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Renal Enlargement: Comparative Autoradiographic Studies of ^{3}H-Thymidine Uptake in Diabetic and Uninephrectomized Rats

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Summary. Incorporation of ^{3}H-thymidine into renal cortical tissue has been studied by light microscopic autoradiography in streptozotocin-diabetic rats, uninephrectomized rats, uninephrectomized diabetic rats, insulin-treated diabetic rats and control rats. The percentage of labelled cortical nuclei (the labelling index) was determined separately in glomeruli, proximal tubules and distal tubules after 2, 4 and 6 days on autoradiographs from 1 μm thick plastic embedded sections. The incorporation of thymidine in glomerular nuclei was consistantly low (< 1%) and no differences were found between the control and experimental groups. In both proximal and distal tubules an increase in thymidine incorporation was seen on day 2 followed by a decline on days 4 and 6. The maximal labelling on day 2 in proximal tubules was 9.1% in the uninephrectomized diabetic group, 3.7% in the diabetic group and 1.4% in the uninephrectomized group. In distal tubules the corresponding values were 5.2, 3.5 and 1.1%. The increase in kidney weight after 6 days was 83, 62 and 37%, respectively. Estimates of the net increase in the number of cortical tubular cells in the different experimental groups showed that the kidney enlargement followed different patterns with respect to the extent of cellular hyperplasia and hypertrophy. The kidney growth in uninephrectomized diabetic rats was dominated by tubular cellular hyperplasia, in the diabetic group hyperplasia and hypertrophy participated to approximately the same extent, whereas cellular hypertrophy was most pronounced in the uninephrectomized animals. Nuclear labelling in the insulin-treated diabetic rats was not different from that of control rats and consequently a hyperplastic effect of streptozotocin can be ruled out.

It is concluded that combined tubular cellular hyperplasia and hypertrophy is involved in the kidney cortical enlargement seen in the present experimental groups and that each group follows different cellular reactions. In glomerular enlargement, however, no cellular hyperplasia is obserbed.

Key words: Streptozotocin diabetes, uninephrectomy, rat kidney, thymidine, autoradiography.

Enlargement of the kidneys can be demonstrated roentgenographically in diabetic patients with recent onset of the disease [1]. A similar increase in kidney size is characteristic in streptozotocin-diabetic rats [2]. Measurements of DNA, RNA and protein content of the whole kidney have suggested that this growth initially is due to cellular hypertrophy followed later by a cellular hyperplasia [3]. Stereological observations have demonstrated that this kidney enlargement is characterized by increased glomerular volume and an increase in proximal tubular length [2]. In addition, glomerular enlargement has been demonstrated in patients with recent onset of diabetes [4].

Renal enlargement following unilateral nephrectomy has been extensively studied as a classical model for compensatory growth [5, 6]. Furthermore, additive effects on renal enlargement of combined experimental diabetes and uninephrectomy have been demonstrated in rats [3, 7]. The mechanisms behind kidney growth in diabetic rats or compensatory growth after uninephrectomy are only partially understood, and results are conflicting concerning the extent to which cellular hyperplasia and hypertrophy are involved in the kidney enlargement.

The present study of the ^{3}H-thymidine incorporation by light microscopic autoradiography was undertaken to provide more detailed information about the cellular reactions involved in renal growth in uninephrectomized rats, streptozotocin-diabetic rats and in rats which were both uninephrectomized and diabetic. The number of labelled nuclei were quantitated separately in three parts of the nephron: glomeruli, proximal tubules and distal tubules of the kidney cortex.

