Experimental Nephritis Induced by Homologous Placental Tissue as Observed with the Light, Fluorescent and Electron Microscope

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ABSTRACT

Toxemia of pregnancy is a common complication of gestation, usually occurring in late pregnancy. Whether toxemia represents an exaggeration of changes incident to pregnancy or depends upon some wholly new factor is a moot point.

Indeed, the cause of the toxemia of pregnancy, despite decades of intensive research, remains the great enigma of obstetrics and constitutes one of an important unsolved problems in the field of human reproduction.

Glomerulonephritis can be induced in various animal species by numerous serums and tissue extracts. Its production by duck immune serum was first described in the rabbit by Masugi (1934). By using a potent standardized nephrotoxic duck serum or its gamma globulin, nephritis has been reproduced in a regular manner by Seegal, et al., (1936).

The experiments recorded here show the results of injecting rabbit antidog-placenta serum into both pregnant and non-pregnant dogs as described by Seegal at al., (1955). The course of the resulting nephritis is compared with that following the injection of rabbit antidog-kidney serum.

The large size of the animal permitted frequent bleeding and the gestation period allowed for observation of nephritis during pregnancy. The findings support the conclusion that rabbit antidog-placenta serum injected in the dog produced an acute nephritis which usually progressed to a chronic state comparable to that which follows the injection of anti-kidney serum. Pregnancy has not been terminated by this antiserum.

Beveans et al. (1965) describe the lesions produced in these pregnant and non-pregnant dogs following injection of either rabbit anti-placenta or rabbit anti-kidney serum.

Acute and chronic phases of the nephritis have been studied over a period of 10 months. The intravenous injection of rabbit antidog-placenta or antidog-kidney serum produced immediate evidence of glomerulonephritis in dogs and rabbits.

The glomerulonephritis so induced may terminate in death within 8 days, may progress to a chronic form or may heal.

Recently, Irino et al. (1967) induced renal lesions in rats by placental extracts. These changes were observed with the electron microscope and the renal glomerular alterations
in rats with a clinical syndrome resembling toxemia of pregnancy showed the characteristic changes consisting of swelling with decreased density of the basement membrane, a dense granular deposition within the along capillary basement membranes, and marked swelling and slight proliferation of glomerular epithelium.

The glomerular lesions, designated endocelial glomerulitis are apparently a result of an antigen-antibody reaction and present further evidence that human toxemia of pregnancy has an immune mechanism as a basis for its production.

Kim(1969) attempted to establish the pathologic changes induced by sensitizing the rat against homologous placental tissue and to compare them with the lesions of the kidney in human toxemia. He found that renal lesions were closely related to that of human toxemia of pregnancy.

The present investigation is aimed to study the lesions in the glomerulus of the pregnant rat kidneys induced by repeated injection of homologous placental tissue as observed with the light, the fluorescent and the electron microscope and adds further evidence for the view that the syndrome, as induced experimentally, constitutes an analog of toxemia of pregnancy as it affects the human.

**MATERIALS AND METHODS**

Male and female albino rats weighing around 200 gms. were divided into Group I and II. Group I is normal control group and was subdivided into non-pregnant, pregnant, and delivery groups. Group II was subdivided into non-pregnant, pregnant, delivery, and abortion groups which were then injected with homologous rat’s placental tissue with complete Freund’s adjuvant mixture.

The placental tissue was injected intraperitoneally once every week in a dose of 0.5 ml and a number of injections was 8 times.

Estrus cycle was determined by the vaginal smear methods of Long and Evans(1922), and mating was achieved by housing a male and female rat together in the pre-estrus cycle and rechecking the vaginal smear for the presence of spermatozoa. In this way the mating was begun from the last injection of placental tissue.

The placental emulsion was prepared as follows. Placentae were removed on the 18th and 20th day of pregnancy. After washing them with sterile isotonic saline solution several times, a 30% saline suspension of placenta was made by the tissue homogenizer and added to the same amount of Freund’s complete adjuvant (Difco laboratory).

Rabbit anti-rat placental serum of IgG fraction of high specificity and potency was conjugated with fluorescein isothiocyanate (FITC) (Microbiochemical associates I.N.C), and was used in the immuno-fluorescent studies.

The animals were sacrificed and necropsied, at one or two week intervals after the last injection of antigen.

After gross examination and the removed kidneys were divided into three parts. One part of the kidney was fixed in 1% osmium tetraoxide in veronal buffer at pH 7.4 for 2 hours each, and was dehydrated at 4°C in a graded series of ethanol solution and embedded in Epon 812, and ultrathin sections were made with glass knife in 400 to 500 Å and were stained with saturated uranyl acetate. Sections were examined in Hitachi HU-IIIE electron microscope.

The other parts of kidney tissue was fixed
in 10% formalin and cut in 6 micron thickness after paraffin embedding. It was stained with routine hematoxylin-eosin and periodic acid Schiff (PAS) stain. And were examined with light microscope. A part of the remainder of kidney tissue was frozen quickly and was sectioned at 5 to 6 microns with the cryostat at -15°C. The sections were thawed and are dried at room temperature for 10 minutes. The sections were overlaid with FITC conjugated rabbit anti-rat placental serum and placed in petridishes containing moistened filter paper for one hour at room temperature. The slides were rinsed in cold 0.01M phosphate buffered saline, pH 7.4 and placed in a 50% solution of glycerol and phosphate buffered saline, and covered with cover glass. It was examined for specific fluorescent light with a A.O. spencer microscope using an Osram HBO 200 mercury arc lamp with appropriate ultraviolet filters.

