Because juxtaglomerular (JG) cells are sprinkled throughout the renal cortex, just as beta cells are sprinkled throughout the exocrine pancreas, the classical extirpation and substitution approach in endocrinology is a limited means for the study of their function. Until they can be isolated and pure extracts made, or until an "alloxan" for JG cells is discovered, their study will depend chiefly upon microscopy. In this presentation, I should like to discuss the following aspects of JG cells: first, morphology and significance of cytological changes; second, factors that control their cytological activity; third, evidence that they produce renin, especially that obtained with the fluorescent antibody technique, and related functional studies with antirenin.

MORPHOLOGY AND SIGNIFICANCE OF CYTLOGICAL CHANGES

The first description of juxtaglomerular cells was in the mouse by Ruyter, a Dutch technician. Of at least 13 species that we have examined, the mouse has the largest and most numerous granules in JG cells. They are, therefore, more easily stained and identified in this animal. As Ruyter's illustrations demonstrate, JG cells are found in the wall of the afferent arteriole, usually just before it enters the glomerulus.

In any discussion of morphology of JG cells, the late Professor Goormaghtigh's beautiful camera lucida tracings should not be forgotten. There is no doubt now that his tracings were accurate in every detail, although for years the fact that his illustrations were considered drawings rather than true outlines of what he saw, led to a certain amount of skepticism. One of Goormaghtigh's camera lucida tracings is shown in figure 1, illustrating JG cells of a rabbit in which the renal artery had been constricted. It demonstrates features of the vascular pole of the glomerulus. Juxtaglomerular cells have a close anatomical relation to both macula densa and also to polkissen or "lacis" cells. Unfortunately, little is known about the function of these structures. McManus pointed out that the position of the Golgi apparatus is reversed in cells of the macula densa and that the basement membrane is absent in this portion of the tubule. He suggested that this arrangement might permit substances to pass back and forth between these two cell types. Recent evidence for a functional relation has been obtained by Fisher, who found that enzyme activity in the macula densa paralleled changes in the granulation of JG cells. The polkissen or lacis cells were once thought to be neural elements.
FIGURE 2
Vascular pole of the glomerulus, showing both efferent (left) and afferent (right) arterioles. "Luci" cells are seen in the triangle formed by the two arterioles. JG cells with dark granules are in the wall of the afferent arteriole, but extend close to where efferent arteriole leaves the glomerulus. Bowie stain. Section from sodium-deficient pig.

FIGURE 3
Hypergranulated and hyperplastic juxtaglomerular cells in a sodium-deficient dog. Glomerulus to the left; macula densa, lower right. Note round vacuoles in some of the JG cells close to the bulging macula densa. Bowie stain.

Electron microscopy has not produced confirmatory evidence. Oberling and Flatt, and Latta and Maunsbach, believe they are continuous with the mesangial cells of the glomerulus and also that they may be transformed into JG cells in conditions of hyperactivity. If true, the occasional appearance of JG granules in efferent arterioles would be satisfactorily explained (Fig. 5).

Electron microscopy reveals that the granules in JG cells are larger and more homogeneous than the mitochondria and that they are surrounded by a limiting membrane. The cytoplasm is richly supplied with endoplasmic reticulum. These features corroborate the concept, based originally on light microscopy only, that JG cells are secretory in nature. However, the interpretation of cytological changes in these cells, as an index of hyperactivity, remains an important question.

Many studies, including our own, have depended upon changes in granulation as seen with the light microscope and assessed by a counting method. Granule-content alone would be a poor indication of secretory activity, but in chronic conditions in which JG granulation is known to increase, evidence for hypersecretion is strong. For example, during the acute phase of sodium deficiency, a significant degree of degranulation of JG cells precedes chronic hypergranulation—a response analogous to that of other secretory cells and quite consistent with stimulation.

