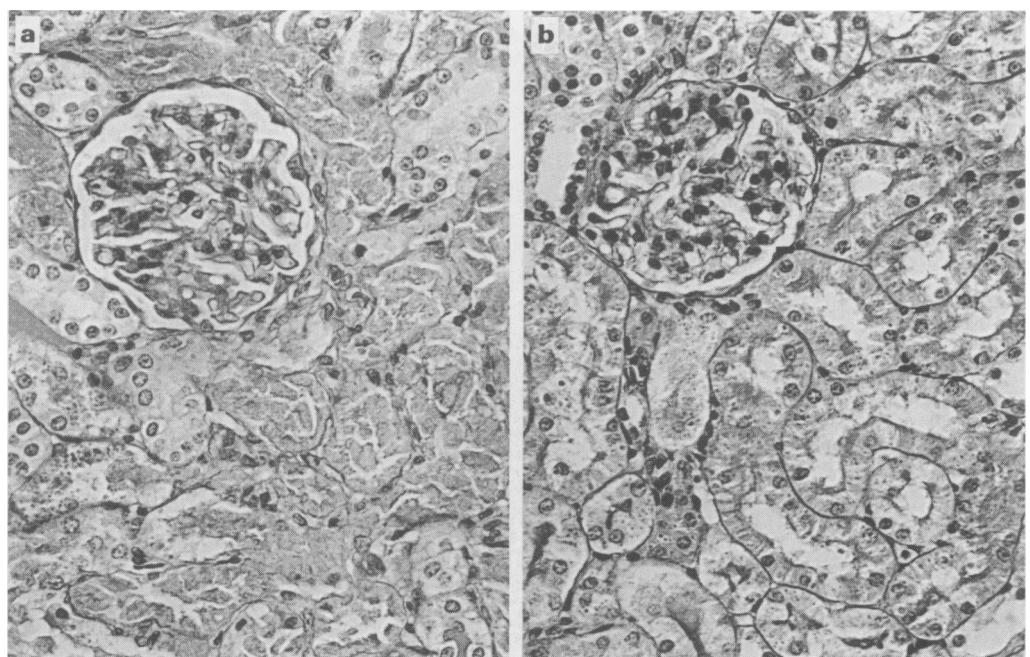


Fig. 4. *a*, Complete necrosis of epithelial cells in most tubuli. Hyaline droplets also occur in the epithelial cytoplasm in rats treated with oestrone + vasopressin. PAS, $\times 300$. *b*, Hyaline droplets in tubular epithelia occur sporadically in animals treated with oestrone + vasopressin + Tamoxifen. PAS, $\times 300$.

