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Cell renewal of glomerular cell types in normal rats. An autoradiographic analysis

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Cell renewal of glomerular cell types in normal rats. An autoradiographic analysis. Normal adult Sprague-Dawley rats received either a single or repetitive injection of the DNA precursor ^{3}H-thymidine (^{3}H-TdR). For autoradiography semi-thin sections were prepared 2 hr to 14 days after labeling. The majority of labeled cells noted in glomerular tufts were endothelial cells. Mesangial cells had a lower production rate. Podocytes revealed no evidence of proliferation. Bowman's capsule cells showed a higher labeling index than tuft cells at all times. Neither the urinary nor the vascular pole was found to be a proliferative zone for Bowman's capsule cells. The flash and repetitive labeling experiments demonstrated a constant rate of cell renewal of about 1% per day, resulting in a long life span for endothelial and mesangial cells as well as Bowman's capsule cells. These data provide a basis for cell kinetic studies in models of glomerular diseases.

Renouvellement cellulaire des cellules glomérulaires chez des rats normaux. Une analyse autoradiographique. Des rats Sprague-Dawley adultes normaux ont reçu une injection soit unique soit répétée du précurseur du DNA, la ^{3}H-thymidine (^{3}H-TdR). Pour l'autoradiographie des sections semi-fines ont été préparées 2 heures à 14 jours après le marquage. La majorité des cellules marquées notées dans les floculus glomérulaires étaient des cellules endothéliales. Les cellules mésangiales avaient une vitesse de production plus faible. Les podocytes n'ont pas révélé de preuve de prolifération. Les cellules de la capsule de Bowman ont montré un plus grand index de marquage que les cellules du floculus à tous les temps. Ni le pôle urinaire ni le pôle vasculaire n'ont été trouvés être une zone proliférative pour les cellules de la capsule de Bowman. Les expériences de marquage unique ou par répétition ont démontré une vitesse constante de renouvellement cellulaire d'environ 1% par jour, entrainant une longue durée de vie pour les cellules endothéliales et mésangiales, ainsi que pour les cellules de la capsule de Bowman. Ces données apportent une base pour des études de cinétique cellulaire dans des modèles de maladies glomérulaires.

The cells of the renal glomerulus in healthy adult mammals have been classified as static cells as suggested by the paucity or absence of mitotic figures noted in normal glomeruli [1, 2]. In recent years various animal models of glomerulonephritis were studied in rats and rabbits, and the uptake of radioactive precursors of DNA was measured as a technique to quantitate glomerular cell proliferation [3–10]. In these studies a low rate of cell production was documented in the control animals. Several authors, however, have indicated only the number of labeled cells noted per glomerular cross section and no accurate labeling index has been calculated [6–8]. Such data can be misleading, especially as in glomerulonephritis, the number of cells per tuft section may change with time. In a previous study [10], we found a labeling index of glomerular tuft cells of 1.9% in normal control rats and recognized differences to the labeling index of cells of Bowman's capsule. Mitotic figures or uptake of ^{3}H-thymidine (^{3}H-TdR) in endothelial and mesangial cells have been described in experimental glomerulonephritis [6–9]. At present, however, it is unclear whether the four recognized cell types in the glomerulus of normal adult animals differ in their rates of proliferation and life spans. In this study the proliferating cells in normal rat glomeruli were characterized on semi-thin autoradiographs by the use of labeling techniques with ^{3}H-TdR.

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Methods

Animals. Male Sprague-Dawley rats obtained from two different breeders (Lippische Versuchstierzucht, Extertal and Zentralinstitut für Versuchstiere, Hannover, Federal Republic of Germany) weighing 190 to 220 g were used in these experiments. The animals had free access to standard laboratory chow and water for 2 weeks at our institution before the experiments were started. They were kept on an automatically controlled light and dark schedule. Forty-four rats from the Extertal breeder were used for the first set of experiments. These studies were repeated with 44 rats supplied by the Hannover breeder. The experimental protocol is shown in Figure 1.