Furthermore, in chronic conditions, hypergranulation of JG cells is almost invariably associated with an increase in size of individual cells, and frequently hyperplasia and metaplasia are observed. Another change, seen most often in the hu-
FIGURE 5
Electron micrograph including a macula densa (MD) cell at the lower right and JG cell upper left. A vacuole is situated just to the right of the nucleus of the JG cell. Between the nucleus and the vacuole a Golgi (GO) element and a secretory granule (G) can be seen. Note intercellular channels, which appear to be continuous with endoplasmic reticulum of both JG and macula densa cells. Osmium-fixed; methacrylate embedded. (From Hartroft and Neicmark: Anat. Record 139: 185, 1961.)

man kidney and to a lesser extent in that of other species, is the presence of vacuoles in JG cells. Goormaghtigh observed these changes in JG cells of rabbits and suggested that they represented part of a glandular cycle. We have seen similar vacuoles in hyperplastic JG cells in dogs (fig. 3); electron microscopy indicates that these vacuoles may be associated with Golgi elements (fig. 5)—another indication of hyperactivity.

From these observations, it seems quite safe to assume that with the possible exception of acute situations, an increase in granulation of JG cells—or in the JG index—indicates hypersecretion. Conversely, in the absence of cytological changes indicative of hyperactivity, as described above, a drop in JGI, or degranulation, can be interpreted as depressed activity.

CONTROL OF JUXTAGLOMERULAR CELLS
I should like to turn now to the question of control of JG cells, or factors that affect
Juxtaglomerular Cells, Liver Disease - Ascites

Graph showing granulation of JG cells in patients dying of liver disease, with and without ascites. The vertical lines represent plus or minus standard error of the mean. Numbers in parentheses refer to the number of cases in each group. JGI—juxtaglomerular granulation index.

their cytological activity. This subject has been reviewed recently, so I will attempt only a brief summary with emphasis on certain aspects. The classification of factors controlling JG cells, described below, is somewhat arbitrary, but has been followed for purposes of presentation. Counterparts in human disease have now been demonstrated for many of the changes in juxtaglomerular cells that have been produced in experimental models.

Pituitary
JG cells probably are not under control of the pituitary. Hypophysectomy in rats neither alters JG cells nor prevents their stimulation by sodium deficiency.

Adrenal
As first shown by Dunihue, JG cells are hypergranulated in adrenalectomized animals. The same change has been observed in Addison's disease and in children with adrenogenital syndrome who die with symptoms of adrenal insufficiency (unpublished). Administration of desoxycorticosterone acetate in animals decreases the granulation of JG cells.

Elevated Blood Pressure
Depression of JG granulation results from an increase in pressure when renal ischemia is not a factor. Experimental examples are injections of naphazoline in rats, high perfusion pressure of isolated kidneys, "untouched" kidney in hypertensive animals with unilateral constriction of the renal artery, and rats with adrenal regeneration or metacorticoid hypertension. In man, JGI is lower in the unaffected kidney in cases of renal hypertension associated with an obstruction in the contralateral renal artery.

Decrease in Blood Pressure or the Presence of Renal Ischemia
Decrease in blood pressure or the presence of renal ischemia are associated with hypergranulation of JG cells. Experimental examples are injections of hydralazine in rats, hemorrhage, and renal ischemia produced by constricting the renal artery, cellophane perinephritis, etc. In man, the same change has been observed in malignant hypertension, hypoxic nephrosis, and in the ischemic "Goldblatt" kidney.

Sodium
As noted above, sodium restriction is followed by an increase in JGI in several species. In man, hyponatremia, regardless of the nature of the disease, is also associated with hypergranulation and hyperplasia of JG cells. In animals, excess sodium, whether in drinking water or added to the diet, decreases JGI. The adrenal zona glomerulosa responds in a parallel way, as first shown by Deane et al, with hypertrophy in sodium deficiency or hyponatremia and atrophy with high-sodium intake. Several years ago, statistical correlations between JGI and the width of zona glomerulosa suggested a functional relationship between these structures; this has been borne out by the accumulating evidence of the last few years.

Potassium
The observations of Deane et al that a high potassium intake produces the same histological changes in the zona glomerulosa as does a low sodium intake, led us to study the effect of potassium on the JG cells. Because of the correlation referred to above, it was expected that JG cells would respond to variations in potassium intake. However, the JG
cells in rats remained unaltered despite dietary deficiency or excess of potassium, although Deane's observations concerning the adrenal were confirmed. In man, the same situation is probably true. We have observed that in conditions with a combination of hypokalemia and hyponatremia, JG cells responded as though hyponatremia alone had been present.