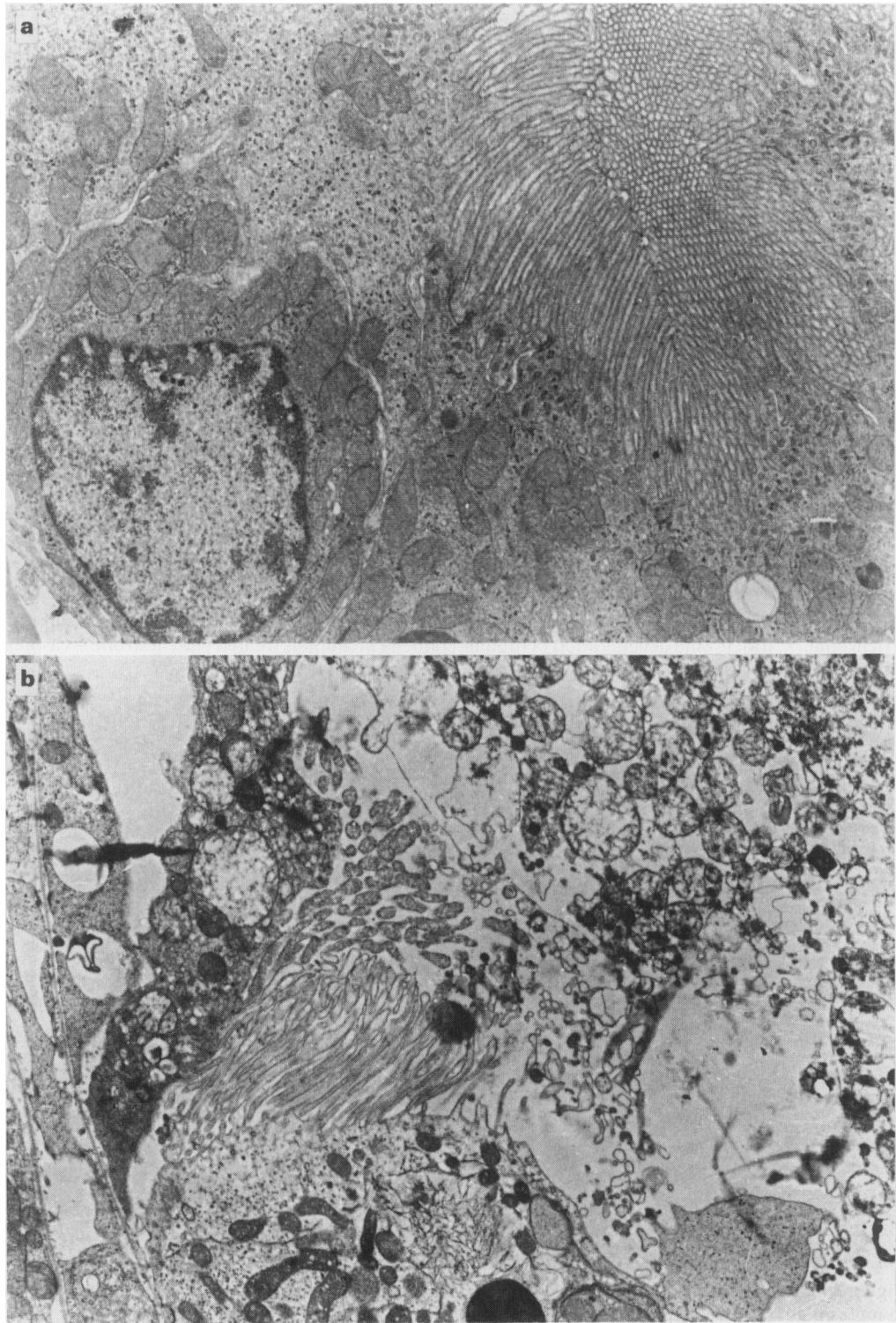


Fig. 5. *a*, Electron-microscopic picture of proximal convoluted tubular epithelia in a control animal. $\times 10200$. *b*, Total necrosis with membrane disruption of tubular epithelia in a rat pretreated with oestrone + vasopressin. $\times 7200$.