Labeling

Flash labeling. In each series 24 rats received a single intravenous injection of ^{3}H-TdR in a lateral tail vein while the animals were kept in a restraining cage. The dose was 1 μCi/g body weight (sp act 5 Ci/mM, Amersham Buchler, Braunschweig, Federal Republic of Germany). The rats were injected in the morning (8 to 10 A.M.) to avoid the influence of circadian variations of the cellular growth cycle [11]. Groups of four rats were anesthetized with ether after 2 hr, and 1, 3, 5, 7, and 14 days, respectively. The abdomen was opened by a midline incision. The aorta was clamped above and below the renal arteries and the superior mesenteric artery was also occluded. The kidneys were flushed with 10 ml of Ringer solution via the aorta while the inferior vena cava was opened by incision. Then 20 ml of 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3)

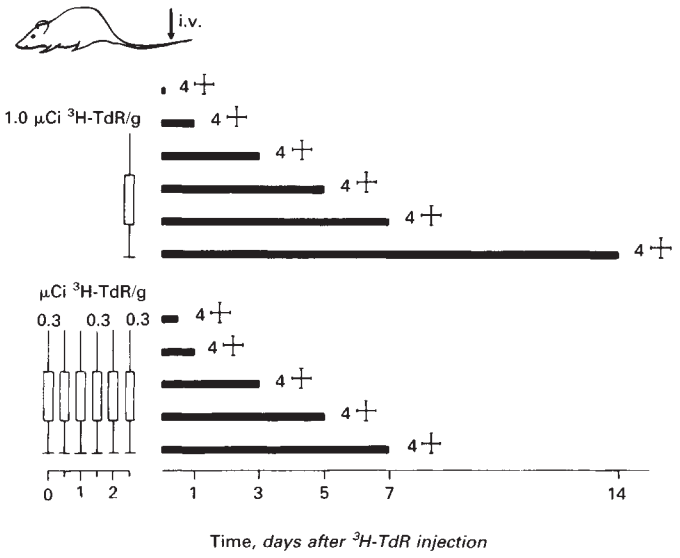

Fig. 1. *Schematic drawing of the experimental protocol for one series of experiments, flash labeling in the* upper portion *and repetitive labeling in the* lower portion. The numbers of rats studied are indicated.

were used for perfusion fixation for 5 min. Small tissue pieces measuring 3 × 6 × 2 mm were excised from both kidneys, including subcapsular and juxtamedullary portions of the cortex and were postfixed in 2% glutaraldehyde for 3 days at room temperature and washed in cacodylate buffer before embedding in epon.

Repetitive labeling. Each injection of ^{3}H-TdR leads to the incorporation into DNA of cells which are in the S phase of their cell cycle at the time of injection. If one assumes an S phase in glomerular cells of 8 to 12 hr, the majority of cells which go through the S phase during this period should be labeled when two injections per day are given over a period of 3 days. Therefore, in each series 20 rats received six intravenous injections starting at 8 to 9 P.M. on day 1 and ending on day 3 at 8 to 9 A.M. The single dose was 0.3 μCi/g body weight, resulting in a total dose of 1.8 μCi/g body weight within 3 days. Groups of four rats were anesthetized 12 hr, and 1, 3, 5, and 7 days, respectively, after the last injection of ^{3}H-TdR, and the kidneys were prepared as described above.

Histology and autoradiography

Semi-thin sections, 1-μm thick, were cut from the epon-embedded kidney specimens. For autoradiography AR10 stripping film (Kodak, Stuttgart, West Germany) was used and developed after 70 to 120 days of exposure in flash labeling and after 130 to 150 days in repetitive labeling with ^{3}H-TdR. The sections were stained with 1% toluidine blue through the emulsion after the developing and fixation procedure of the autoradiographs. Longer exposure times of 200 to 250 days were used for photographic documentation. A minimum of 100 subcapsular and juxtamedullary glomeruli on at least three sections of the right and left kidney was evaluated, the number of all cells per glomerulum was counted, and the labeling index of tuft cells was calculated. More than 1000 cells of Bowman's capsule were evaluated to determine the relative number of labeled cells. Cells were considered labeled if there were five or more grains over their nucleus. The average autoradiographic background was 0.3 grains per nucleus. Most labeled cells had a grain count of about 20. Semi-thin sections enable classification of radioactively labeled cells. When the differentiation between endothelial and mesangial cells was difficult, the same cell was followed in two to three adjacent sections, leading in most cases to a definite characterization of either endothelial or mesangial cell type. The relative frequency of labeled endothelial and mesangial cells and podocytes was determined on the basis of the total number of labeled cells in glomerular tufts. The data are given as mean ± SD. Student's *t* test was used for statistics and a *P* value of less than 0.05 was taken as significant.

