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THE GRANULATION OF JUXTAGLOMERULAR CELLS IN RENAL HYPERTENSION, DESOXYCORTICOSTERONE AND POST-DESOXYCORTICOSTERONE HYPERTENSION, ADRENAL REGENERATION HYPERTENSION, AND ADRENAL INSUFFICIENCY

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Research Article

THE GRANULATION OF JUXTAGLOMERULAR CELLS IN RENAL HYPERTENSION, DESOXYCORTICOSTERONE AND POST-DESOXYCORTICOSTERONE HYPERTENSION, ADRENAL REGENERATION HYPERTENSION, AND ADRENAL INSUFFICIENCY [1, 2]

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The pathogenesis of renal hypertension remains a mystery. Renal disease can be severe enough to produce terminal uremia without producing hypertension. On the other hand, hypertension can be induced by partial constriction of the renal artery, and the kidney in question may retain good excretory function. The excretory activities of the kidney seem not to be directly related to its hypertensive or antihypertensive functions. It is therefore inviting to consider aspects of renal anatomy other than those directly and obviously concerned with excretion. Such a structure is the juxtaglomerular apparatus. Certain cells of this juxtaglomerular complex containing discrete secretory granules are found in the wall of the afferent glomerular arteriole, just before it joins the glomerulus. Goormaghtigh studied them many years ago in relation to renal hypertension (2). These cells are in a position which would be particularly affected by changes in intraluminal pressure, such as occurs with Goldblatt clamping. In the classic Goldblatt experiment, the renal artery is partially constricted, producing a decrement in blood pressure downstream. Such a decrement in pressure would reduce the stretch of the walls of the entire renal arborization, including the juxtaglomerular cells residing in the arteriolar walls. These cells, then, could be stretch receptors which change their rate of secretion in response to changes in stretch. With this in mind, the juxtaglomerular apparatus was studied in relation to the Goldblatt type of experimental hypertension. If the juxtaglomerular apparatus has any fundamental role in renal hypertension it might also be altered in other forms of experimental hypertension. Therefore, rats with desoxycorticosterone hypertension, postdesoxycorticosterone hypertension, and adrenal regeneration were also studied.

METHODS

This study was greatly facilitated by the use of the staining technique developed by the Hartrofts for the juxtaglomerular (JG) apparatus (3). Each granule is discretely stained in this method. A 40x fluorite oil immersion objective enabled us to see the individual secretory granules much better than the usual 43x achromatic "high dry" objective, yet provided a large field for rapid scanning. Compensating eyepieces (12x) were used in conjunction with this objective. The degree of granulation in the juxtaglomerular apparatus was estimated using the system devised by the Hartrofts (3). Their weighted juxtaglomerular index (JG index) was utilized, giving a total index number per 100 glomeruli. This system is advantageous in that it counts each JG complex and weighs it according to its degree of granulation. Experience indicated that whenever a large number of juxtaglomerular complexes were to be seen, the number of granules per complex were usually also increased. Even though this is a semiquantitative method, it has considerable precision. Two different observers scanning the same slide rarely differed by more than 10 per cent. Figure 1 shows various groups of juxtaglomerular cells.

Renal hypertension was produced in our rats by partially constricting the renal artery, using a silver ribbon clip. This is the method developed by Wilson and Byrom (4) and used extensively in recent years by Floyer (5). In the rat, hypertension can be produced by constricting only one renal artery and leaving the other

[1] This investigation was supported by a grant from the American Heart Association and by Grant No. H2008 from the National Heart Institute, United States Public Health Service.

[2] This article is a full report of studies presented at the meeting of the American Federation for Clinical Research, May, 1957 (1).

[3] This work was started during the tenure of an Established Investigatorship of the American Heart Association.