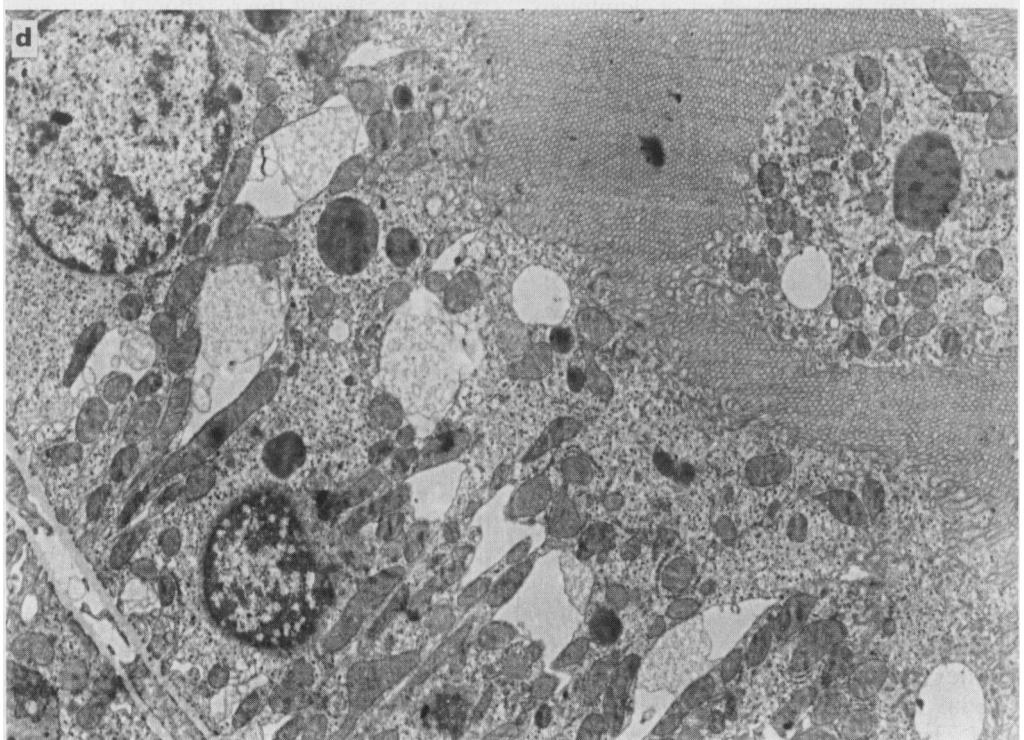
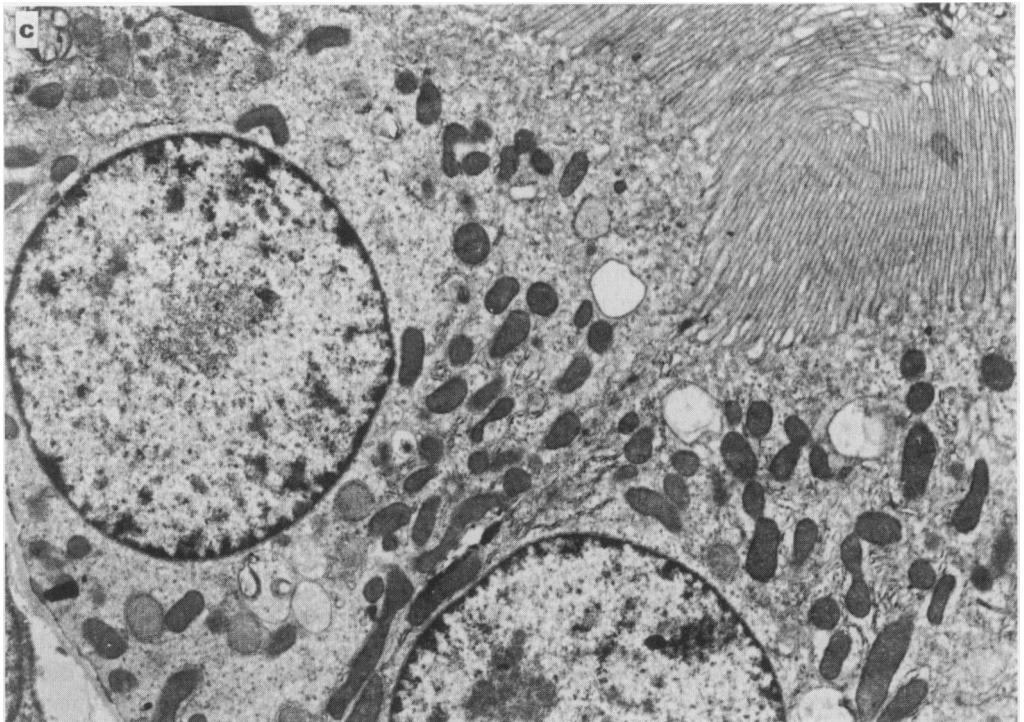


Fig. 5. c. Almost complete prevention of damage to epithelial membranes in an animal treated with oestrone + vasopressin + Tamoxifen. $\times 7200$. **d.** Incomplete prevention of damage to cell structure with dilatation of intracellular spaces and basal membrane invaginations in a rat treated with oestrone + vasopressin + Tamoxifen. $\times 7200$.

Discussion

Human bilateral renal necrosis is most common in pregnancy (Duff & More 1941; Sheehan & More 1953; Deutsch *et al.* 1971; Matlin & Gary 1974; Di Paolo & Facchini 1982; Hault *et al.* 1982). At the present stage of our knowledge, the development of bilateral renal cortical necrosis can not be prevented with certainty in clinical practice. We have constructed a renal cortical necrosis model with the aim of studying the prevention of renal cortical necrosis (Kovács *et al.* 1964; László 1981). After the administration of vasopressin to rats pretreated with oestrone, bilateral renal cortical necrosis occurred in all these animals. It appeared probable that the anti-oestrogen Tamoxifen would prevent the vasopressin-induced renal cortical necrosis in this model.

The non-steroid anti-oestrogens have been used in clinical practice for 20 years. The ovulation-induction effect of clomiphene citrate was reported first in women with fertility disturbances (Greenblatt *et al.* 1961; 1962; Adams *et al.* 1971; Huppert 1979). Reports were later published about the similar action of Tamoxifen (Klopper & Hall 1971; Williamson & Ellis 1973; Gerhard & Runnebaum 1979). The anti-oestrogen effect of Tamoxifen has been described in many species (Fromson *et al.* 1973). Oestrogen-induced uterus growth was blocked with the use of the compound (Jordan *et al.* 1977; 1978).