Results

Normal adult rat glomeruli show a low frequency of mitotic figures which are found in endothelial, mesangial, and Bowman's capsule cells (Fig. 2). The relative incidence of labeled endothelial and mesangial cells and podocytes in glomerular tufts varied. The results of the studies using flash or repetitive labeling indicated that the majority of labeled cells were endothelial cells (Fig. 3). Mesangial cells were labeled less frequently (Fig. 4). Of note was the almost complete absence of labeled podocytes. Only one labeled podocyte was found in the approximately 10,000 glomeruli evaluated in this study. The differential labeling of tuft cells with time after ^{3}H-TdR injection is illustrated in Figure 5. After flash labeling there tended to be less labeled endothelial cells. The rate of proliferation of tuft cells is indicated by the ^{3}H-TdR labeling index as shown in Figure 6.

An unexpected finding was the higher labeling index found up to day 3 in the second series of experiments, when the Sprague-Dawley rats were supplied by a different breeder. The overall pattern, however, was comparable, showing an increase from 2 to 24 hr after thymidine injection. An interpretation of this increase in the labeling index may be that the cells labeled after 2 hr went through the G_2 and M phase of the cell cycle and produced two daughter cells which were seen 1 day later. The gradual decline of the labeling index from 1 to 14 days after ^{3}H-TdR injection indicates a long life span of the newly formed cells.

After repetitive doses of ^{3}H-TdR significantly higher labeling indexes were noted in both series than after flash labeling (Fig. 7). Seven days after the end of thymidine injection, the labeled cells continued to have high mean grain counts comparable to those found on day 1. Often two or more labeled cells were noted in adjacent positions (Fig. 3).

The comparison of the labeling index of Bowman's capsule cells and glomerular tuft cells revealed a constantly higher cell proliferation rate in Bowman's capsule (Fig. 8). This was particularly remarkable because of the lack of signs of proliferation of the visceral epithelial cells, the podocytes. The labeled Bowman's capsule cells were seen near the urinary pole, in the central part (Fig. 9) and near the vascular pole at all times after flash or repetitive labeling. Thus, there was no evidence of a special cell proliferation zone in Bowman's capsule. Similar findings were seen in the second set of experiments (data not shown).

Discussion

In recent years several authors have examined cell proliferation in glomeruli of rats with various types of glomerulonephri-