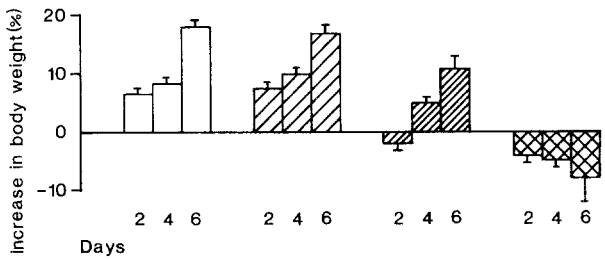

Fig. 1. Mean changes in body weight during the experimental period. ☐ control animals, ▨ uninephrectomized animals, ▨ diabetic animals and ▩ uninephrectomized diabetic animals. Bars show SEM

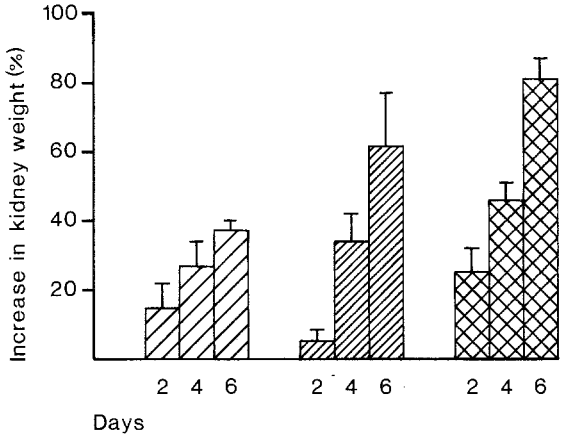

Fig. 2. Increase in kidney wet weight expressed as percentage of control kidneys. ▨ uninephrectomized animals, ▨ diabetic animals and ▩ uninephrectomized diabetic animals. Bars show SEM

Materials and Methods

A total of 78 female Wistar rats weighing 115 ± 5 g (mean ± SD) were used. Right-sided nephrectomy was performed at 09.00–12.00 h by a dorsal approach under pentobarbital anaesthesia (25 mg/kg body weight). Streptozotocin was given IV at the same time in a dose of 90 mg/kg body weight. The uninephrectomized diabetic animals were injected with streptozotocin immediately after the operation. Blood glucose was measured daily with Dextrostix (Ames) and a reflectance meter (Ames, Japan). Urine was tested for ketone bodies every day with Ketostix (Ames). Body weight was determined at the beginning and at the end of each experimental period.

Autoradiography

For autoradiography, animals were injected IP at 08.00–11.00 h, with ^{3}H-thymidine (Amersham International, Amersham, Bucks, UK; 1 mC/kg body weight) before retrograde aortic perfusion fixation of the kidneys with 1% glutaraldehyde in 0.1 mol/l cacodylate buffer. Small blocks of mid-cortical tissue were cut in a standardized fashion from the lateral border of the left kidney, postfixed in osmium tetroxide, dehydrated in alcohol and embedded in Epon. Sections (1 µm thick) were cut (Ultrotome III, LKB, Stockholm Sweden) and covered with photographic emulsion (Kodak NTB 2). After 6 weeks of exposure the sections were developed (Kodak D 19) and stained with toluidine blue.

Experimental set-up

The following five groups of animals were used: (1) diabetic, (2) uninephrectomized, (3) uninephrectomized and diabetic, (4) control and (5) insulin-treated diabetic animals. Each of the first four groups consisted at each experimental time of five to seven animals. Non-diabetic rats with two intact kidneys served as controls. The kidneys from the diabetic and the uninephrectomized diabetic groups were injected with ^{3}H-thymidine and perfusion fixed after a diabetes duration of 2, 4 and 6 days, counted from the onset of glycosuria. The uninephrectomized animals were correspondingly injected with isotope and fixed 2, 4 and 6 days after the operation. The left kidney was weighed after the perfusion fixation in all animals, including control rats, and processed for autoradiography. Furthermore, a group of six diabetic animals were treated to normoglycaemia with a non-commercial, very long acting heat treated insulin (Monocomponent Ox Ultralente, pH 5.5, Novo, Copenhagen, Denmark) from the onset of glucosuria [23]. These animals were injected with isotope 1 h before perfusion fixation of the kidneys 2 days after the onset of diabetes, at which time the labelling index was maximal in the non-treated diabetic animals (see Results). The left kidney was weighed and processed for light microscopic autoradiography.

