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The Bivalence of Juxtaglomerular Cells in the Maturing Rat Kidney

A Comparative Study of Secretory and Contractile Potential

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Summary. A comparative immunofluorescence and light microscopical study of the three cell types of the juxtaglomerular apparatus (pure muscle cells, pure granular cells and mixed cells) was performed on the growing and maturing kidney of the rat. Mixed cells, containing contractile protein and secretory granules, are detectable on the first postnatal day in about one third of the JGAs. From the third week, the number of bivalent cells increases, while the proportion of pure muscle or pure granular cells decreases. Morphological and functional maturation, achieved by 3 to 4 weeks, is associated with increasing numbers of bivalent cells and a shift in the main site of renin production from the inner to the outer cortical zone.

The divergent internal structure of JGA cell types expresses the range of varied differentiation expressed by one cell line. Pure muscle or granular cells are at the extremes of the range and mixed cells take up an intermediate position.

Key words: Juxtaglomerular apparatus – Granularity of afferent arterioles – Immunofluorescence of contractile protein – Maturation of kidney.

Introduction

Previous studies on the functional and structural changes in the juxtaglomerular apparatus (JGA) of kidneys of rabbits subjected to blood loss or repeated orthostatic collapse, have disclosed three cell types: Pure smooth muscle cells, pure granular epithelioid cells and mixed or so-called bivalent cells. These latter cells contain myofilaments, a well developed rough endoplasmic reticulum and specific granules (Cain and Kraus, 1971a). It has been suggested that, dependent upon varying physiological and pathological demands, the activity of bivalent

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cells may be predominantly contractile or secretory, however, both activities may be accomplished simultaneously. Similar observations have been made on the JGA of the rat kidney during the course of maturation, growth and ageing (Cain and Kraus, 1971b) but the quantitative relationships of the three cell types to each other have not been investigated further. It has been shown, in both man and experimental animals, that the juxtaglomerular granulation index varies with age and various pathological conditions (Cain and Kraus, 1976).

Previous comparative immunofluorescence and light microscopical studies have been concerned with the quantitative changes in the different cell types of the murine JGA from birth to maturity. In contrast with the view that afferent arteriolar muscle cells and granular epithelioid cells differ from each other (Bucher and Reale, 1962; Reale, Marinozzi and Bucher, 1963), we would like to suggest that smooth muscle cells are transformed into epithelioid cells when there is increased demand for renin and, as bivalent cells, participate in the synthesis of secretion products. The histological features of the JGA cell types apparently reflect diverse functional states, expressing endogeneously determined capacities of an otherwise uniform cell population.

Materials and Methods

Animals. Rats of either sex of the Wistar strain BD were used. Five rats each were killed at the age of 1, 11 and 21 days; six animals were sacrificed on the 31st day of life. The rats were anesthesized with ether and the kidneys removed and weighed. Samples of the left kidney were snap frozen.

Antiserum. Antiserum to chicken smooth muscle contractile protein was kindly provided by Dr. U. Gröschel-Stewart of the Institute of Zoology, Darmstadt Technische Hochschule. A myosin enriched fraction was prepared by ammonium sulphate precipitation of an actomyosin extract of gizzards (Gröschel-Stewart, Schreiber and Mahlmeister, 1976). The myosin was separated by chromatography on Sepharose 48 and purified on DEAE-Sephadex A-50. Antisera were raised in rabbits by sub- and intracutaneous injections of 2 mg of myosin emulsified in Freund's complete adjuvant followed, after an interval of three weeks, by three intravenous injections of 4 mg of myosin, administered on alternate days. Blood was drawn ten days after the last injection. Antibody specificity was found to be directed against the heavy chains of the myosin molecule. The antibodies were tissue but not species specific, cross reacting immunohistologically with smooth muscle of rat, rabbit and human origin.

Histological Examinations. Kidney sections, cut at 4 μ in a cryostat, were incubated with 1:10 diluted anti-chicken myosin serum (ACMS) or rabbit anti-human albumin serum. Incubations were carried out in a moist chamber for 30 min at room temperature. The sections were washed in phosphate buffered saline (PBS), stained with 1:20 diluted fluoresceinated goat anti-rabbit gamma-globulin serum (Hyland, FITC to protein molar ratio 2.8), washed in PBS, counterstained with 0.1% Evans blue and covered with glycerol in glycine buffer. They were examined under a Zeiss incident fluorescence microscope.

