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THE EFFECTS OF OESTROGEN, ACTH AND CORTISONE ADMINISTRATION AND HYPOPHYSECTOMY ON HISTOLOGICAL CHANGES INDUCED BY UNILATERAL RENAL PEDICLE CLAMPING

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Summary.—A study was made in rats of the histological changes occurring in the kidney after temporary application of clamps to the renal pedicle following oestrogen, ACTH and cortisone treatments and removal of the pituitary. It was found that the tubular damage is increased to a small extent by oestrogen administration and to a more significant degree by ACTH and cortisone treatments. In rats hypophysectomized for several weeks the tubular necrosis either did not occur at all or only to an extremely mild degree.

The investigations suggest that the change in sensitivity of the tubular epithelial cells to hypoxia may play a role in the development of the renal cortical necrosis induced by hormone treatment.

FOLLOWING oestrogen or ACTH treatment, renal cortical necrosis can be induced in rats by the administration of vasopressin preparations (Byrom, 1938; Byrom and Pratt, 1959; Kovács and Dávid, 1963*a*,*b*). Study of the pathogenesis has shown that both hormones increase the sensitivity of the renal vessels to the vasoconstrictive effect of vasopressin (Lloyd, 1959; Lloyd and Pickford, 1962; Deis, Lloyd and Pickford, 1963; Kovács, Csernay and Dávid, 1964*a*; Kovács *et al.*, 1965; Kocsis *et al.*, 1965; László, Csernay and Kocsis 1973*b*). Thus, a prolonged O_2 deficiency develops and the kidney tissue necrotizes. The possibility cannot be discounted, however, that oestrogen and ACTH change the sensitivity of the tubular epithelial cells to hypoxia. The present experiments were made in an effort to answer this question.

Examinations were also carried out on hypophysectomized rats. It had previously been observed that after the removal of the pituitary the tubular necrosis normally induced by oestrogen + vasopressin either did not result at all, or to only a very mild extent (Kovács *et al.*, 1964*b*), although the renal vasospasm could be demonstrated even in these animals (László *et al.*, 1966, 1973*a*).

The sensitivity of the kidney tissue to hypoxia was investigated by the temporary application of clamps to the renal pedicle.

MATERIALS AND METHODS

The studies were carried out on 130 male albino rats of the R-Amsterdam strain, weighing 170–200 g, kept on a standard diet. The animals were divided into 5 groups: (1) Non operated, untreated control rats were divided into 4 subgroups according to the duration of the kidney pedicle clamping. In 10 animals the pedicle clamping lasted for half an hour, in a further 10 for 1 hour and in a third 10 for 2 hours, while the sham operation was performed

on the 10 animals in the fourth subgroup. (2) The rats were treated subcutaneously with 1·0 mg of oestrone acetate (Hogival, Chinoin) daily for 10 days, and the renal pedicle was clamped in 10 animals for half an hour and in 10 animals for 1 hour. (3) The animals were given subcutaneous injections of 0·02 mg of 24-aminoacid-containing synthetic retard ACTH (Cortrosyn, Organon) daily for 10 days, and pedicle clamps were applied in 10 animals for half an hour and in 10 animals for 1 hour. (4) Twenty rats were given subcutaneous injections of 10 mg of cortisone acetate (Adreson, Organon) daily for 10 days, and on the tenth day the renal pedicle was clamped for half an hour (10) or 1 hour (10). (5) In the hypophysectomized group the duration of the pedicle clamping was deliberately longer. Our earlier observations (Kovács *et al.*, 1964*b*) had indicated that it could be expected that the ischaemia-induced renal injury would be moderated by the hypophysectomy. Accordingly, the pedicle clamping was maintained for 1 (14) or 2 (16) hours.

