by the fact that embryos in FCS (2nd control group) did not differ from embryos bathed in saline with regards to their capacity to form teratocarcinomas. The most likely explanation for the low yield of teratocarcinomas from in vitro grown embryos lies in the decreased viability of certain cells in these embryos. We have shown previously that only 27% of all in vitro grown egg cylinders developed into somitic stage embryos if cultured for an additional four days in vitro. Since the number of teratocarcinomas was in the same range it seems that teratocarcinomas develop only from most viable embryos. Although the number of teratocarcinomas was small, it nevertheless proves that the in vitro conditions have not anulled the capacity of the embryonic cells to form teratocarcinomas. On the other hand, this potential for transition into stem cells of teratocarcinomas can be fully realized only upon transplantation into living animals, since we have never seen teratocarcinomas developing in vitro from embryos kept for extended periods of time in culture.

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## Streptozotocin induced diabetic nephropathy and renal tumors in the rat

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*Summary.* Adult Wistar rats rendered diabetic by a single dose of streptozotocin develop renal morphological changes which show subtle differences compared to those seen in human diabetic renal disease. The early tubular degeneration is sited in the distal rather than the proximal convoluted tubule and subsequent glomerular lesion shows linear deposits of IgG and albumin in the basement membrane rather than in the mesangium. The carcinogenicity of streptozotocin in the rat is reconfirmed.

Streptozotocin [2-deoxy-2-(3 methyl-3-nitrosamines)-D-glucopyranose] is a drug produced by the soil organism *Streptomyces achromogenes*[1] which is commonly used experimentally for the induction of diabetes mellitus[3]. It selectively damages pancreatic beta islet cells but in addition to its diabetogenic effect it is a potent carcinogen in many animal species[4–6]. This paper briefly reports the main morphological renal changes in the experimental diabetic rat and draws comparisons with renal lesions of human diabetes.

50 male adult Wistar rats of b.wt 180–225 given a single i.v. dose of 65 mg/kg b.wt of streptozotocin (Upjohn, USA) were compared to control rats treated with normal saline. All rats were housed in galvanized wire cages in air conditioned rooms and fed on a diet of stock pellets (Allied Feeds) with water ad libitum. Following administration of streptozotocin rats were initially tested daily and then at weekly intervals for presence of glycosuria and hyperglycaemia as indicators of diabetes. 30 streptozotocin treated rats were considered diabetic when they showed random urinary glucose levels in excess of 500 mg/dl and blood glucose levels 300–400 mg/dl. Rats became diabetic within 2 weeks following administration of streptozotocin and in addition to glycosuria and hyperglycaemia exhibited polyuria and weight loss but failed to develop hypertension. Groups of diabetic and control rats were therefore sacrificed at intervals from 2 to 30 weeks and examined for evidence of renal diabetic nephropathy and tumor development. Kidney tissues from both groups were processed routinely for examination by light, electronmicroscopy as well as for the localization of immunoglobulins and albumin using a modified immunoperoxidase technique[7].

Morphologically, the earliest visible light microscopic changes in kidneys of diabetic rats in the initial 2 weeks comprised swelling and vacuolation of epithelial cells in the macula densa particularly affecting those cells lying opposite but not immediately adjacent to the glomerular stalk. Subsequently such epithelial degenerative changes became more marked or extensive with progression of diabetes so that by 3–4 weeks almost the entire distal tubular epithelium was affected (fig. 1). Histochemical staining showed that the swelling and vacuolation of epithelial cell cytoplasm was primarily due to an accumulation of fluid and glycogen granules within the cytoplasm rather than to an accumulation of lipid as is the case in human diabetes. The tubular degenerative changes although similar to those reported in man nevertheless differed in that the distal tubule is affected in the rat whereas the proximal tubule is involved in the human. The tubular degenerative changes are considered to be due to the diabetic state rather than to a direct toxic effect of streptozotocin because the changes were not seen immediately following streptozotocin administration.

Various glomerular lesions comparable with those found in human diabetic glomerular disease were noted. These changes included formation of 'fibrinoid caps', linear electron-dense deposits in the basement membrane and focal segmental mesangial sclerosis, however glomerulosclerosis of the nodular (Kimmelsteil-Wilson) type were not found. Fibrinoid caps (fig. 1) seen on light microscopy, corresponded in ultrastructural studies to electron-dense deposits in a subendothelial location adjacent to which there was fusion of foot processes of podocytes. Such lesions first appeared at day 30 and became increasingly more frequent thereafter. The linear deposits in the basement membrane, present after 14 days, were shown by immunoperoxidase staining to comprise both IgG and albumin and contrasted with several previous reports in which the deposits in the experimental rat were localized in the mesangium[8–11].