Light microscopic autoradiographs were prepared from sections of cortical tissue taken from three random blocks from each animal. The number of labelled nuclei and the total number of nuclei were counted on photographic prints with a final magnification of × 900 for tubular counts and × 2,000 for glomerular counts. The tubular labelling index (i.e. percentage labelled nuclei of the total number of nuclei) was calculated separately for proximal and distal tubules from nuclear counts within a grid frame [8]. The glomerular labelling index was based on nuclear counts from all glomeruli in the sections. In the majority of cases it was possible to identify the labelled glomerular cells (epithelial, endothelial or mesangial cells). Average nuclear counts for each experimental group were 810 for proximal tubules, 480 for distal tubules and 307 for glomeruli at each time. The total net increase in kidney cortical tubular cells was estimated according to Johnson and Vera Roman [9] based on curves for the labelling index of tubular cells. Statistical analysis was performed using Student's t-test for comparison of unpaired samples.

Results

The diabetic and uninephrectomized diabetic animals developed diabetes with blood glucose values in the diabetic group of 18.7 ± 1.3 mmol/l (mean ± SD) and in the uninephrectomized diabetic group 17.2 ± 1.0 mmol/l (mean ± SD). Glycosuria could be demonstrated on the first day after injection of streptozotocin and none of the animals showed ketonuria. No statistically significant differences were noted in body weight between control and uninephrectozimed animals (Fig. 1). Diabetic animals showed an initial small decrease in weight, whereas uninephrectomized diabetic animals exhibited a gradual increase of weight loss.

The kidney weight of the control animals did not change significantly during the experimental period (0.67 ± 0.05 g; mean ± SD) and consequently the average kidney weight for all control rats was used as base value. The kidney weight of the insulin-treated animals after 2 days of diabetes was 0.69 ± 0.07 g (mean ± SD) and not statistically different from control animals. The kidney weight in the remaining experimental groups increased gradually (Fig. 2). The increase was 37% in the uninephrectomized group, 62% in the diabetic group and 83% in the uninephrectomized diabetic group after 6 days.