The hypophysectomy was performed under ether anaesthesia by parapharyngeal approach, 3 weeks before the intervention. The effectiveness of the operation was decided on the basis of a careful inspection of the peripheral endocrine glands and the sellar region. In doubtful cases serial sections were prepared from the region for histological examination, and those animals in which a remnant could be detected were excluded from the evaluation. The surgical trauma caused by the pedicle clamping was endured badly by the hypophysectomized rats, 4 animals from the 1-hour group and 7 from the 2-hour group not surviving for 24 hours. The temporary renal ischaemia was induced with a special automatic clamp, with a length of 12 mm and a compression surface of 2 × 5 mm. Laparotomy was carried out under ether anaesthesia and the left renal pedicle clamped. The abdominal wall was then closed. A half, 1 or 2 hours later, the clamp was removed through the existing laparotomy incision, again under ether anaesthesia, and the abdominal cavity was closed. By means of the injection of Indian ink into the carotid artery and the tetracycline fluorescence method, it was confirmed in a number of the animals that the clamp stopped the renal circulation totally and that complete ischaemia resulted. It was always the blood vessels of the left kidney which were clamped. In the case of sham operation the procedure was fairly similar: laparotomy was performed under ether anaesthesia, the left renal pedicle was prepared, the abdominal wall was closed without renal clamping and a half, 1 or 2 hours later the laparotomy was repeated. One day after the clamping the animals were killed by decapitation and both kidneys were immediately removed, fixed in 4% formalin and embedded in paraffin. The 4–6 μm sections were stained with haematoxylin and eosin. No histological changes were observed after sham operations, or in the right kidney. For easier observation of the morphological changes, microphotographs are shown from the 1-hour pedicle clamping experiments only.

RESULTS

The extent of the histological changes in the left kidney of the control rats is proportional to the duration of clamping of the renal pedicle. After half-hour clamping mild degenerative alterations were observed: cloudy swelling involving mainly the primary convoluted tubules with occasional hyalin degeneration. Following one hour's clamping, necrobiosis and focal necrosis of the epithelial cells were found, together with hyalin and granular casts originating from the necrotic and desquamated epithelial cells in the lumina of the tubules, corresponding to the Henle loops and the convoluted tubules on the clamped side (Fig. 1). In the 2-hour group, diffuse necrosis of the epithelia of the Henle loops and the convoluted tubules, desquamation of the necrotic epithelial cells and formation of granular casts in the lumina of the tubules were observed. The glomeruli for the most part were contracted and focal capillary loop necrosis had developed in one or 2 glomeruli. In smaller areas the entire renal tissue, including the glomeruli and the medullary substance, had necrosed, and a picture corresponding to infarct had arisen.

After oestrogen treatment more marked histological alterations were found in the renal cortex. After the half an hour's clamping extensive necrobiosis could

be observed in the epithelial cells of the primary and secondary convoluted tubules and the Henle loops, with focal necrosis in places, and eosinophil casts in the lumina of the tubules. In the 1-hour clamping group tubular necrosis predominated over the necrobiosis of the renal epithelial cells, and only the collecting tubules and the glomeruli had not necrosed (Fig. 2).

The most severe renal injury was found in the ACTH-treated group. Following renal pedicle clamping for half an hour, focal necrobiosis and necrosis could be seen, moderate in the primary and secondary convoluted tubules and considerable in the Henle loops. In 3 rats from this group the necrosis and necrobiosis of the tubular epithelium were much more diffuse and uniformly affected the epithelial cells of the convoluted tubules and the Henle loops. In the majority (8 rats) of the ACTH-treated animals the 1-hour clamping gave rise to extremely marked renal lesions; severe, diffuse tubular necrosis developed in the renal cortex. The picture corresponded essentially to the change seen in the untreated rats after the 2-hour clamping, with the difference that focal glomerular lesions too occurred in places in the controls (Fig. 3).

The histological changes observed in the clamped kidney after cortisone administration agreed with those for the ACTH-treated group.

In the hypophysectomized rats the ischaemia induced by the renal pedicle clamping gave rise to much more moderate histological changes than for the controls. Following one hour's clamping, only mild degenerative alterations, cloudy swelling and lesions indicative of necrobiosis in a few small foci, could be seen in the epithelial cells of the convoluted tubules (Fig. 4). The difference is striking between the untreated controls and the hypophysectomized group after 2 hour's clamping too. The glomeruli in the hypophysectomized animals proved to be intact. Diffuse degenerative alterations and focal necrobiosis could be observed in the convoluted tubules, with necrobiosis of the epithelial cells in the Henle loops, and minor focal necrosis in one or 2 rats.