Ascites and Edema

Conditions associated with ascites and edema cause hypergranulation of JG cells. Experimental examples are seen in dogs in which the thoracic vena cava has been constricted and in rats in which ascites was subsequent to aminonucleoside nephrosis. In man, hypergranulation is seen in heart disease with edema and in liver disease with ascites (fig. 6).

RELATION OF JG CELLS TO RENIN AND ANTI-RENNIN

Evidence that renin is elaborated by JG cells, as reviewed previously, is based on correlations of renin content and index of granulation of JG cells, microdissection techniques, and the fluorescent antibody technique. Only the latter is discussed in this presentation.

This work was first done in our laboratories at Washington University in collaboration with Robert Edelman; it is being continued in Toronto with Dr. L. Sutherland. Localization of renin in sections of kidneys from rabbits and dogs was demonstrated by means of...
Hyperplastic JG cells in a sodium-deficient dog. Glomerulus to the left. Different arteriole is cut in cross section, with small clusters of JG granules showing bright specific fluorescence. Red cells in glomerulus show autofluorescence. Macula densa shows no staining. Frozen-dried section stained with fluorescein-labeled antihog renin. fluorescein-labeled canine serum which contained antibodies of hog renin. The species cross reaction of antihog renin was advantageous in these studies. Since the renin extracts used to produce antirenin are not pure (sp. acty: 10 units/mg of protein); the resulting antisera must contain antibodies to small amounts of kidney protein in addition to renin. Since antihog renin also neutralizes dog and rabbit renin,33 antibodies to renin in these two species are effective while antibodies to other hog protein are less likely to interfere. When either frozen-dried or cold acetone fixed sections were incubated with the fluorescein-labeled antiserum, the result was very specific staining of JG cells in the rabbit and dog (figs. 7 and 8). Contrary to results of others using microdissection techniques,51 no specific staining has been observed in the macula densa.

Recently, we have stained JG cells of the pig with this antiserum. The purpose was first, to determine whether structures other than JG cells would stain in the kidney of this species and second, to determine whether the staining would be altered by adsorbing the conjugated antiserum with hog kidney powder rather than in the conventional way with liver and bone marrow powder. Two three-week-old pigs were used: one was fed a high-salt diet for two weeks to provide a kidney powder of low renin content; the other pig was fed a low-salt diet for two weeks to make JG cells more prominent for staining purposes. The adrenals and JG cells of these two animals responded to the diets as well, or better, than other species we have studied (figs. 9, 10). With the fluorescent antibody technique, JG cells stained brilliantly in sections incubated with labeled antirenin. However, brushborders and droplets in proximal tubules also stained with antiserum adsorbed in the conventional way with liver and bone marrow powder. Evidence for the specificity of the stain was obtained in a significant reduction in staining of proximal tubules when the conjugated antiserum was adsorbed with hog kidney powder, while the staining of JG cells remained unaffected (fig. 11).

Since the fluorescent antibody studies indicate that antirenin localizes in JG cells in vitro, the possibility that localization would occur in vivo was investigated. We were interested in the possibilities that antirenin would affect sodium excretion and that JG cells would be damaged by exposure to antirenin.

In dogs immunized with hog renin, excretion of sodium remained normal, as did histological appearance of zona glomerulosa, despite the fact that the antibodies these animals produced were capable of neutralizing their own renin. The explanation may lie in the hypergranulation and hyperplasia of the JG cells (fig. 12), which indicate that the circulating antirenin is compensated for by an increased renin secretion. This change in JG cells has also been observed by Schmid.34 Similar changes occurred with passive immunization, when serum containing antirenin was injected subcutaneously for several days in dogs and rabbits although in several instances a suggestive inhibition of sodium retention resulted when the animals were fed a sodium-deficient diet. On the other hand, single large
intravenous doses of antirenin produced a very definite effect on sodium excretion.