The blocking test was carried out in each set of sections by unconjugated anti-sera followed by fluorescent staining.

RESULT AND DISCUSSION

Body weight, blood pressure, and urine albumin were within normal range and no other pathological alterations were observed in the kidney by histologically, histochemically, immunologically, or electron microscopically in group I animals.

The Group II, that had received numerous placental emulsion injections, showed a marked raise of blood pressure and an increase in urine albumin not only in the pregnant and delivery groups but also the non-pregnant group.

At necropsy, the kidneys of all group II animals showed increased perirenal fat tissue, pale appearance, and no adherent renal capsule and cortex.

On cut sections, they revealed a pale and widened cortex, the renal pelvis had no gross alterations. The light microscopic findings are seen mainly in the glomeruli, with variable and non-specific changes in the other structures. The glomeruli were all affected and enlarged. They are characteristically bloodless glomeruli, with the capillary lumen reduced by cytoplasmic swelling of endothelial cells. The number of cells in the glomerular tuft is either normal or slightly increased, and inflammatory infiltrates are sparse or absent. The lobular pattern of the glomerular tuft is often accentuated in the late stage of pregnancy of Group II animals, and visceral epithelial cells are large in size because of cytoplasmic swelling.

The PAS positive material is present in the visceral epithelial cells and basement membrane of the glomerular capillary.

Endothelial cells showed swelling of their cytoplasm with the result that they may fill the capillary lumen partially or almost completely. The mesangium showed focal thickening present in some lobules. Those findings are more prominent from two weeks gestation and early delivery animals in Group II.

The capillary basement membrane of the glomerulus is thickened in all of the animals but the third week gestation group revealed characteristic thickening of the capillary walls.

The tubules showed no characteristic alterations. The PAS positive materials are contained often in the proximal convoluted tubules but the tubule usually appeared relatively normal. And renal blood vessels and the interstitial tissue showed no remarkable finding.

Intense apple-green fluorescence was obser-
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ved in the basement membrane of the glomerular capillary and in the cytoplasm of the endothelial cells. The endothelial cytoplasmic fluorescence revealed more prominence in the basement membrane of capillary as linear or spotlike appearance. The fluorescence was proved to be specific anti-placental serum by the blocking test. This specific fluorescence was observed only in the Group II animals with multi-injected placental emulsion and the most intense findings showed in the late period of pregnancy and in the two weeks from last injection of placental emulsion of non-pregnant animal. No specific fluorescence is evident in the group I.

The endothelial cells appeared increased in size. In some instances the increase in size was accompanied by a decrease in the relative density of the cytoplasmic ground substance and a reduced concentration of organelles. This findings were believed to represent cellular swelling. Some areas showed numerous caveolae intracellularis and multiple adjacent intracytoplasmic vesicles.

The endothelium showed the development of multiple small, cytoplasmic folds termed cytofolds. These cytofolds related vacuoles contained aggregates of dense, flocculent material. Occasional single membrane-limited cytoplasmic inclusion bodies containing homogenous dense material were seen.

These findings are more prominent in pregnant and delivered animals of Group II.

The basement membrane appeared relatively unaltered in Group I. The Group II showed unevenness in shape and density. Other in focal areas and on occasion diffusely, the lamina densa exhibited a patchy increase in density and marked widening. In some cases the lamina rara interna was frequently obliterated or occupied by deposit material or abnormally widened. Occasionally the complete disappearance of the lamina rara interna in the late pregnant animal of Group II was noted. The lamina rara externa was relatively unaltered without the finding of mild increased density.

The epithelial cells were also enlarged but microvilli were less apparent. The foot process was frequently preserved, with only focal areas that showed the portion of thickened basement membrane. Some of the epithelial cells were many and large with single membrane-limited inclusions containing various density of dense material and occasionally showing the non-membrane-limited dense bodies, and vesicles.

The data presented here indicated that there are general similarities in the structure of glomeruli of human preclampsia or eclampsia, previously described by many authors. The dense material deposits are largely intracapillary and subendothelial portion as lamina densa and lamina rara interna of capillary basement membrane.

In systemic lupus erythemasosus they may be intracapillary, subendothelial, or subepithelial and in acute glomerulonephritis they are almost entirely subepithelial (Faith and Trump, 1966). This findings suggest that certain differences do exist between eclampsia and other renal disease in histological basis.

The appearance of the deposited material was different in other renal disease. With such a mechanism, immunologic specificity may be essential to glomerular localization as in the immunofluorescent microscopic findings.

SUMMARY

Homologous placental emulsion injected into
pregnant rats induced perirenal fatty changes, bloodless glomeruli, and massive PAS positive material deposited in the basement membrane and epithelial cells. Specific anti-placental antibody was observed in the basement membrane and endothelial cell cytoplasm. Ultrastructurally, the dense material deposits are largely intracapillary and lamina densa and lamina rara interna of capillary basement membranes, and swelling and slightly proliferation of glomerular endothelium and epithelium.

The glomerular lesions, designated endothelial glomerulitis and basement membrane alterations are apparently a result of an immunological reaction and present evidence that human toxemia or eclampsia has an immune mechanism as a role for its production by autoantiplacental antibody.

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