Twenty to 25 preglomerular arterioles were evaluated in 1 to 4 sections of the left kidney of each rat. The total number of cells per JGA and the number of cells exhibiting specific fluorescence, which was arbitrarily graded as moderate (+) or strong (++), were determined. The coordinates were recorded and schematic drawings prepared for later comparison with the distribution patterns of granulated cells, selectively demonstrated in the afferent arterioles. To this end, the cryostat sections were fixed in Helly's solution and reacted by Bowie's technique for the staining of granules

(1935). The JGAs, previously evaluated immunohistologically, were located by their coordinates and cells containing a few (+), several (++) or many (+++) granules were counted. The distribution patterns of granulated and specifically fluorescent cells were compared with one another, using the drawings prepared previously.

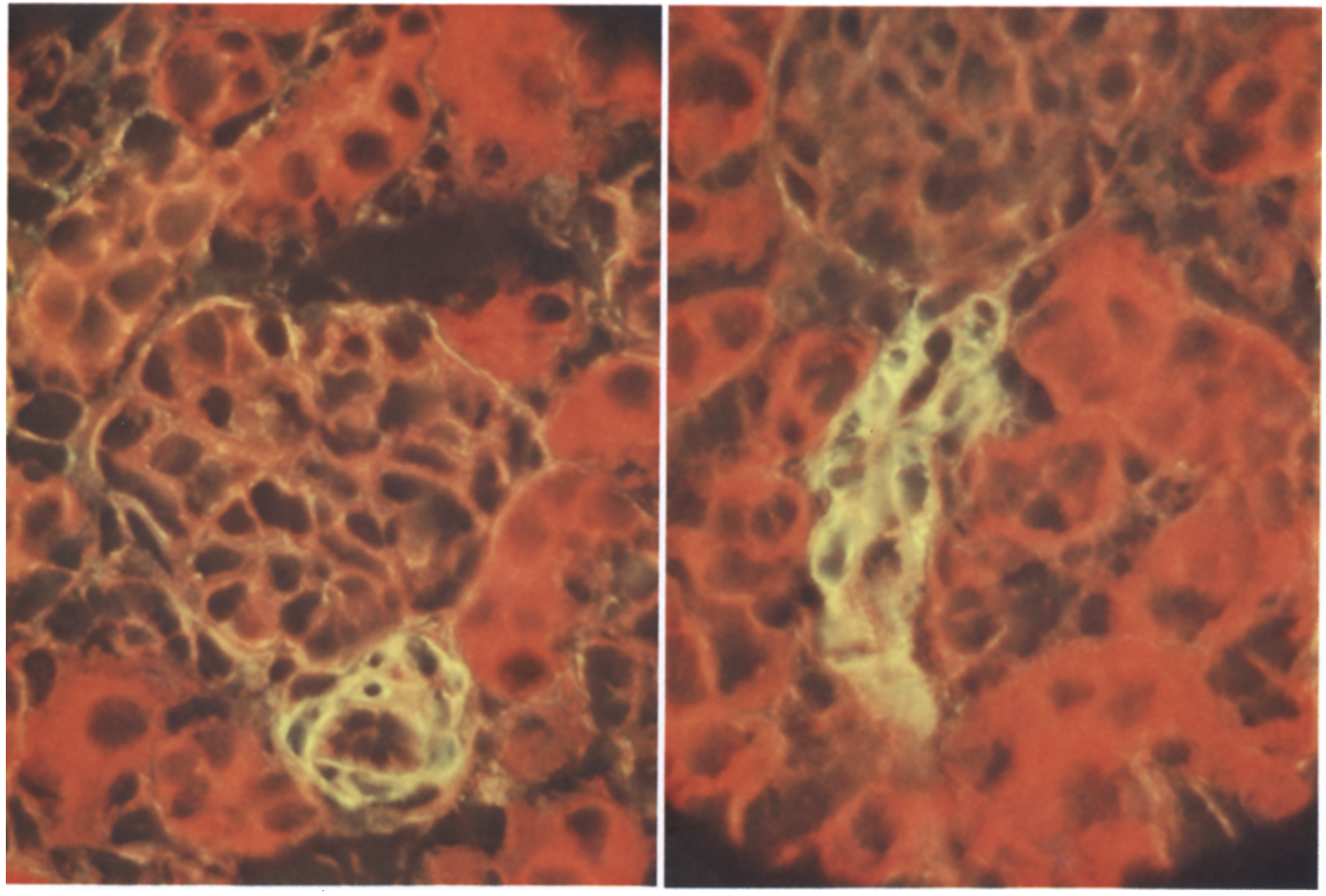
The results obtained by immunofluorescence and light microscopical examinations of 17 to 22 JGAs in the kidney of each rat were recorded separately. A few structures evaluated immunohistologically were excluded for various reasons after studying the Bowie stained sections (e.g., failure to recognize a structure on fluorescence microscopy, technically poor preservation of a JGA). The number of the JGAs in the final computations was, therefore, smaller than that originally examined. From these data, the mean number of cells per JGA and the mean number of cells with either cytoplasmic appearance were calculated, firstly, for the individual animal and, secondly, for each of the four age groups.

