Juxtaglomerular Cells as the Source of Renin: Further Studies with the Fluorescent Antibody Technique and the Effect of Passive Transfer of Antirenin

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ALTHOUGH Goormaghtigh postulated in the early 1940's that juxtaglomerular (JG) cells secreted renin,[1] evidence was lacking until recent years. Results from Tobian's group and our own laboratories[3] showing correlations between renin content and degree of granulation of JG cells under different experimental conditions in rats strengthened this hypothesis. Similar correlations have since been observed by others.[4,5] More direct evidence that renin is concentrated in the vascular pole of the glomerulus was provided by the microdissection studies of Bing and Kazimierczak[6] and separation of glomeruli by magnetic iron as carried out by Cook and Pickering.[7] Application of the fluorescent antibody technique to this problem was first attempted by Nairn, Fraser and Chadwick.[8] These workers, using the "sandwich" technique (sections incubated with unlabelled antirenin followed by fluorescent labelled antiglobulin), observed specific staining in the glomerular tuft and concluded that renin was not secreted by JG cells. Their apparently conflicting results could possibly be explained on two bases. First, the "sandwich" technique, which is useful in enhancing brightness of specific staining, also gives rise to more non-specific or background staining and, secondly, the renin extracts used to produce antibodies were impure, containing other kidney proteins in addition to renin.

Later, Robert Edelman, working in our laboratory, was successful in applying the direct fluorescent antibody technique to localize the histologic site of renin,[9] by using fluorescein-labelled antirenin without the addition of antiglobulin, in contrast to the "sandwich" or indirect method. Specific staining was found in JG cells but not in glomerular elements or in other structures of the renal cortex. Glomerular staining was observed only when the "sandwich" technique was used and, even then, the staining was much less intense than in JG cells and, furthermore, did not satisfy blocking tests. Continuation of our original work has shown consistently that specific staining with fluorescein-labelled antirenin is limited to JG cells. In addition to use of the direct technique, both very thin sections (0.5-1.0 μ) and blocking tests are essential in these studies.[10]

The other objection to use of the fluorescent antibody technique for histologic localization of renin, i.e. the use of impure renin to produce antibodies, has not been overcome completely. However, species cross-reactions in the ability of antirenin to neutralize pressor activity of renin can be utilized both in the staining procedure and in blocking tests, thereby minimizing the effect of antibodies to other kidney protein. In our studies, antibodies to hog renin, produced in the dog, stained JG cells of hog, dog and rabbit, corroborating results of others regarding the pressor-neutralizing characteristics of canine anti-hog renin.[11] Non-specific staining can be detected by direct blocking tests, again taking advantage of species cross-reactions; for example, fluorescent staining of rabbit JG cells by labelled anti-hog renin is blocked if the antirenin is incubated with dog renin before being applied to the section, while non-specific staining remains. Other tests, in addition, provide strong evidence for specificity of the reaction.[9,10,12]

Despite increasing evidence in favour of the JG cell as the source of renin, it has been suggested that more intensive study of the embryo is essential before this concept can be accepted; specifically, renin is known to be present in the kidney of the fetal pig, but JG cells have not been adequately demonstrated.[13] We are studying this problem with both the Bowie stain and the fluorescent antibody technique. So far, we have not obtained suitable material from the mesonephros of the pig embryo, but in the developing metanephros JG cells have been observed by both staining techniques, even in the less mature glomeruli of the outer cortex. Admittedly, JG granules are smaller and more difficult to find in the embryo than in the young adult pig, but their presence can be demonstrated (Figs. 1 and 2). As a corollary to this study, JG cells can be detected by the Bowie stain in the fish mesonephros. In both cases, JG cells are definitely an arteriolar component and distinct from a prominent macula densa, the other main component of the juxtaglomerular complex.[14]