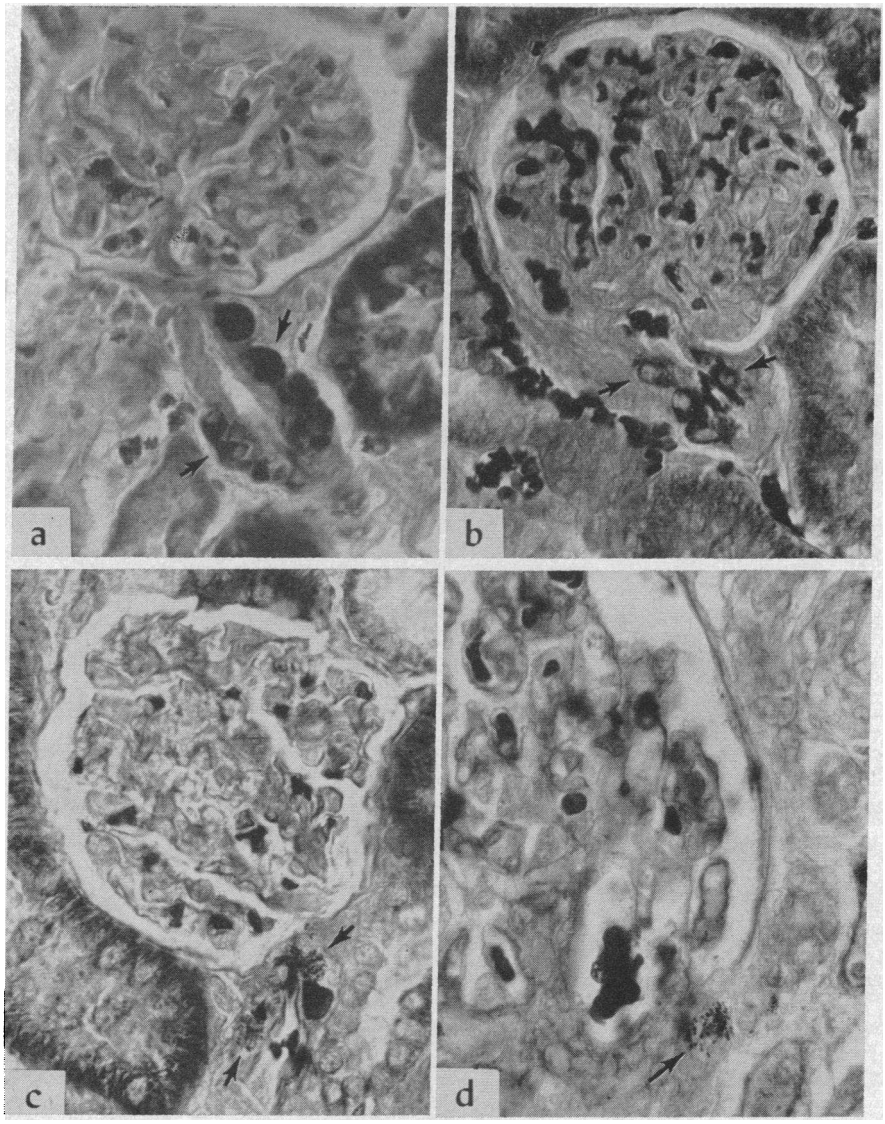

FIG. 1. VARIOUS GROUPS OF JUXTAGLOMERULAR CELLS

In picture a, eight juxtaglomerular (JG) cells loaded with granules are seen in the wall of the afferent arteriole. In b four JG cells laden with granules are seen in the wall of the afferent arteriole. The unstained nuclei of these cells are surrounded by the black granules. In c, two cells are seen which are only moderately filled with granules. Pictures a, b, and c are sections from rat kidney. Picture d shows one granular JG cell in a section from the kidney of a patient with malignant hypertension.

kidney untouched (4, 5). This form of hypertension was utilized in these studies. Floyer's useful terminology is also employed. The "clipped" kidney is one with the renal artery partially constricted; the "untouched" kidney is the unmanipulated kidney opposite to the "clipped" one (5).

The level of arterial blood pressure in rats was determined with the Friedman microphonic method (6). The rats were lightly anesthetized with U.S.P. ether during the blood pressure measurements.

Only male rats were utilized in these experiments. They were all of the Wistar strain except those used in the adrenal insufficiency and adrenal regeneration experiments. Except in the postdesoxycorticosterone acetate (DCA) group, the rats usually weighed between 200 and 400 Gm. Within this range, the JG counts did not noticeably vary with weight or age. The rats at all times were either eating Purina laboratory chow (0.24 per cent sodium) or the special "synthetic" diets mentioned below.

RESULTS

A. *Renal hypertension*

Normal rats. The average juxtaglomerular index for 12 normal Wistar rats weighing between 200 and 300 Gm. is 36, with a standard deviation of ± 8. The mean blood pressure for 90 normal Wistar rats is 99 (S.D., ± 7) mm. Hg (Table I).