Certain tissues, and tumours developing from them, have very high affinities for labelled oestradiol; in both experimental animals and humans, this affinity can be successfully suspended with synthetic non-steroid compounds, among them Naphoxidine, ethamoxo-trifethol (Tamoxifen), clomiphene and Norgestrel (Edgren *et al.* 1966; 1967; Jensen *et al.* 1967; 1971; Sander 1969; Terenius 1971 *a,b*).

Renal tumour induced with oestrogen could be favourably influenced with Naphoxidine (Bloom *et al.* 1967). The development of DMBA-induced, hormone-

dependent rat mammary carcinoma was inhibited (Jensen *et al.* 1967). Encouraging results have also been achieved in human mammary carcinoma (Bloom *et al.* 1967; Jensen *et al.* 1971; Antonio *et al.* 1974; Bloom & Boesen 1974). Tamoxifen, with a structure similar to that of clomiphene has recently been used with good results for the reduction of human mammary carcinoma (Cole *et al.* 1971; Ward 1973; Mouridsen *et al.* 1978; Furr *et al.* 1979). Numerous oestrogen receptors were found in mammary carcinoma cells (Folca *et al.* 1961; Sander 1969; Johnson *et al.* 1970; Korenman & Dukes 1970; Feherty *et al.* 1971; Jensen *et al.* 1971; Le Clerq *et al.* 1973; McGuire *et al.* 1974; Borgna *et al.* 1982). The oestrogen receptor-blocking feature of Tamoxifen is well known (Skidmore *et al.* 1972; Lippman *et al.* 1976; Jordan *et al.* 1977; Capony & Rocheft 1978; Katzenellenbogen *et al.* 1978; Borgna & Rocheft 1980); this compound has been shown to prevent the reaction of oestrogens with oestrogen-specific receptors of target cells (Hahnel *et al.* 1973; Jordan 1975) and it decreases the oestrogen-receptor substance of the uterus cytoplasm (Clark *et al.* 1973; 1974).

In our studies, oral administration of the oestrogen-receptor blocking agent Tamoxifen provided considerable protection against the development of the renal cortical necrosis caused by oestrone + vasopressin treatment. This observation suggests that the vascular effect of oestrogen may be manifested through the oestrogen receptors. The possibility of a correlation between the responsiveness of the renal vessels and the sex-steroids is supported by some published data (Byrom 1937; 1938; Lloyd & Pickford 1961; 1962; Honoré 1962 *a,b*; Deis *et al.* 1963; László 1981). However, the presence of oestrogen receptors in the kidney has long been a subject of debate. The kidneys were earlier considered to be an 'oestrogen-unresponsive' tissue (Jensen *et al.* 1966). Later, a specific oestradiol-macromolecule complex was demonstrated in the embryonal kidney of the guinea-pig (Pasqualini *et al.* 1973).

and a 17β -oestradiol receptor was found in the rat kidney, localized in the nuclear chromatin (King & Mainwaring 1974).

Our experiments indicate that the necrosis-averting effect of Tamoxifen does not amount to total prevention. This can be explained in that Tamoxifen (in addition to its specific receptor-blocking effect) influences hormone metabolism in various ways (Jordan 1984). The anti-oestrogen acts on the oestrogen-induced prolactin synthesis (Lieberman *et al.* 1983 *a,b*), progesterone-receptor production (Horwitz *et al.* 1978; Eckert & Katzenellenbogen 1982), specific protein synthesis (Westley & Rochefort 1979, 1980; Edward *et al.* 1980), and the binding of tritiated thymidine by protein (Lippman & Bolan 1975). Overall, these effects together could impair the main, receptor-blocking action of Tamoxifen.

The question thus arises as to whether anti-oestrogens may be used in future as a means of preventing human renal cortical necrosis. For the time being, the observations do not lead to many practical conclusions. The influence on maternal and the fetal physiology of compounds antagonizing the 'physiologically' high oestrogen level during pregnancy is not known and has not yet been tested. The experience gained both in the intensive research work on anti-oestrogens which is being done elsewhere and in our own investigations gives hope that the prevention of this extremely serious disease will eventually become possible.

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