An outgrowth of studies with the fluorescent antibody technique has been an attempt to determine what effect antirenin itself might have on JG cells *in vivo*. As mentioned above, our source of antirenin was from serum of dogs immunized with hog renin, and this antirenin is capable of reacting with the dog's own renin as well as with the antigen, hog renin. We found,[12] as did Schmid and Graham,[4] that such dogs compensate for circulating antirenin by hyperplasia and hypergranulation of JG cells (Fig. 3), the degree of response being

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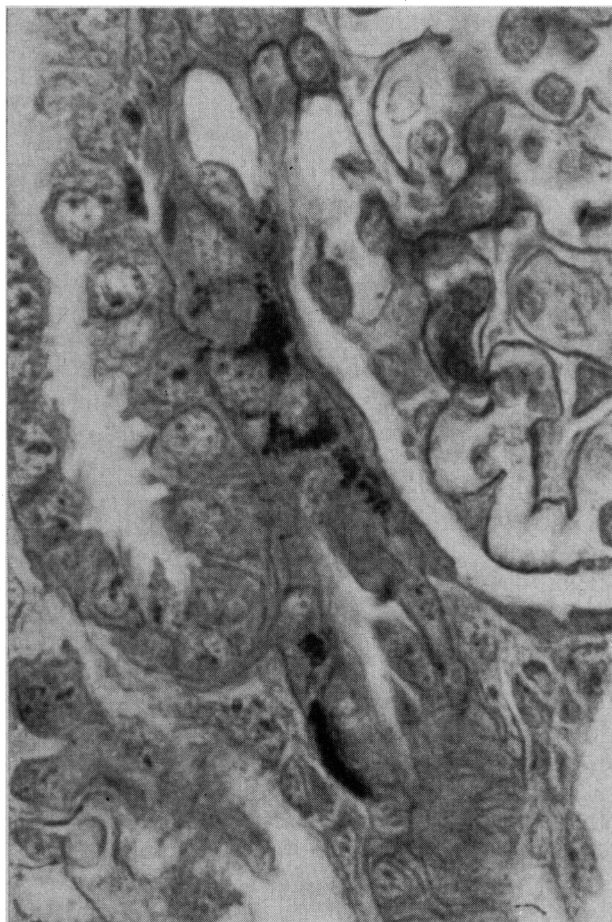

Fig. 1.—Juxtaglomerular (JG) cells in a 5-week-old pig. Glomerulus at the upper right; macula densa, left centre. JG cells in the afferent arteriole contain darkly staining secretory granules. At this site, the arteriole has been cut tangentially (lumen can be seen above and below). Bowie stain; original magnification, × 900.

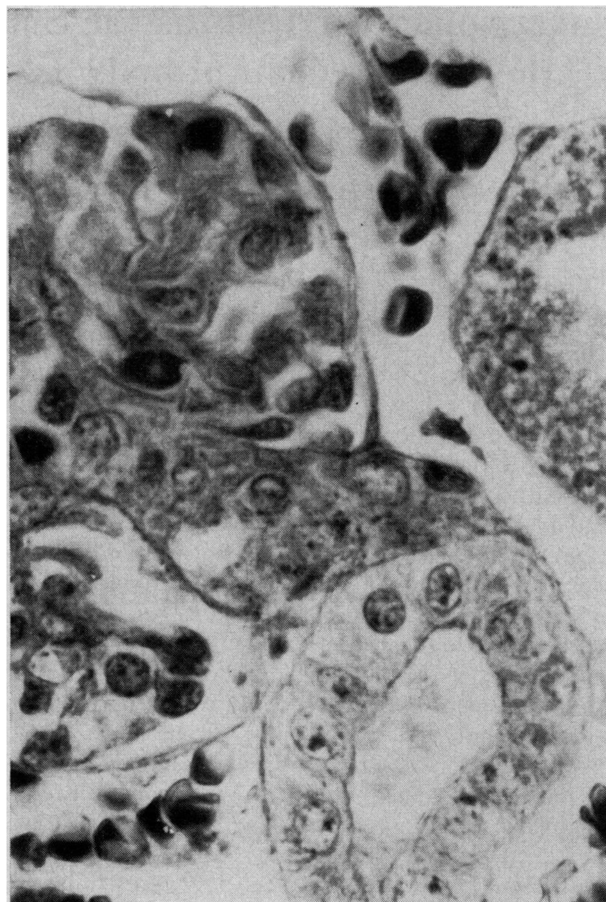

Fig. 2.—JG cells (centre) in a fetal pig metanephros. A prominent macula densa is seen adjacent to and just below JG cells; glomerulus is to the left. JG granules are smaller and less numerous than in Fig. 1. Bowie stain; original magnification, × 900.