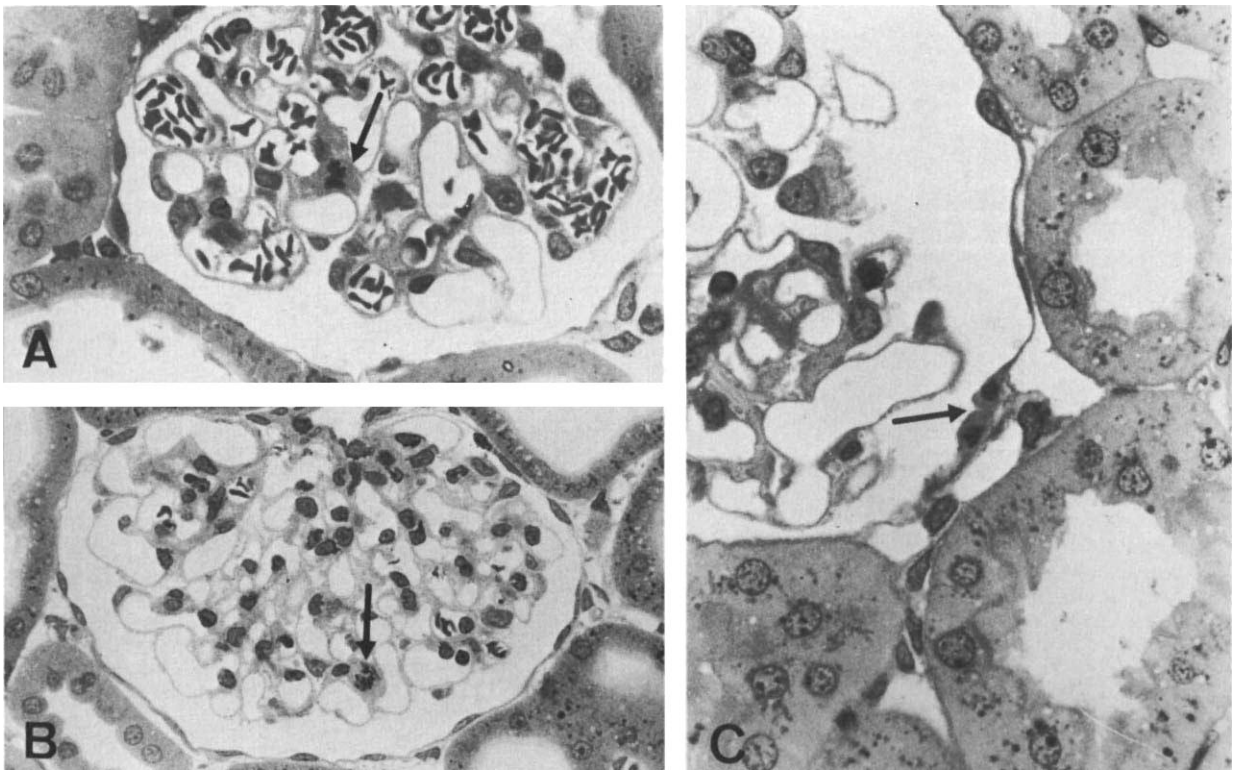

Fig. 2. *Mitotic figure of a mesangial cell* **(A)**, *an endothelial cell* **(B)**, *and a cell of Bowman's capsule* **(C)** *(indicated by* arrows*) in normal rat glomeruli.* **(A**, ×580; **B** ×500; **C** ×750)

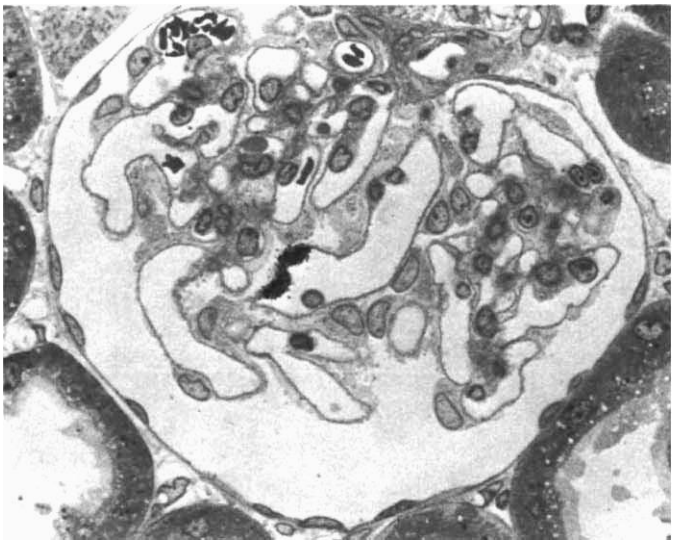

Fig. 3. *Histological section of a rat glomerulus 1 day after* ^{3}*H-TdR labeling, showing two radioactively labeled endothelial cells.* (×560)

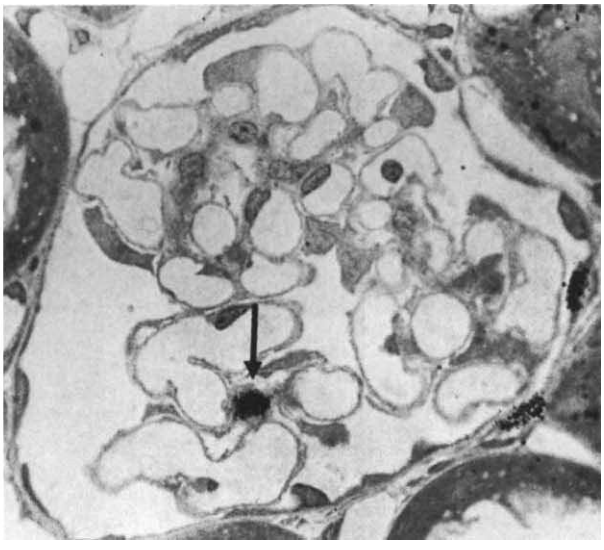

Fig. 4. *A* ^{3}*H-TdR labeled mesangial cell indicated by an* arrow *on this histological section of the glomerulus 1 day after thymidine injection.* Two labeled cells of Bowman's capsule can also be seen. (×600)