Results

The average weights of the kidneys were 0.013, 0.084, 0.132 and 0.242 g and the average body weights 5.29, 14.15, 26.19 and 46.67 g at the age of 1, 11, 21 and 31 days, respectively. The kidney to body weight ratios were 0.02, 0.06, 0.05 and 0.05 at the respective time intervals. As expected, the kidneys of the neonates and 11 days old animals were immature, the outer cortical zone comprising primitive glomeruli and embryonal tubules. While identification of the parenchymatous components posed little difficulties by light microscopy, unequivocally differentiating certain outer cortical structures from each other on fluorescence microscopy was not always easy. Since we selected only clearly identifiable structures for immunohistological evaluation, examination of the JGAs was practically restricted to the inner cortical zone of the kidneys of 1 and 11 days old rats.

Strong specific fluorescence of vascular smooth muscle was evident in kidney sections incubated with ACMS followed by fluoresceinated anti-rabbit gamma-globulin serum. The subpelvic smooth muscle layer was also specifically stained, being especially prominent in the newborn kidneys. There was no specific fluorescence in sections treated with anti-albumin serum and labeled goat antiserum. Further controls of the ACMS specificity were published previously by Gröschel-Stewart and her associates (1976).

Three hundred and sixty three JGAs were evaluated light and immunofluorescence microscopically. The number of specifically fluorescent cells varied greatly from one apparatus to another. Thirty one JGAs (8.5%) were devoid of specifically stained cells. Four to 10 fluorescing cells were counted in 255 JGAs (70.2%). Thirty four (9.4%) and 32 (8.8%) JGAs contained 2–3 and 11–16 stained cells, respectively. A single fluorescing cell per JGA was encountered three times (0.8%), while as many as 17–20 cells were stained in 8 JGAs (2.2%). The different arterioles could generally be clearly distinguished from one another, although we occasionally mistook an efferent for an afferent vessel, as shown during the study of Bowie stained sections. The red fluorescence of the tissues in the Evans blue counterstained sections greatly facilitated the recognition of structural details. The JGAs were characterized by a circumscribed thick arteriolar wall composed of relatively large, rounded or polyhedral cells. Strongly fluorescing spindle shaped cells appeared to correspond to the usual smooth



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Figs. 1 and 2. Juxtaglomerular apparatuses of 11 (*left*) and 21 (*right*) day old rats. Note specific fluorescence of the cytoplasm of the large cells in transversally (*left*) and longitudinally (*right*) cut arterioles. Cryostat sections incubated with rabbit and chicken myosin serum and fluoresceinated goat anti-rabbit gammaglobulin and counterstained with Evans blue. Fig. 1: ×100; Fig. 2: ×160