Sham operated rats. In nine rats a sham operation on one kidney was performed. The renal artery of this one kidney was dissected out, a silver clip was applied to it, and the clip was then immediately removed and the incision sutured. Five weeks after this sham operation, the operated kidney had a mean juxtaglomerular index of 34 (S.D., ± 12) and the contralateral unmanipulated kidney had a mean JG index of 35 (S.D., ± 13) (Table I).

Rats with unilateral constriction of a renal artery. In sixteen Wistar rats weighing between 150 and 250 Gm., a clip was applied to one renal artery in such a way as to constrict it partially; the other kidney was "untouched." Ten weeks later the blood pressure of these rats was ascertained and eight were found to be definitely hypertensive, with a blood pressure of over 140 mm. Hg (Table II). In these eight rats the "clipped" kidneys had an average JG index of 76, which is significantly higher than that of normal or sham operated kidneys ($p = 0.0001$). The "untouched" contralateral kidneys of these rats showed striking

TABLE I

The mean juxtaglomerular indices for several groups of Wistar rats with renal hypertension along with appropriate control groups*

Group	No. of rats	Mean blood pressure at end of experiment (*mm. Hg*)		Data concerning JG† indices		
				Mean	Range of values	Standard error of the mean
a. Normal Wistar rats	12	99	One kidney examined	36	23 to 53	± 2.5
b. Sham operated rats	9		Sham operated kidney	34	23 to 65	±13.0
			Contralateral untouched kidney	35	21 to 65	±13.7
c. Hypertensive rats 10 weeks after unilateral clipping	8	172	Clipped kidney	76	40 to 129	±10.2
			Contralateral untouched kidney	1	0 to 4	± 0.7
d. Hypertensive rats 6 weeks after unilateral clipping	11	159	Clipped kidney	44	21 to 76	± 5.8
			Contralateral untouched kidney‡			
e. Hypertensive rats 7–14 days after unilateral clipping	7	145	Clipped kidney	46	28 to 63	± 4.1
			Contralateral untouched kidney	9	4 to 14	± 1.4
f. Rats with little or no hypertension 7 weeks after unilateral clipping	13	96	Clipped kidney	48	26 to 80	± 4.3
			Contralateral untouched kidney	6	0 to 20	± 1.9
g. Normal rats on a basic diet containing 0.002% Na	8	110	One kidney examined	48	29 to 62	± 4.0
h. Normal rats on the basic diet plus 0.4% Na	8	111	One kidney examined	30	19 to 54	± 3.9
i. Rats with unilateral clipped kidney on a basic diet containing 0.002% Na	22	169	Clipped kidney	66	39 to 98	± 3.5
			Contralateral untouched kidney	6	0 to 33	± 1.8
j. Rats with unilateral clipped kidney on the basic diet plus 0.4% Na	13	181	Clipped kidney	58	22 to 112	± 7.8
			Contralateral untouched kidney	1	0 to 4	± 0.3

* Both kidneys were present in all rats.
† Juxtaglomerular.
‡ This kidney was left intact for subsequent studies (Group b, Table III).

degranulation of the juxtaglomerular apparatus. From a normal value of 36 the index fell to an average of 1 in these kidneys, and the JG index ranged from 0 to 4, far below normal JG indices. These findings are consistent with observations by others that renal ischemia may be associated with an increase in the number of granular JG cells (2, 7) and also with observations that the contralateral "untouched" kidney has a great decrease in the granular JG cells (8, 9).

In another group of hypertensive rats with a similar operation, the ischemic kidney was examined six weeks after constricting the renal artery. The JG index averaged 44 in these kidneys, a value not as high as was seen in constricted kidneys 10 weeks after clipping (Table I).

Another group of seven hypertensive rats was sacrificed 7 to 14 days after a similar constriction of only one renal artery. The "clipped" kidneys had an average JG index of 46 with a range of 28 to 63, and the contralateral "untouched" kidney had a mean JG index of 9 with a range of 4 to 14. Even this brief interval after clipping one renal artery allowed for considerable degranulation of the "untouched" kidney and hypergranulation of the ischemic kidney (Table I).