In this study, four small dogs were used, weighing 1.8 to 3.6 kg. Three had been hypophysectomized as puppies; the other was a toy terrier with a full-grown weight of 3.6 kg. Since the responses of the intact dog were similar to those of the hypophysectomized dogs, the results on all four dogs will be presented together. In each case, the volume of serum injected was 8 ml/kg, but the antirenin titer of the serum varied from 90 to over 100

*Mr. Lawrence Lynch, medical student at Washington University, collaborated with the author on portions of this study during the summer months of 1961 and 1962, while working on a Summer Research Fellowship awarded by Washington University.

units per ml. Each animal served as its own control. They were maintained on a sodium-deficient diet, and after urinary sodium was stable at a low level, the serum was injected intravenously in a single dose. Although the injected serum contained about 10 times the amount of sodium excreted in 24 hours, only a slight and insignificant increase in urinary sodium occurred following the injection of control serum. However, when serum containing antirenin was injected, there was a striking increase in sodium excretion during the next 24 to 48 hours. No consistent changes were observed in urinary volume, potassium excretion, or in serum electrolytes. The combined results of 15 experiments (8 antirenin; 7 control) are shown in figure 13.
Sections of kidneys from same animals as in figure 9, showing typical fields of JG cells. (Left) high-salt diet. (Right) sodium deficiency. Bowie stain.

Cold acetone fixed section of kidney from sodium-deficient pig, stained with fluorescein-labeled anti-hog renin. Only a small portion of the glomerulus is included in the field. JG cells show bright specific fluorescence. Macula densa (just below and to the right of JG cells) does not stain. The conjugated antirenin was adsorbed with hog kidney powder before staining.

Hypergranulated and hyperplastic JG cells in a dog with a high titer of circulating antirenin following immunization with hog renin. Glomerulus, to the left; macula densa, to the right. Bowie stain.
Effect of Antirenin on Urinary Electrolytes in Na-deficient Dogs

As noted, the antirenin titer of the serum used in these experiments varied. In four of the eight antirenin experiments, the recipients' own serum was assayed for antirenin one day after the injection. These values were plotted against the change in urinary Na/K with good correlation. JG granules in the kidneys of these animals could still be stained by fluorescein-labeled antirenin, indicating that intracellular renin was not blocked. This observation is not too surprising since the effect of antirenin was transient, lasting for only two days.

Summary

Morphological and ultrastructural characteristics of JG cells are consistent with the concept of secretory or endocrine activity. At least in the chronic situation, evidence indicates that hypergranulation of JG cells—or elevated JGI—means hyperactivity. Factors that control cytoplasmic activity of JG cells are concerned with volume or pressure changes and with sodium. Despite the influence of potassium in the adrenal zona glomerulosa, potassium does not affect JG cells. The fluorescent antibody technique in the rabbit, dog, and domestic pig, demonstrates that renin is located in the JG cells, but not in the macula densa or other structures of the renal cortex. Inhibition of sodium retention by antirenin in sodium-deficient dogs provides further evidence that renin, secreted by JG cells, is concerned in the regulation of sodium excretion, whether by its influence on aldosterone or by an intrarenal mechanism.

References


Discussion

Dr. Louis Tobian, Minneapolis: From a historical perspective, it should be pointed out that the juxtaglomerular cells were given great prominence in the late 1930's and early 1940's, only to become almost moribund in terms of scientific interest until their recent resurrection by Dr. Phyllis Hartroft. Her studies have stimulated others, and it is now evident that this resurrection has indeed been a fruitful one. I was impressed with the antirenin studies on sodium excretion. Indirect evidence has shown that the juxtaglomerular cells and their secretion of renin are very important regulators of sodium excretion, but not one of us, I think, has seen a direct demonstration of this. From the results on these slides, one would have to be utterly convinced that the juxtaglomerular cells play an important part in this regulation. These cells, situated where they are, seem to have the effect of regulating arteriolar pressure, possibly in the body as a whole, but certainly in the afferent arteriole. Through their responsiveness to changes in pressure or volume in the afferent arteriole, and through whatever mechanism they respond to, the juxtaglomerular cells not only cause increased reabsorption of sodium—and presumably water also—but perhaps in addition cause the humoral effect on blood vessels of the angiotensin indirectly produced. These two effects, by changing the caliber of vessels and by regulating volume, would tend to keep arteriolar pressure in the body, and certainly in the kidney, at a normal level. I think Dr. Hartroft's talk has certainly provided very solid evidence of the importance of these cells as a sort of prime volume receptor or space receptor causing this regulation.