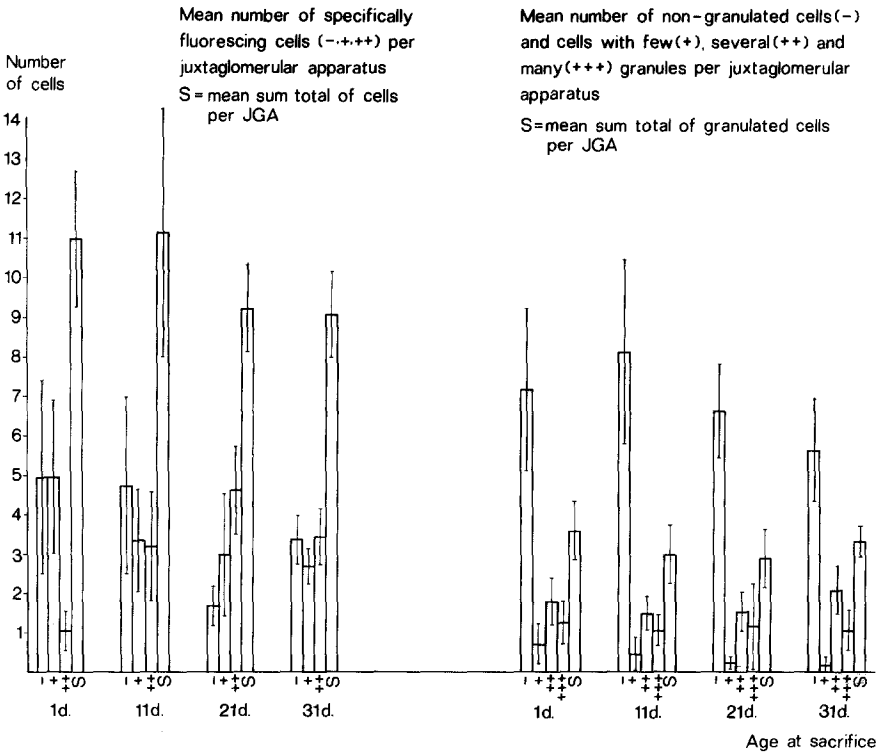

Fig. 3. Histogram showing the mean numbers and standard deviations of specifically fluorescing and granulated cells of the JGAs as compiled for the age groups of 1, 11, 21 and 31 days

muscle elements. Many large cells, being epithelioid in appearance, were also specifically stained (Figs. 1 and 27). The cytoplasm of these cells was either diffusely fluorescent or, more often, showed a delicate granular or stippled pattern.

The results are presented diagramatically in Figure 3, which illustrates the mean number and standard deviation of the specifically fluorescent cells of the JGAs studied at the four time intervals. From this graphic representation it is readily apparent that most afferent arteriolar cells were specifically stained, denoting the widespread occurrence of contractile protein in the juxtaglomerular apparatuses. That the number of specifically fluorescent cells varied considerably from one JGA to another in the same kidney is reflected by the rather large standard deviations.

The results of the examination of Bowie stained sections are summarized in Figure 3. There were, on the average, more nongranulated than granulated cells per JGA, though in an occasional apparatus the latter predominated. A comparison of the schematic drawings made by light or fluorescence microscopy showed clearly the dual reactivity of some cells with both techniques. The average number of granulated cells was about 3 per JGA with a range from none to as many as nine. The JGAs in the inner cortical zone of the

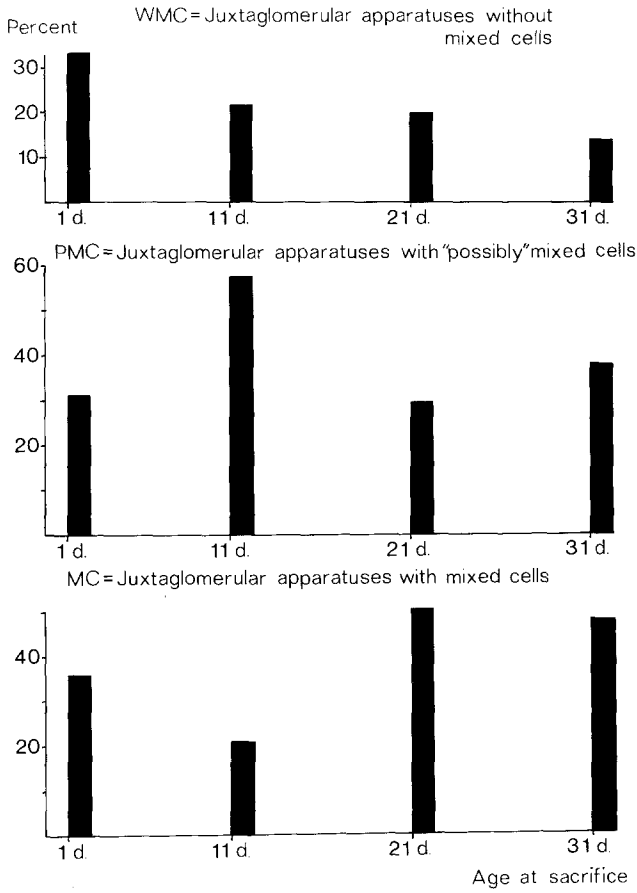

Fig. 4. Diagramatic representation of the percentage of JGAs with mixed cells, "possibly" mixed cells and without mixed cells at the age of 1, 11, 21 and 31 days