The kidney cortex appeared normal on light micro-

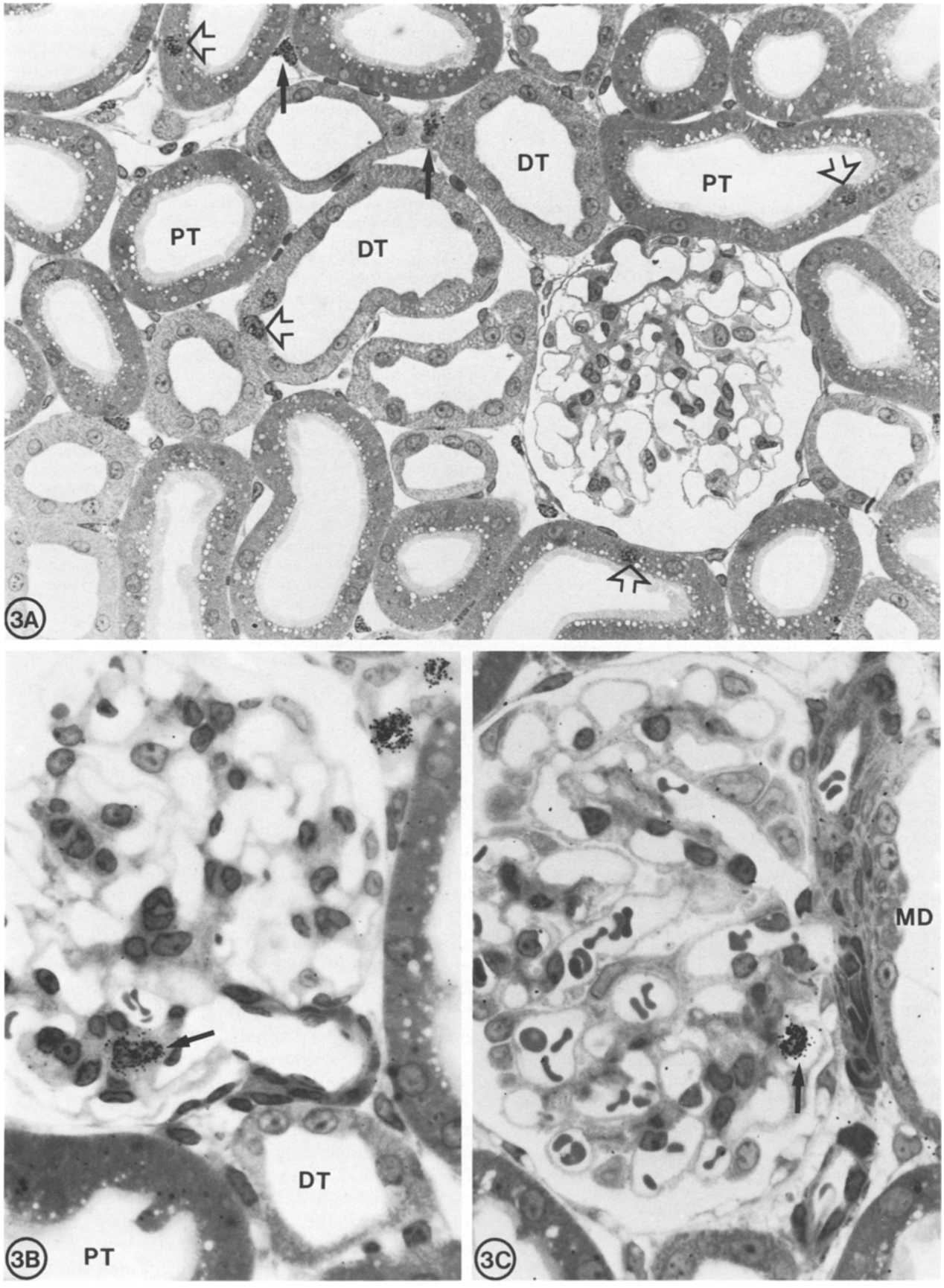

Fig. 3 A–C. Light microscopic autoradiographs of Epon embedded sections of kidney cortex (1 μm thick) stained with toluidine blue. **A** shows a representative survey section of renal cortex from a diabetic rat with a well preserved structure and no signs of tissue damage. Open arrows indicate tubular cells labelled with ^{3}H-thymidine. Arrows show labelled interstitial cells (×400). **B** is a larger magnification of a glomerulus with a labelled mesangial cell (×900) and **C** shows a glomerulus with a labelled endothelial cell (×900). PT: proximal tubules, DT: distal tubules, MD: macula densa