tis, which are characterized by glomerular hypercellularity and are commonly termed "proliferative." The main interest of such studies, however, has focused upon the question whether the increase of cells in the glomeruli was due to augmented proliferation of intrinsic glomerular cells or due to infiltration of cells of extrarenal origin [4–10]. These experiments have shown that a small proportion of glomerular cells in normal rats or rabbits takes up ^{3}H-thymidine. The labeling index observed ranged from 0.1% to nearly 3% [6–10]. However, no information, to our knowledge, is presently available with regard to classification of the newly formed cells and only few data are available to extrapolate the life spans in normal adult animals [12, 13]. The present study clearly shows that the great majority of proliferating cells in the normal rat glomerulus is of endothe-

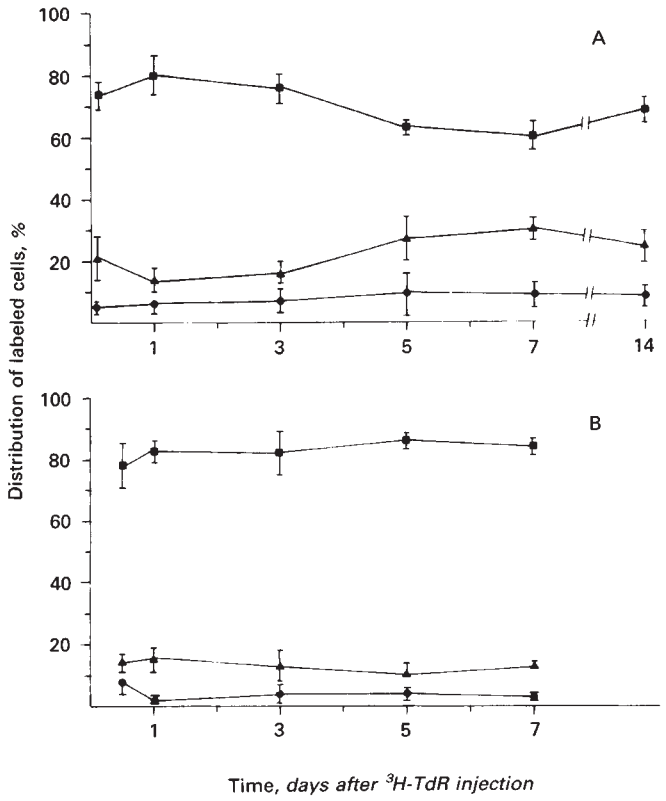

Fig. 5. *The relative frequency of labeled endothelial* (■), *mesangial* (▲), *and unclassified cells* (●) *at different times after a single* (**A**) *or six repetitive* (**B**) *injections of* ^{3}*H-TdR.* In both experiments the endothelial cells had at all times a significantly higher labeling index ($P < 0.001$) than the mesangial cells.

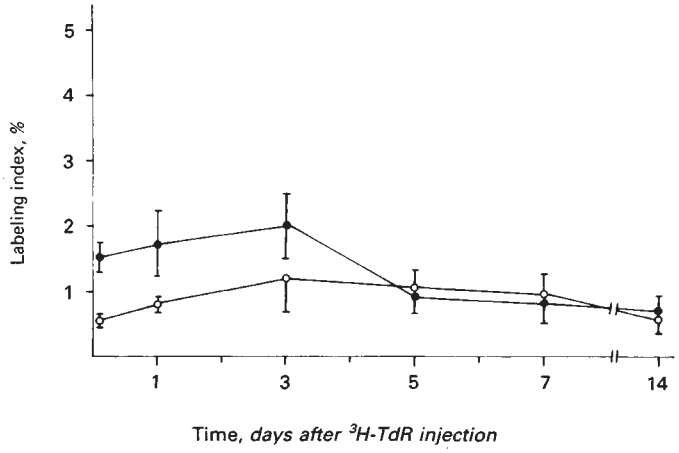

Fig. 6. *Percentage of all labeled cells in glomerular tufts after flash* ^{3}*H-TdR labeling.* Symbols are: ○, first series; ●, second series.