newborn rat kidney appeared morphologically mature, as shown by the ratios of granular cells to the total number of JGA cells (R) or to the number of specifically fluorescent cells (R') at the four time intervals: R was 0.33, 0.27, 0.31 and 0.36 and R' was 0.60, 0.46, 0.38 and 0.54 on the 1st, 11th, 21st and 31st postnatal day, respectively.

In order to corroborate further the observation on the dual reactivity of some JGA cells, the following computations were carried out. Theoretically, the juxtaglomerular apparatus might comprise cells endowed solely with one characteristic, i.e., cells with contractile protein only (A) and cells with granules only (B). Assuming that this is indeed the case, then either $A+B=C$ or $A+B<C$, where C is the sum total of the JGA cells. If $A+B<C$, then at least X cells have neither characteristic ($A+B+X=C$). If another possibility, however, is realized, namely that $A+B>C$, then at least Y cells must have both characteristics ($Y=A+B-C$). The calculations, based on the mean number of A-, B-

and *C*-cells, are as follows:

$A_1 + B_1 = 6.07 + 3.85 = 9.92 < C_1 = 11.01$
$A_{11} + B_{11} = 6.59 + 2.89 = 9.57 < C_{11} = 11.16$
$A_{21} + B_{21} = 7.69 + 2.90 = 10.59 > C_{21} = 9.25$, hence $Y_{21} = 1.34$
$A_{31} + B_{31} = 6.18 + 3.33 = 9.51 > C_{31} = 9.11$, hence $Y_{31} = 0.40$.

This value of Y is obviously a minimal one. Many more cells may have been of the Y type, having both contractile protein and granules, and the number of X cells would thus be correspondingly larger. An accurate estimate cannot be made because the study was not designed to determine exactly the number and nature of the X cells.

The JGAs evaluated histologically were assigned to one of three types: 1. Those in which only contractile protein or only granules were detected, i.e., JGAs without mixed or Y cells (referred to as JGA-WMC), 2. JGAs in which, according to the formula $A+B \lesseqgtr C$, mixed or Y cells were certainly present (JGA-MC) and 3. JGAs possibly comprising mixed or Y cells (JGA-PMC). The percentage of juxtaglomerular apparatuses pertaining to each of these three types was calculated for the four time intervals. The results are graphically represented in Figure 4, from which two interesting facets of JGA development emerge. Firstly, at birth about one third of the apparatuses contained cells equipped with contractile protein as well as granules. Secondly, the percentage of apparatuses comprising cells with one component decreased from 33 at birth to 14 at the age of one month.

Discussion

The present observations, based on morphological examinations and a quantitative analysis, demonstrate that the afferent arterioles of the young rat comprise endothelial and adventitial cells and three elements differing from one another in their shape, size and internal structure. Kidney sections treated first with antimyosin and fluoresceinated antisera and then stained by Bowie's technique disclose in the preglomerular segment of the afferent arterioles (1) pure smooth muscle cells, (2) pure secretory cells and (3) cells of the mixed type, reacting positively with both methods. The concurrent presence of contractile protein and specific granules in the same JGA cells has important functional implications: Under physiological conditions, afferent arteriolar muscle cells of the neonate and young animal are capable of contractile as well as secretory activities. Since the techniques used here are of relatively low sensitivity, cells containing small amounts of contractile protein and/or very few and tiny granules could probably not be distinguished from cells without one or another of these components. Hence the actual proportion of mixed cells in the JGA population is probably higher than our findings would lead one to assume. Nevertheless, the proposition that the structurally different JGA cells are and remain distinctly separate elements (Bucher and Reale, 1962; Reale et al., 1963) can be refuted. Our observations extend those of other investigators (Bohle and Sitte, 1966;

Latta and Maunsbach, 1962) who contend that in emergency situations, with increased demand for renin, smooth muscle cells are transformed into epithelioid cells.