5 months following administration of streptozotocin 6 of the residual rats developed renal tumors. Tumors were

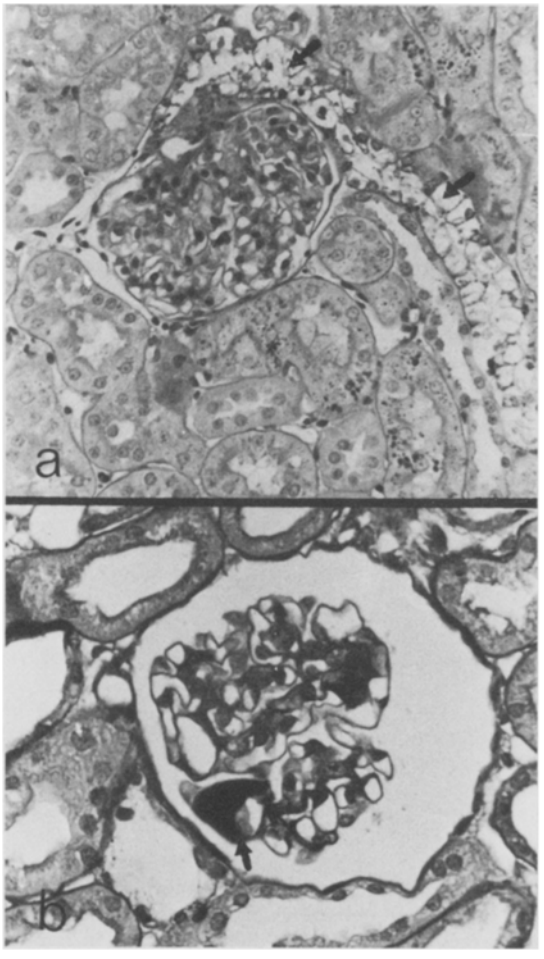


Figure 1. *a* Part of kidney of rat 4 weeks following streptozotocin treatment showing distal tubular degeneration (arrow). *b* Glomerulus from diabetic rat of 3 months duration showing fibrinoid cap formation (arrow).

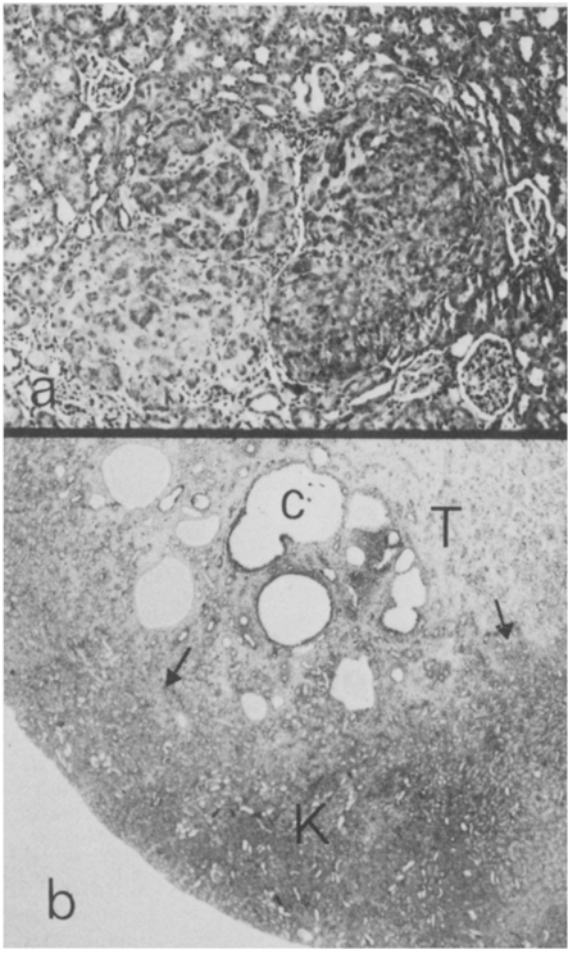


Figure 2. *a* Part of kidney showing 2 small adenomas from rat 5 months following streptozotocin treatment; *b* part of kidney from rat 5 months post streptozotocin treatment showing nephroblastoma-like tumor (T) with invasive edge (arrow) into adjacent compressed kidney tissue (K). There are also several cysts (C) in the tumor.

either cortical adenomas and/or malignant tumors with histopathological features consistent with nephroblastoma (fig. 2). Both renal and extra renal tumors induced by streptozotocin are well documented[12–14] and the present renal findings did not differ from those previously reported. That the development of renal tumors is not causally related to the diabetic state but is a direct result of streptozotocin is supported by the recent work of Mauer et al.[15] who showed that non-tumorous kidneys from streptozotocin treated rats develop tumors within 4 months of their transplantation into healthy recipients. The present work once again highlights the potent carcinogenicity of streptozotocin in the rat although streptozotocin has been used in the treatment of some human neoplasms[16,17] the proven carcinogenic effect of the drug in the rat warrants reconsideration of its use in man.

1 Acknowledgments. We wish to thank Mr S.G. Watkins and Mrs D. Greening for technical assistance.
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