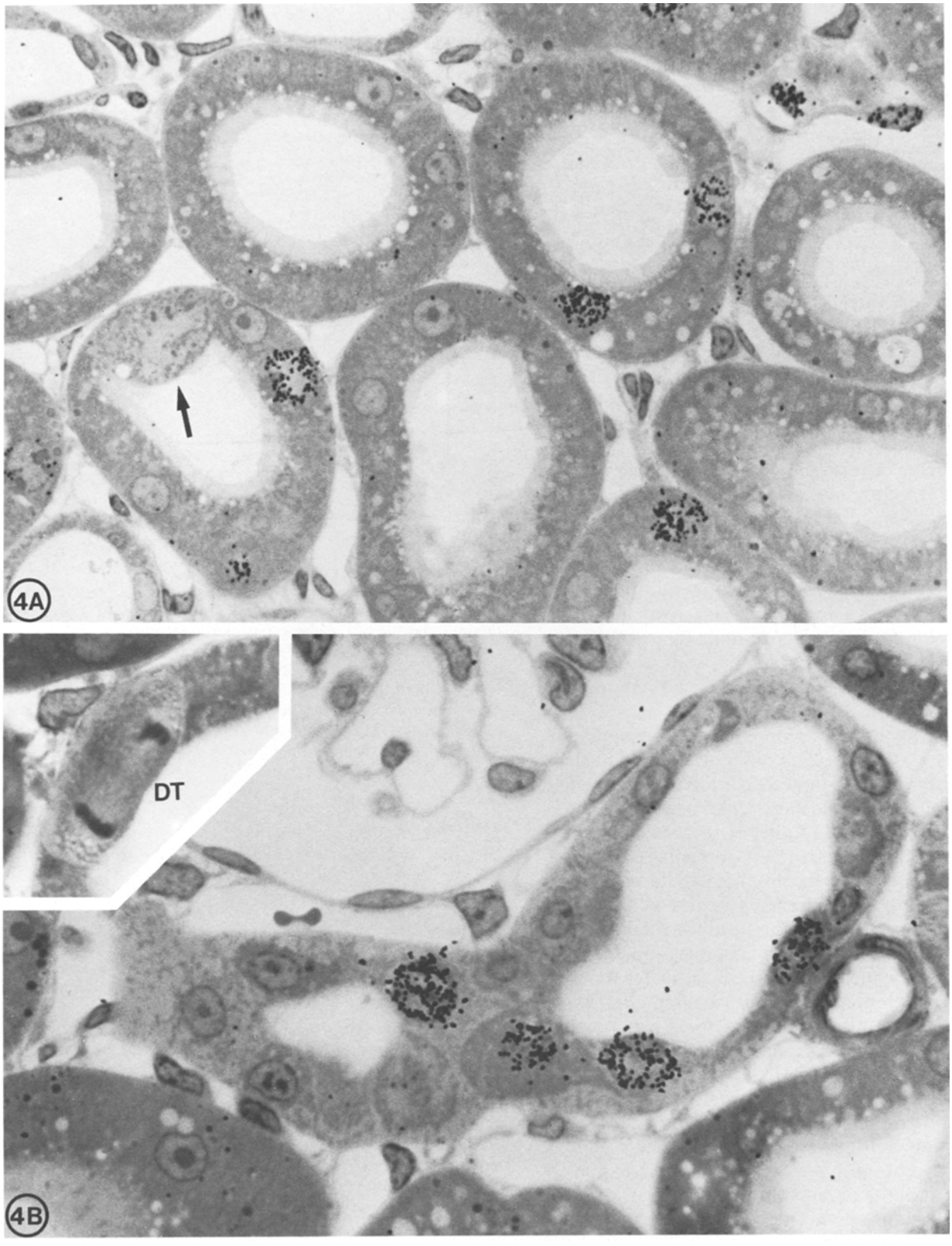

Fig. 4 A and B. Light microscopic autoradiographs of labelled tubular cells. In **A** grains are located over nuclei from proximal tubular cells. Arrow indicate a cell undergoing mitosis (× 900). **B** shows distal tubular cells with labelled nuclei. Inset shows a cell from a distal tubule (DT) with a mitotic figure (× 1000)

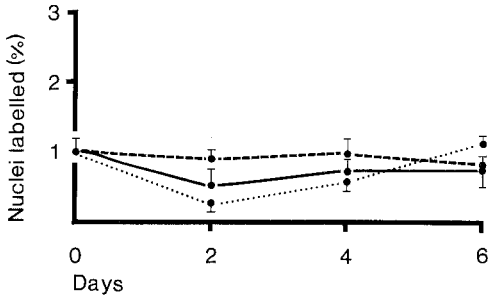

Fig. 5. Labelling index of glomerular cells (percentage labelled nuclei of the total number of nuclei counted) during the six experimental days. Control labelling is shown at day 0. Values are given as mean ± SEM. Solid line: uninephrectomized diabetic animals, dashed line: diabetic animals and dotted line: uninephrectomized animals.

scopy in the 1 μm thick sections in all experimental groups and no sign of tissue damage was observed (Fig. 3 A). The labelled glomerular cells consisted almost equally of mesangial (Fig. 3 B) and endothelial cells (Fig. 3 C). Only once was a labelled visceral epithelial cell observed. Labelling of parietal epithelial cells occurred also but was not quantitated. Proximal tubular cells usually showed the most intense labelling and mitotic figures were most frequently observed in these cells (Fig. 4 A). Distal tubules also contained cells with labelled nuclei as well as mitotic figures (Fig. 4 B). As seen from Figure 3 A interstitial cells also had labelled nuclei. This labelling, however, was not quantitated.

The labelling index of glomeruli was constantly low (approximately 0.9%) and no differences could be demonstrated between the three experimental groups and controls throughout the experimental period (Fig. 5).

The tubular labelling index of the control kidneys did not vary throughout the experimental period and consequently the values for proximal and distal tubular labelling were pooled separately (0.4 and 0.7%). The labelling index on day 2 for the insulin-treated diabetic group was 0.5 ± 0.06 for proximal tubules and 0.6 ± 0.03 for distal tubules (mean ± SEM). Both values were not statistically different from control labelling.