The results of a mathematical analysis reveal that the mean sum total of the mean number of specifically fluorescing cells plus the mean number of granulated cells in the JGAs of the 21 and 31 day old rats is larger than the mean number of JGA cells actually counted. The explanation is, of course, that some cells, containing both contractile protein and granules, were counted twice in that they were recognized by both morphological techniques. Similar results were obtained when each individual JGA was analyzed separately. Indeed, we have calculated that 36, 22, 50 and 48 per cent of the apparatuses at the age of 1, 11, 21 and 31 days, respectively, contain cells with both qualities (Fig. 4). Since, for the reasons given above, we probably underestimated the number of mixed cells on the one hand, and took no account of the number of "possibly" mixed cells on the other, it appears to us that the occurrence of JGA cells with both contractile protein and granules is the rule rather than the exception.

Juxtamedullary, mediocortical and subcapsular layers may be distinguished in the kidneys of neonates and 11 day old animals. For the first two weeks of life, nephronogenesis persists in the outer cortical zone. In contrast to this, well developed glomeruli and easily recognizable proximal and distal tubules are present at birth in the juxtamedullary zone. In addition, there are distinct maculae densae and afferent arterioles with granulated epithelioid cells. In the 1 and 11 day old rats, the JGAs of the inner cortical zone, particularly of the juxtamedullary layer, were counted. It is of interest, if not surprising, that about one third of the inner cortical JGAs at birth contain cells with both contractile protein and granules. This observation agrees with the observation that maturation of the nephrons at this developmental stage is well advanced in the juxtamedullary layer. It may be concluded that cellular metabolism in the juxtamedullary layer, unlike the mediocortical and subcapsular layers, provides for cellular proliferation, growth and differentiation as well as functional activities of a more specialized nature.

The juxtaglomerular apparatuses of the newborn rat are apparently equipped to handle physiological demands for renin production and release. Assuming that the number of JGAs consisting of cells endowed with both components is a measure of maturity, our findings are indicative of structural and functional maturation of the JGAs during the first month of life. This is evident from the decrease of JGAs without mixed cells from 33% at birth to 14% at the age of one month (Fig. 4), that is, by a factor of 2.4. We are not aware of comparable data published by other investigators. In any event, with maturation of the kidney, there is a notable decrease in JGAs with cells with one component only while JGAs with mixed cells become prevalent. The present observations extend previous work on quantitative alterations of the juxtaglomerular granulation index from the neonatal period to maturity (Cain and Kraus, 1971b). Perinatally, the first secretory granules are synthesized exclusively in mural cells of the juxtamedullary arterioles. Thereafter, the primarily secretory activity is taken over by the cells of the mediocortical and finally outer cortical afferent

arterioles. We feel that these findings indicate the existence of functional differences of the inner cortex during the neonatal period and adulthood, the inner cortex possibly being subject to different regulatory mechanisms at different times.

The presence of filaments and attachment bodies typical of smooth muscle cells in the cytoplasm of granular juxtaglomerular cells (Adebahr, 1962; Barajas and Latta, 1963; Cain and Kraus, 1976) and the present observations all support the thesis of the bivalent character of afferent arteriolar cells. Furthermore, changes in physiological and pathological demands for renin appear to be associated with predominance of either contractile protein or granules in the JGAs (Hatt, 1961; Hatt, Dvojakovic and Cornet, 1962). We believe that rather than constituting two separate cell populations, the cells of the afferent arterioles, and those of other renal arterioles and arteries (Cantin, Araujo-Nascimento, Benchimol and Desormeaux, 1977), are characterized by their inherent capacity for synthesizing both contractile protein and renin. The histological features of pure smooth muscle and pure granulated cells probably represent the two extremes of a spectrum of possible morphological appearances, expressing the momentary functional state of this regulatory apparatus. It seems inappropriate, therefore, to consider the differentiation of granular cells in the normal kidney and their increase in number under pathological conditions a "metaplasia of smooth muscle cells into juxtaglomerular cells" (Cantin et al., 1977), since mobilization of specialized muscle cells for the enhanced synthesis of renin is probably taking place. That the afferent arteriolar cells of the ischemic kidney do not take up tritiated thymidine (Cantin et al., 1977) is further evidence against metaplastic development of juxtaglomerular cells, since metaplasia is closely linked to cellular proliferation (Wilhelm, 1971). Lastly, the occurrence of intermediate cell forms (Cantin et al., 1977) is in agreement with our contention that an increase in the number of granulated cells reflects a change from one functional state to another.

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