Dr. Jacques Genest, Montreal: I should like to support the concept put forward by Dr. Hartroft concerning the role of angiotensin on sodium excretion. As you all know, angiotensin infused into normal subjects stimulates aldosterone secretion and excretion and this is accompanied by marked sodium retention. However, we have infused angiotensin in hypertensive subjects and have observed in these patients the same degree of aldosterone stimulation, but in contrast to normal subjects, this is accompanied by a very marked natriuresis. Because of these effects of angiotensin on the renal regulation of sodium, we became interested in the determination of arterial blood angiotensin levels in patients with severe generalized edema. Preliminary results indicate that in most of the patients studied, there is greatly increased concentration of angiotensin in arterial blood. All these patients with edema were normotensive and this finding brings up a very interesting question as to why these patients have a high arterial angiotensin level without any diastolic hypertension. One patient with nephrotic syndrome secondary to disseminated lupus erythematosus had the most refractory type of edema we have ever seen. This edema was resistant to very high doses of natriuretic agents (thiazides, chlorothalidone, spironolactone, triamterene and mercurials), used singly or in various combinations. The patient had a normal urinary aldosterone but the arterial blood taken on two different occasions contained excessive amounts of angiotensin. These findings may be in line with the concept put forward by Dr. Bartter, three years ago, concerning the increased "sympathoadrenal tone" in kidneys of dogs with cardiac failure. This suggests that intrarenal circulatory factors may be quite important, in addition to hormonal factors, in the regulation of salt excretion.

Dr. Meyer Friedman, San Francisco: Every year someone attempts to prove that the juxtaglomerular apparatus is producing renin. Anything that has to be proved every year gives one a little doubt, particularly when there has been proof otherwise in the early 1940's that this apparatus might not be the
site of renin formation. We too used associative data (which mostly you have seen today) obtained mainly from the fetal pig. The mesonephros is a very early portion of the fetal pig and has very little glomerular structure. Nor could we find anything that even looked like a juxtaglomerular cell in the 18 mm fetus of the pig. Yet per gram of weight, there was adequate renin in such kidneys, and as the tubular phase of such fetuses degenerate, so did the renin content diminish. In the metanephros (the final kidney) whose initially there are plenty of glomeruli but little tubular mass, the renin content increased along with the tubular mass. Braun-Menendez also thought he was able to get singular portions of the proximal glomerular tubule as distinguished from the juxtaglomerular apparatus, and he too found that seemingly the proximal tubule contained renin. When we felt we had damaged the proximal tubule in the mature rabbit, we observed a marked diminution in the renin content of that kidney. Investigators who claim that the JG cells produce renin should go to an embryonic animal—dog or pig—obtain the mesonephros, demonstrate the presence of the juxtaglomerular structure there, and our arguments would cease. But as long as these fetal kidneys assay for renin, and no one has found juxtaglomerular cells, I think it is very difficult to positulate that renin is coming from nothing in the fetal kidney.

Dr. Alberto C. Taquini, Jr., Buenos Aires: I should like to ask Dr. Hartroft why the increase in the juxtaglomerular index in the clip kidney does not keep up with the increase of renin or circulating substances in chronic experimental renal hypertension. Some studies have shown that in the chronic phase of experimental renal hypertension, there is no increase in renin or angiotensin. I understand that in your studies, as well as in those of Dr. Tobian, in the clip kidney there is an increase in the JG index.

Dr. Oscar Helmer, Indianapolis: Both Dr. Peart and I now can definitely find renin in the chronic hypertensive animal. Both of us, using similar techniques, can show that in the chronic hypertensive animal there is renin present.

Dr. Alberto C. Taquini, Jr., Buenos Aires: If there is a pressor substance circulating in the hypertensive animal, one would expect that with cross circulation experiments with a technique capable of detecting a pressor substance when it is infused in the recipient rat, one would find it in the circulation. We also were able to show that if one destroys the central nervous system of rats in which one had previously infused angiotensin, or norepinephrine, the floor pressure reached after pithing is higher than that of normal or hypertensive animals. If the infusion is discontinued, they fall to the same level of normal and hypertensive animals. In any case, it is a matter of who looks at the problem whether one is convinced that the pressor substance can be increased.