Labelling of tubules in the remaining experimental groups followed a characteristic pattern. In proximal tubules a significant increase in labelling index was seen on day 2, with significant differences between the three experimental groups (Fig. 6). The thymidine incorporation in the uninephrectomized diabetic group was more than twice that of the diabetic group and approximately six times that of the uninephrectomized group. A sharp decline in labelling occured at day 4 in all experimental groups, but the labelling index was still significantly higher than in control tubules. After 6 days, thymidine uptake remained elevated in the two diabetic groups but had fallen to control values in the uninephrectomized group.

A slightly different pattern of labelling was seen in distal tubules (Fig. 7). A similar peak of labelled cells

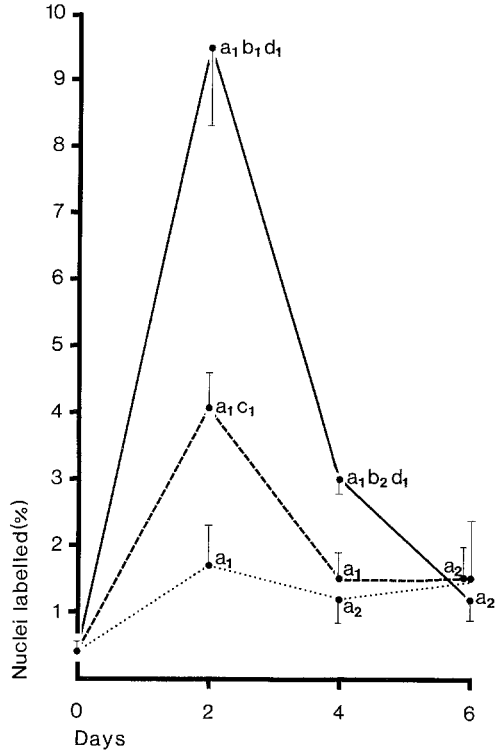

Fig. 6. Labelling index of cortical proximal tubular cells given as mean ± SEM. Solid, dashed and dotted lines represent uninephrectomized diabetic rats, diabetic rats and uninephrectomized rats, respectively. Subscripts give differences of significance: *a*: experimental groups different from control group. a_1: $p < 0.01$, a_2: $p < 0.05$; *b*: uninephrectomized diabetic rats different from diabetic rats. b_1: $p < 0.01$, b_2: $p < 0.05$; *c*: diabetic rats different from uninephrectomized rats. c_1: $p < 0.01$, c_2: $p < 0.05$; *d*: uninephrectomized diabetic rats different from uninephrectomized rats. d_1: $p < 0.01$, d_2: $p < 0.05$

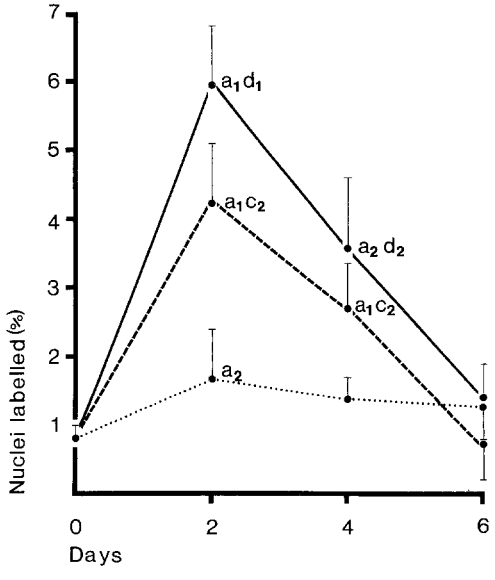

Fig. 7. Labelling index of cortical distal tubular cells given as mean ± SEM. Solid, dashed and dotted lines indicate values for uninephrectomized diabetic, diabetic and uninephrectomized animals, respectively. Differences of significance as indicated in Fig. 6.

was seen on day 2, although with lower maximal values. The decline in labelling index on day 4 was relatively smaller compared to proximal tubules in the two diabetic groups, whereas the uninephrectomized group had reached control levels at this time. On day 6 no differences from control values were noted.