DISCUSSION

The results show that the sensitivity of the epithelial cells of the tubules and the Henle loops to hypoxia is enhanced by prolonged oestrogen treatment. It was demonstrated in our previous experiments that the administration of oestrogen increases the sensitivity of the renal vessels to the vasoconstrictive effect of posterior pituitary extracts (Kovács *et al.*, 1964*a*, 1965). It was assumed that the sensitizing effect of oestrogen is exerted in a complex way. The formation of the renal cortical necrosis can be promoted by both the change in the contractability of the renal vessels and the change in the sensitivity of the tubules to oxygen deficiency. This may explain the fact that most bilateral renal cortical necroses in human

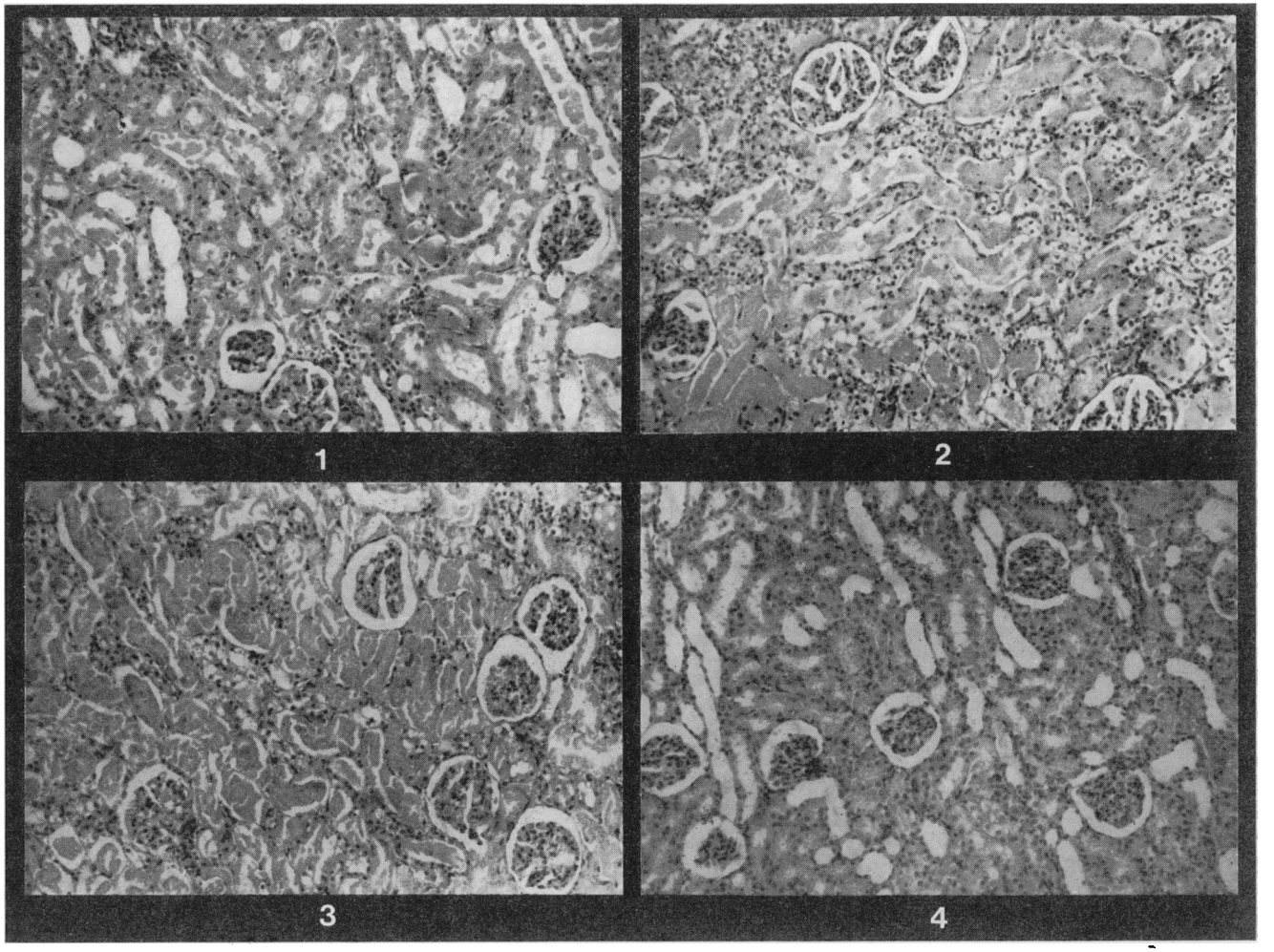
EXPLANATION OF PLATES

FIG. 1.—Kidney of untreated control rat after 1 hour's renal pedicle clamping. Necrotic tubular epithelial damage and cast formation. H. and E. ×81.

FIG. 2.—Kidney of rat treated with oestrone acetate for 10 days, after 1 hour's renal pedicle clamping. Tubular necrosis. H. and E. ×81.

FIG. 3.—Kidney of rat treated with ACTH for 10 days, after 1 hour's renal pedicle clamping. Extensive tubular necrosis. H. and E. ×81.

FIG. 4.—Kidney of untreated rat, hypophysectomized 3 weeks previously, after 1 hour's renal pedicle clamping. Mild degenerative tubular injury. H. and E. ×81.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pathology have been described at the end of pregnancy (Sheehan and Moore, 1952; Heptinstall, 1966; Strauss and Welt, 1971).

The possibility has also been raised that the oestrogen does not act *via* a true increase of the sensitivity to hypoxia, but as a result of the treatment the clamped vessels open subsequent to the clamping and thus the duration of the ischaemia is in effect lengthened. By means of our rat angiorenographic method (László *et al* 1964) it was possible to exclude this possibility. Angiorenography 5, 10 and 20 minutes after the removal of the clamp did not reveal any noteworthy difference in the dimensions of the renal vessels of the controls and the oestrogen-treated animals.