proportional to the titre of antirenin. When Freund's adjuvant (complete) is included in the immunization procedure, changes in JG cells are accompanied by infiltration of plasma cells around the glomerular vascular pole adjacent to both macula densa and JG cells (Fig. 4). The latter two structures, however, remain intact with no separation between them. It is quite possible that, rather than infiltrating, the plasma cells may be contained in dilated lymphatic vessels normally draining the glomerular pole. If true, it might be speculated that, under these conditions, renin or angiotensin would not be present in thoracic lymph.

In otherwise normal dogs, immunization with hog renin does not produce obvious changes in blood pressure, in sodium excretion or in the adrenal cortex, despite high titres of antirenin. In contrast, we found that passive transfer of antirenin, if given in large single doses, blocks sodium retention in sodium-deficient dogs,[12] indicating the importance of the renin-angiotensin system in electrolyte handling by the kidney. Schmid[15] came to the same conclusion in studying the effect of exogenous renin; sodium excretion following renin injections was greater in dogs with circulating antirenin than in control dogs. These observations can be explained, at least in part, on the basis of aldosterone secretion. Ganong *et al.*[16] found inhibition of the aldosterone-stimulating activity of renin in nephrectomized dogs, if the renin was first incubated with antirenin. Thus, studies with antirenin have provided further evidence for the dual role of the renin-angiotensin system.

Cytologic changes in JG cells have already been described in dogs immunized with hog renin (above). Passive transfer of antirenin leads to similar changes if injections are given repeatedly by the subcutaneous route over a period of time. JG cells compensate by becoming hyperactive, presumably to secrete excess renin. Changes following single large doses of antirenin, injected intravenously, are best shown by fluorescence microscopy. In sections obtained by biopsy or at autopsy, JG granules were still capable of being stained with fluorescein-labelled antirenin, suggesting that neutralization of renin did not occur intracellularly (i.e. in the JG cell)—that the reaction occurred only intravascularly.[12] Further studies, however, revealed that antirenin, or at least the globulin fraction of plasma protein, was indeed entering the JG cells in these experiments. Anti-canine globulin, conjugated with fluorescein, was used to demonstrate the histologic site of globulin. For control,