Based on Figures 6 and 7, the total net increase in kidney cortical tubular cells was estimated according to Johnson and Vera Roman [9]. In these calculations, the area under the curves, corrected for control labelling, is used as an expression for the additional number of cells entering the S-phase of the cell cycle in which the incorporation of thymidine occurs. Furthermore, the length of the S-phase must be estimated as it is not known, and we used 8 h for both proximal and distal tubules in all experimental groups as a reasonable estimate [9]. Furthermore, it was assumed that all tubular cells entering the S-phase in the three experimental groups would undergo mitosis. Given these premises the net increase after 6 days in the number of cortical tubular cells was 11% in the uninephrectomized group, 32% in the diabetic group and 71% in the uninephrectomized diabetic group. In the uninephrectomized group, the increased cell mass was due to an approximately equal increase in proximal and distal tubular cells, in the diabetic group the increase was somewhat more pronounced in distal tubular cells, whereas a proximal tubular hyperplasia was dominating in the diabetic uninephrectomized group. Figure 8 is a diagram of the relationship between the relative net increase in kidney wet weight and the calculated net increase in cortical tubular cells.

Discussion

This report has demonstrated that kidney enlargement in short-term diabetic rats and rats subjected to both diabetes and uninephrectomy involves a major component of cortical cellular hyperplasia, indicated by a burst of thymidine incorporation in both proximal and distal tubules 2 days after induction of diabetes. The rise in labelling index is more pronounced in both diabetic groups compared to the uninephrectomized group.

The combination of uninephrectomy and diabetes shows an enhancement of renal growth response compared to the isolated effect of these conditions. The growth seems to follow different patterns of cellular hyperplasia and hypertrophy in the experimental groups. Estimations of the net increase in the number of cortical tubular cells after 6 days (Fig. 8) shows that hyperplasia can account for about one-third of the total kidney growth in the uninephrectomized group, whereas approximately equal amounts of hyperplasia and hypertrophy are seen in the diabetic group. In the uninephrectomized diabetic group most of the renal growth is due to hyperplasia.

The mechanisms by which cells are altered from a non-growing to a growing state are poorly understood and are primarily studied in vitro. From such experiments [10–12], it has been suggested that two main regulatory mechanisms initiate DNA synthesis and mitosis. According to the 'critical mass' theory, DNA synthesis is induced after a certain cell mass has been reached. Proliferation is thus secondary to cell hypertrophy. However, cultured cells can be stimulated to DNA synthesis without concomitant cellular enlargement. It is furthermore found that cells stimulated to divide without prior enlargement progress only through one single cell cycle thus leading to disturbed cell size homeostasis [13].

The present study suggests that the compensatory growth following uninephrectomy is in good accordance with the 'critical mass' theory, with cellular hypertrophy as a prerequisite for DNA synthesis. This theory is also suggested by another study [9] showing an increase in RNA and protein synthesis 1 h after the operation followed by a sharp rise in DNA synthesis with a maximum after 48 h. This very early hypertrophic response after uninephrectomy is found also by other workers [3, 14–16] followed later by a peak in DNA synthesis after 2–3 days [17–19]. A 7% net in-