The histological changes induced by clamping the renal pedicle after ACTH pretreatment are even more severe. This mechanism may play a considerable role in the fact that after prolonged ACTH administration vasopressin gives rise to renal cortical necrosis (Kovács and Dávid, 1963*b*). It was found in our previous angiorenographic examinations that the renal vasoconstriction brought about by vasopressin after ACTH treatment is not so marked as after oestrogen administration (Kocsis *et al.*, 1965). Accordingly, only a moderate renal blood flow decrease was observed (László *et al.*, 1973*b*). It was assumed that the effect of the ACTH is exerted by the stimulation of the adrenal cortical function; a similar effect could be produced by employing cortisone in place of ACTH.

Our results prove convincingly that the renal ischaemia in rats hypophysectomized for several weeks leads to less severe histological damage. These studies can be used to give an answer to the question of why a marked renal cortical necrosis does not result in the hypophysectomized animals despite the renal vasoconstriction (László *et al.*, 1966) and the renal blood flow decrease following oestrogen + vasopressin administration (László *et al.*, 1973*a*). After removal of the pituitary the oxygen demand of the kidneys is more moderate and less sensitive to renal cortical hypoxia, and hence they tolerate the oxygen deficiency resulting from the renal vascular spasm better.

The results can be correlated with, and to a certain extent explain, the observations that some renal injuries do not occur, or to only a low degree, in the event of hypophysectomy. It has been shown that the development of nephrocalcinosis due to the administration of NaH_2PO_4, parathormone and dihydrotachysterol is inhibited by hypophysectomy (Selye, 1958; Selye, Gabbiani and Jean, 1962; Selye, Gabbiani and Tuchweber, 1962). Certain data (Girard *et al.*, 1957; Farnsworth, 1959; Salgado and Mulroy, 1959; Girard and Rassaert, 1962, 1963) indicate that the renal and vascular damage otherwise induced by the injection of DOCA does not result if the pituitary is previously removed. More severe renal lesions cannot be caused by hexadimethrine bromide in hypophysectomized rats (Kovács *et al.*, 1967).

If apart from (or besides) the primary toxic damage, the ischaemia also plays a part in the development of the renal lesions in the above experimental models, then it may be assumed that the decreased sensitivity to hypoxia in the hypophysectomized animals may be an important factor in the prevention of the renal lesion.

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