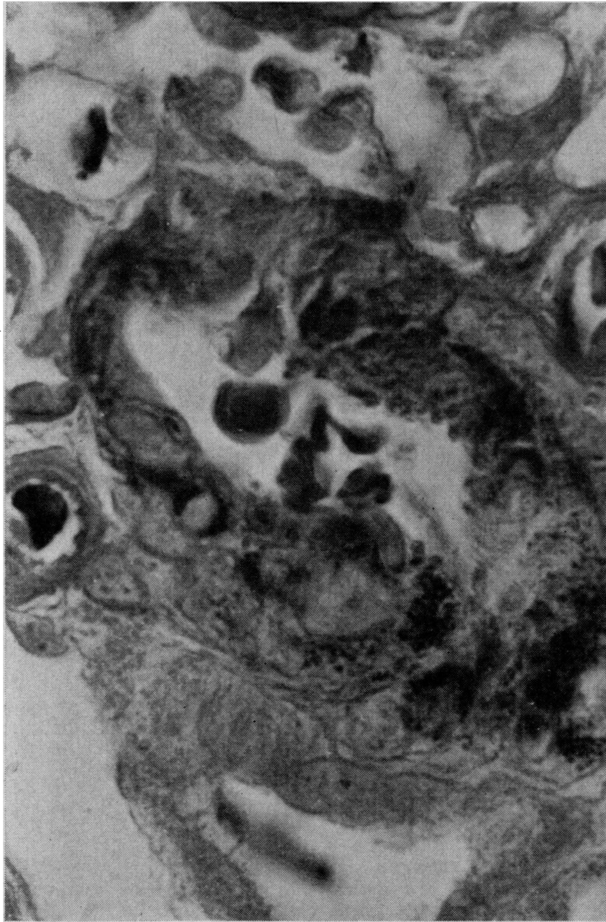

Fig. 3.—Hypergranulation and hyperplasia of JG cells in a dog with a high titre of antirenin following immunization with hog renin. Lumen of the afferent arteriole, centre; glomerulus, above; macula densa, below. Bowie stain; original magnification, × 900.

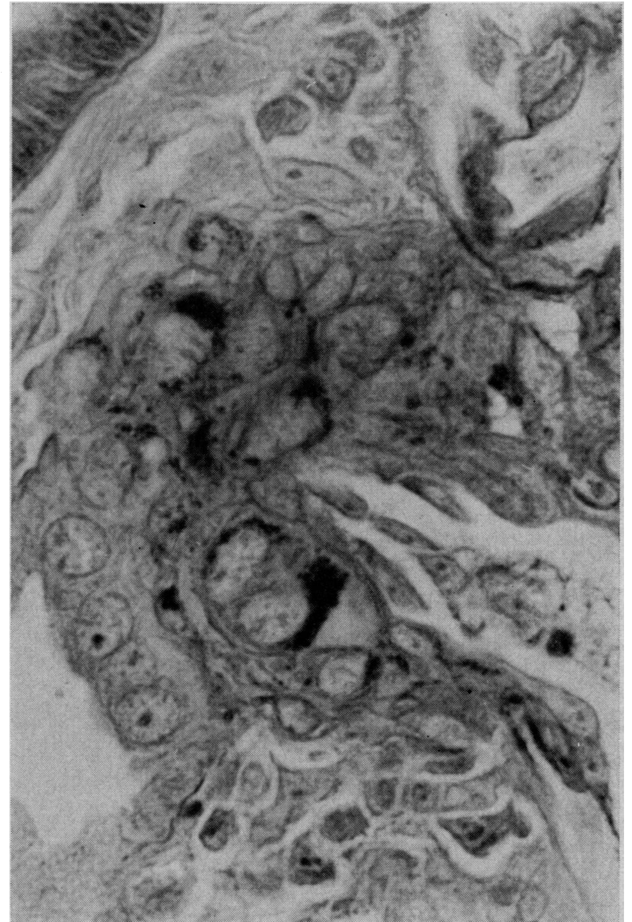

Fig. 4.—JG cells in a similar dog as in Fig. 3, but injected with Freund's adjuvant (complete) as well as hog renin in the immunization procedure. In addition to hyperplasia and hypergranulation of JG cells, plasma cells have accumulated adjacent to both JG cells and macula densa (note especially lower centre). Glomerulus above and to the right; macula densa, below and to the left. Bowie stain; original magnification, × 900.

dogs never injected with canine serum, including those immunized with hog renin which had high titres of antirenin, were compared to dogs injected with normal canine serum and to dogs injected with canine serum containing anitbodies to hog renin. Results are summarized in Table I. Of the experimental models studied, significant staining with fluorescein-labelled antiglobulin was found in JG cells *only* in dogs previously injected with antirenin. Although confirmation and further work to elucidate the fraction or fractions of globulin present are necessary, the possible significance of this observation at present is twofold. First, the observation suggests that globulin (presumably gamma globulin containing antirenin) is capable of entering the JG cells from the blood, and, secondly, with modifications in procedure, it may provide a future tool for damaging JG cells selectively.