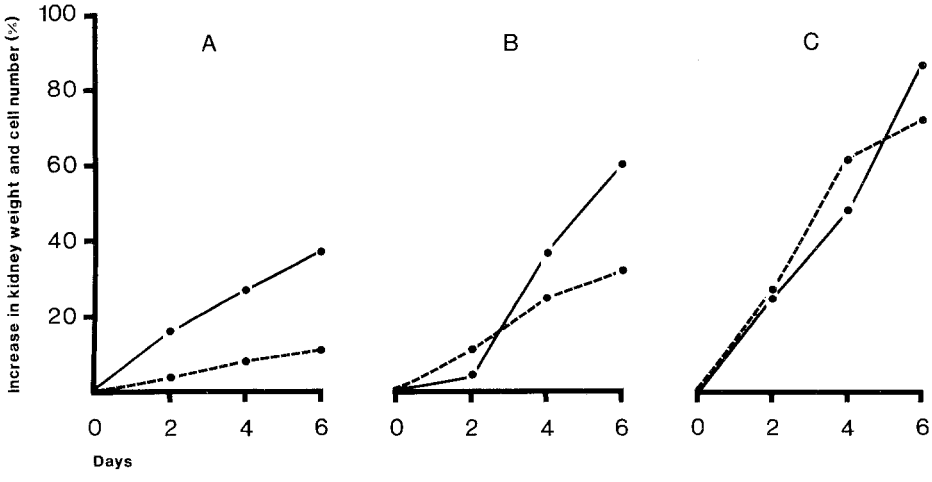

Fig. 8 A–C. The increase in kidney weight expressed as percentage of controls (●—●) and calculated net increase in cell number of cortical tubuli (●-----●). **A** shows values for uninephrectomized animals. After 6 days the increase in cell number is approximately 30% of the increase in kidney weight. **B** indicates diabetic animals and shows an initial increase in cell number with only a small weight increase on day 2. On day 6 the cell number has increased about 50% compared with the increase in kidney weight. **C** shows curves for uninephrectomized diabetic animals. The increase in kidney weight and cell number is almost parallel

crease of tubular cells 5 days after uninephrectomy has been calculated by Johnson and Vera Roman [9] and a 4.3% increase in proximal tubular cells 3 days after the operation was found by Williams [19]; both reports are in good agreement with the present study.

In the present study, a hyperplastic renal cellular response was observed after 2 days in diabetic animals, even without a significant increase in kidney weight, thus resembling the cellular response seen in vitro with DNA synthesis without previous increase in cell size. Whether these newly formed cells create cell size heterogeneity as seen in vitro remains to be clarified. After day 2 the diabetic kidney enlargement appeared to be dominated by hypertrophy with a rapid kidney weight increase followed by a relatively smaller increase in cell number. In the uninephrectomized diabetic group, the increase in kidney weight and the estimated increase in tubular cells was almost parallel. This kidney growth was different from both the uninephrectomized and diabetic group and not a simple addition of these two stimuli for kidney enlargement.

Biochemical analysis of kidneys from diabetic rats has shown an early increase in renal RNA content [3, 20, 22]. However, a significant increase in renal DNA content could not be demonstrated biochemically in diabetic animals after 3 days [22], whereas the amount of DNA after 5 days was increased by about 10% in both diabetic and uninephrectomized diabetic groups [3]. Thus, the autoradiographical findings and the biochemical analysis do not show temporal agreement. These differences could be due to the differing ages of the experimental animals, as older animals show decreased proliferative capacity [18], or might be due to different sensitivity of the methods applied. An effect of streptozotocin on diabetic kidney enlargement is unlikely as the present study has shown that insulin treatment can prevent both cellular hyperplasia and kidney enlargement. Furthermore, in long-term insulin-treated diabetic rats, no increase in renal weight is seen compared to control rats [23].

Enlargement of glomeruli is well documented in short-term experimental diabetes in rats [2] and in man [4] applying stereological methods. The present study has not demonstrated an increased proliferative capacity of the diabetic glomerulus for the first 6 days of diabetes in rats and consequently this glomerular growth is probably due to cellular hypertrophy. This is in good accordance with glomerular growth following uninephrectomy studied by stereological methods [24]. No increase in glomerular cell number in mid-cortical glomeruli could be shown in spite of an increased glomerular volume of 30% compared to adult control rats.

The underlying mechanisms leading to kidney growth after uninephrectomy and especially after diabetes in experimental animals are not yet fully established. In addition, kidney growth can be induced by other experimental procedures such as high protein diet [26] or administration of ammonium chloride [25]. Different hormones and the nutritional state also influence this growth [26]. However, it does not seem likely that experimental kidney enlargement induced by different stimuli, as seen in the present study, is a process which follows the same general growth pattern.

In early human diabetes the mechanism behind kidney enlargement and its possible connection to the development of long-term lesions of diabetic nephropathy is unknown. It remains to be clarified if the same renal cellular reactions operate both in man and experimental animals with acute onset of diabetes.

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