lial origin. Moreover, it is possible that some of the cells classified in our light microscopic evaluation as mesangial cells could be endothelial cells. In serial electron microscopic sections a high proportion of cells originally classified as mesangial cells were clearly identified as endothelial cells by reconstruction of the whole glomerulus [14]. To minimize this potential error, the labeling of mesangial cells was verified on consecutive sections which unequivocally allows distinction of these two cell types. Of particular note in the results obtained was the virtual absence of cell proliferation of podocytes and the high rate of proliferation in Bowman's capsule. Both layers of the glomerular epithelium, the visceral and the parietal portion, are of the same embryologic origin. The reasons that underlie these strikingly different rates of proliferation of both cell types remains to be elucidated. It is of interest, however, that in various forms of glomerulonephritis characterized by increased numbers of glomerular epithelial cells, the parietal cell type is much more conspicuously involved than its visceral counterpart. This is most obvious in crescentic glomerulonephritis [1]. In cultures of isolated glomeruli proliferating cells were described and classified as visceral epithelial cells [15]. These in vitro data are different from the in vivo results obtained in the present study, which may be a result of the cell culture technique. The unrestricted topographic distribution of labeled cells of Bowman's capsule strongly argues against the assumption that a regenerative zone exists at the vascular or urinary pole [2], comparable to the crypts in intestinal cell proliferation.

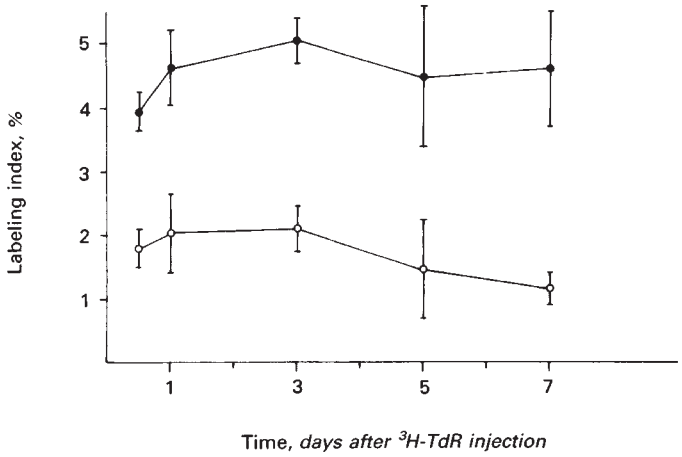

Fig. 7. *Labeling index of cells in glomerular tufts after repetitive intravenous injections of* ^{3}*H-TdR.* Each symbol represents one series of experiments with four rats each. Symbols are: ○, first series; ●, second series.

The extent and the time course of the observed labeling indexes as well as the labeling intensity indicate a low but constant rate of cell renewal in endothelial, mesangial, and Bowman's capsule epithelial cells. The observed pattern of cell proliferation was characterized by a labeling index three times higher after repetitive ^{3}H-TdR injections within 3 days than after flash labeling and the slow decrease of the labeling index from 1 to 7 or 14 days, respectively. This suggests the presence of one or few cell divisions of glomerular cells with a self-renewing stem cell system and random loss of the newly formed cells.

The life span of glomerular cells can be estimated from the following two findings: *(1)* The labeling index of 1 to 2% after flash labeling and 1.8 to 3.8% after 6 doses of ^{3}H-TdR within 3 days indicate a mean life span of about 50 to 100 days. *(2)* The gradual decrease in the labeling index from day 3 to days 7 or 14, respectively, implies a life span of a few weeks. It cannot be excluded that there are subpopulations in each cell group, some of which are short-lived and some long-lived glomerular cells,