TABLE I.—DISTRIBUTION OF GLOBULIN IN DOG KIDNEYS AS SHOWN BY THE FLUORESCENT ANTIBODY TECHNIQUE

Experimental	*JG cells*	*Endothelial cells and blood vessel lumina*
(1) Non-injected....................	Trace	Trace
(2) Immunized with hog renin.......	Trace	Trace
(3) Injected with normal dog serum..	Trace	Dense
(4) Injected with dog serum containing antibodies to hog renin..........	Dense	Moderate to dense

Commercial conjugated anti-canine globulins were used to stain dog kidney sections prepared in the same way as for antirenin.[9, 10] Sequential sections were stained with conjugated antirenin in order to identify fields and compare the staining.

SUMMARY AND CONCLUSIONS

As previously reported,[9, 10, 12] use of the fluorescent antibody technique indicates that the source of renin is the juxtaglomerular (JG) cell and not macula densa or other structures of the renal cortex. This concept is strengthened by the demonstration of JG cells, containing granules, in the fetal pig metanephros and fish mesonephros by fluorescent staining and/or the Bowie stain.

High titres of antirenin in dogs immunized with hog renin are associated with hyperplasia and hypergranulation of JG cells.[4, 12] When Freund's adjuvant (complete) is used in the immunization procedure, plasma cells, indicative of antibody formation, are found in abundance around the glomerular vascular pole, possibly in dilated lymphatics. The significance of this latter finding can only be based on speculation at present.

Passive transfer of antirenin to sodium-deficient dogs blocked sodium retention, illustrating the importance of the renin-angiotensin system in the handling of sodium by the kidney.[12] In these animals, staining with fluorescein-labelled antiglobulin suggests that antirenin entered the JG cell itself, although irreversible damage did not result.

Additional photomicrographs and graphs, illustrating other aspects of the text, may be found in references 9 and 12.

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Discussion

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The location of renin in the kidney is of such importance that another and more direct approach may be mentioned. Cook and I showed that glomeruli could be filled with particles of magnetic oxide of iron. After passing the kidney through a sieve the fragmented glomeruli and tubules are suspended in saline and passed in front of an electromagnet. The glomeruli collect near the pole; the tubules pass on. Assay of the two samples shows that nearly all the renin is contained in the glomerular fraction. The small amount in the tubular fraction can be attributed to contamination with cells and other fragments, as was shown by direct experiment. The glomeruli from the outer part of the cortex contained much more renin than those from the inner part. It was noted that the glomeruli could be separated into those with attached tubules and arterioles, and those in which the glomerular tuft was naked, by passing them through a suitable sieve. Assay showed that the glomeruli with attachments contained more renin from milligram of nitrogen than the naked glomeruli. It, therefore, seemed that renin was located in one of the attachments. Cook proceeded to cut glomeruli in half. The renin was found in the proximal fragment containing the afferent and efferent arterioles and macula densa. None was found in the distal fragment to which the proximal tubule was attached. Finally, we have found that by breaking up the cells mechanically, the renin can be spun out in graded sucrose in the same fraction as the mitochondria. It is not found in the microsomal fraction.

Summary of Discussion—Session 1

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DR. DAHL'S PAPER

The main point of the discussion was related to species differences in response of the blood pressure to high salt intake. Dogs after six years on a high salt diet showed only slight hypertension, in contrast to the relative ease with which hypertension could be produced by renal artery constriction. Rabbits on a high salt intake for two to three years had shown no hypertension, but chicks were highly responsive. In reply to numerous questions, Dr. Dahl stated that no information was available on differences between the salt-sensitive and salt-resistant groups in regard to: changes in intracellular sodium content, plasma volume or extracellular fluid volume; ability of the two groups to handle an acute sodium load; any other change in renal function; differences in the size of the kidneys or adrenals; correlation between persistent hypertension after diminishing high salt intake and structural lesions in the kidneys.

DR. TOBIAN'S PAPER

Morris described nerve endings in relation to the JG cells which he believed acted as chemoreceptors and others as baroreceptors. These, he suggested, might provide a nervous basis for renal responsiveness to changes in perfusion pressure. Tobian agreed that such a mechanism might magnify the signal produced, for example, by slight changes in blood volume.