Dr. George E. Wakerlin, New York: In 1948, Dr. John Marshall (at the University of Illinois) showed that when one produces a high titer of antirenin to hog renin in dogs, one not only obtains a marked increase in the renin content of the kidney, but also a considerable increase in the granules in the juxtaglomerular apparatus. Later, in 1956-1958, Dr. Herman Schmid extended this work and produced high titers of antirenin in a large number of dogs, as has been reported by Dr. Hartroft today, and found a large increase in the amount of renin in the kidneys and a considerable increase in the granules in the.
cells of the juxtaglomerular apparatus. Indeed, there was an excellent correlation among the amount of renin in the kidney, the titer of antirenin and the granularity of the juxtaglomerular apparatus. I do not know what the explanation of Dr. Friedman's earlier results is, but for me the findings of Dr. Hartroft and her group, and of Dr. Tobian, are very convincing. I feel now we have the evidence that renin is secreted by the juxtaglomerular apparatus.

Dr. Sydney M. Friedman, Vancouver: I think a good case has been made for the origin of renin from JG cells, although the fetal kidney findings remain unexplained and most tantalizing. I do not feel we should reject the conclusion that renin is formed in the juxtaglomerular region. It is possible, however, that the formation of renin shifts from a primitive generalized locus in the fetus to a more specialized region as maturation occurs. Also, and more importantly, the pituitary should not be denied consideration on the basis of negative evidence obtained from experiments of the type in which the pituitary is removed. When dealing with distribution of salt and water and the regulatory mechanisms for blood pressure, much more detailed and thorough examination must be made of the individual discrete functions of the pituitary before one can rule out a role for it. I think the interrelationship of the posterior pituitary, the adrenal cortex, and the juxtaglomerular cells must be taken into account when considering how this machinery actually works. I should like to ask that the pituitary extirpation experiment cited should not be taken as critical evidence. Similarly, general feeding experiments, where one deprives salt or adds potassium, likewise cannot be considered critical because there are too many steps intervening between the experimental manipulation and what actually happens in the animal body.

Dr. Phyllis M. Hartroft, Toronto: As Dr. Meyer Friedman has pointed out, it is difficult to demonstrate juxtaglomerular cells in the embryonic kidney by the usual staining techniques, and I have to agree with him that the embryology of JG cells needs intensive study. Possibly, the fluorescent-antibody technique might help in that respect. I should like to take issue with the remark that evidence for JG cells as the site of renin elaboration is based on " associative data." It is true that earlier evidence was only associative and even direct correlations between JG granularity and renin content might be put in that classification, but more recent methods are much more direct. In addition to the fluorescent-antibody technique which I have discussed, other supporting evidence can be cited. Both Bing's results with microdissection techniques, and Cook's results with microdissection techniques, and Cook's results with glomeruli isolated with magnetic iron, have shown that renin is concentrated in structures of the vascular pole of the glomerulus. With regard to Dr. Taquin's question, in chronic experimental renal hypertension, JG granularity is not always increased. In our experience with hypertensive rats in which one
renal artery is constricted, changes in JG granulation in the clipped kidney are inconsistent while degranulation of JG cells in the opposite kidney is a usual finding. In other types of renal ischemia (e.g., Dunihue's studies on cellophane perinephritis) the increase in JG granulation may be only temporary.

In answer to Dr. Hoobler's questions, when sodium deficiency does produce a drop in serum sodium, there is an inverse correlation between granulation of juxtaglomerular cells and the level of serum sodium. We have shown this in cats and dogs as well as in rats and, as I mentioned briefly, these parameters can also be correlated in man. On the other hand, dietary sodium deficiency may produce a good increase in granulation without a drop in serum sodium, although changes in JG cells are never as severe as when hyponatremia is present. In this case, the stimulus for JG cells might be termed a tendency for a drop in serum sodium, and the resulting increase in granulation of JG cells would represent a compensatory mechanism which has succeeded in maintaining normal serum sodium.

References

Juxtaglomerular Cells
PHYLLIS M. HARTROFT

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