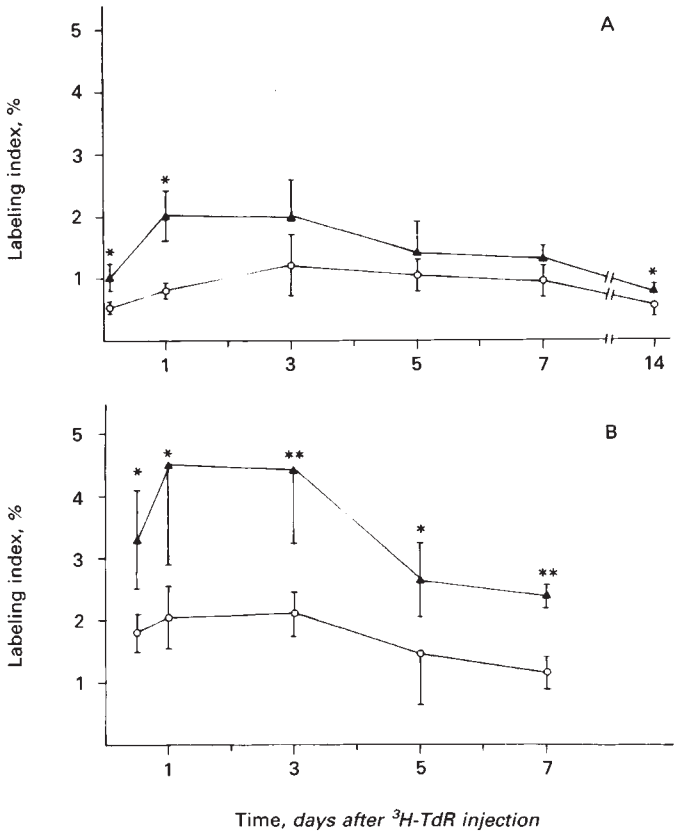

Fig. 8. *Comparison of the proliferation of Bowman's capsule cells* (▲) *to that of tuft cells* (○). **A** *Flash.* **B** *Repetitive* ^{3}*H-TdR injection.* Symbols are: * $P < 0.05$, ** $P < 0.005$.

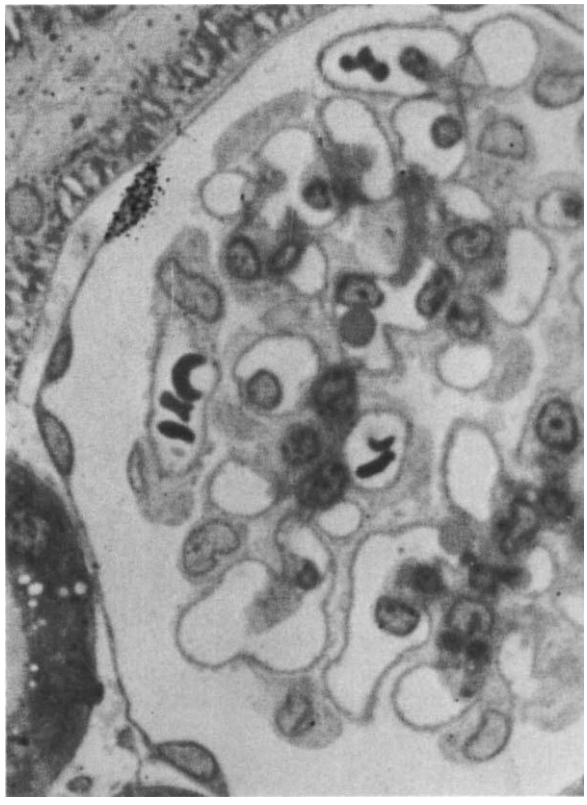

Fig. 9. *A labeled cell of Bowman's capsule 7 days after a single injection of thymidine.* (×1000)

as is acknowledged for lymphocytes. Based on the data of Olivetti et al [16] and Helmchen et al [14], there are about 600 cells in the whole rat glomeruli. Applying these data, one can estimate an absolute number of newly formed cells of 6 to 12 per glomerulus per day.

It is remarkable that different labeling indexes were found in the two sets of experiments carried out in rats obtained from different breeders, indicating wide individual variations in animals of the same strain. While the overall labeling pattern in these two sets of studies was comparable, the findings point to the problem of how misleading it may be comparing data obtained in animals with experimental glomerulonephritis to "normal data" from the literature, especially if only one time point is examined and no kinetic studies are performed.

In conclusion, the present data demonstrate that it is possible and meaningful to classify proliferating cells in the glomerulus on the light microscopic level using autoradiographic techniques. Application of such methods should prove useful in studies on cell kinetics in glomerulonephritis. Such experiments are in progress on rats with an AGBM nephritis, as in the model used previously [10].

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