Thirteen other rats had the same clipping procedure as the foregoing groups of rats. However, they failed to develop hypertension during seven weeks of constriction of one renal artery and all had blood pressures under 110 mm. Hg. Even these rats had significant hypergranulation of the ischemic kidney. In this group the mean JG index of the constricted kidney was 48 (S.D., ± 15), with values ranging from 26 to 80. The "untouched" kidney had a mean JG index of 6 (S.D., ± 6), with values ranging from 0 to 20 (Table I). Even though the "untouched" kidney here showed considerable degranulation, it was not nearly as complete as that seen in truly hypertensive rats with a similar operation.

The degranulation of the "untouched" kidneys after unilateral clipping cannot be the result of renal hypertrophy, since rats with a single fully hypertrophied kidney have normal JG indices (Table III).

TABLE II

The juxtaglomerular indices for eight individual hypertensive rats 10 weeks after a clip had been applied to one renal artery

Rat no.	Blood pressure at end of experiment (*mm. Hg*)	JG* index of ischemic kidney	JG index of contralateral untouched kidney
1	163	68	0
2	174	105	0
3	175	129	3
4	185	40	0
5	150	53	1
6	185	68	4
7	178	62	0
8	168	84	1
Mean values for the group	172	76±27†	1±2

* Juxtaglomerular.
† ± Indicates standard deviation.

TABLE III

The mean juxtaglomerular indices for certain groups of Wistar rats with only one kidney present, including groups with past or present hypertension and control groups

Group	No. of rats	Mean blood pressure at end of experiment (*mm. Hg*)	Data concerning JG* indices		
			Mean	Range of values	Standard error of the mean
a. Normal rats with one kidney, on Purina chow	11		34	9 to 50	±3.6
b. Previously hypertensive rats that became normotensive 6 weeks after the excision of a clipped kidney (eating Purina chow)	11	96	28	16 to 39	±2.2
c. Rats with one clipped kidney on a basic diet containing 0.002% Na	14	174	55	37 to 82	±4.3
d. Rats with one clipped kidney on the basic diet plus 0.4% Na	8	184	45	27 to 94	±7.5

* Juxtaglomerular.

Partial constriction of the renal artery of degranulated kidneys. In five rats, one renal artery was partially constricted with the subsequent development of hypertension. As noted above, the "untouched" contralateral kidney seven weeks later has almost complete degranulation of the JG cells with an average JG index of 1, and values ranging from 0 to 4. At this time the renal artery of this degranulated "untouched" kidney was also "clipped." Four weeks later both kidneys were examined. In two of the five rats, the kidney which may be assumed to have been previously degranulated was still degranulated despite the clipping procedure. However, in the three other rats the previously degranulated kidney had definitely regranulated as a result of the second clipping operation. The JG indices were 36, 28, and 14 in the previously degranulated kidneys of these rats.

The effect of a low sodium diet on the granulation of the juxtaglomerular apparatus. Three experiments were done to assess the influence of the amount of sodium in the diet on the degree of juxtaglomerular granulation. In these studies the diet consisted of purified materials, including 20 per cent casein, 70 per cent sucrose, 5 per cent corn oil, 5 per cent sodium-free salt mixture made with reagent-grade chemicals, and vitamin supplementation. This constituted our "low sodium diet" and contained 0.002 per cent sodium. Our control diet with 0.4 per cent sodium was made by adding pure sodium chloride to the basic low sodium diet. Water was given *ad libitum.*

The two diets were offered to two groups of normal Wistar rats. A group of eight rats on the diet containing 0.4 per cent sodium for five weeks had a mean JG index of 30 (S.D., ± 10). The group of eight rats on the diet containing 0.002 per cent sodium for five weeks had a mean JG index of 48 (S.D., ± 10). Thus the rats on a very low sodium intake had a significantly higher degree of JG granulation ($p = 0.001$). This confirms the earlier observations of the Hartrofts (3) (Table I).

The next experiment deals with hypertensive rats with a clip on one renal artery, the other kidney remaining "untouched." Sixteen weeks after the clip was applied, these hypertensive rats were divided into two groups which were evenly matched for blood pressure. These two matched groups were placed on the experimental diets described above for an additional eight weeks. There was a slight reduction in blood pressure in the group of 22 rats on the 0.002 per cent sodium diet. Before going on the diet, the mean blood pressure of this group was 177 mm. Hg. After eight weeks on the diet, this mean had fallen to 169 mm. Hg. The other group of 13 hypertensive rats on the 0.4 per cent sodium diet had an initial blood pressure of 173 mm. Hg which rose to 180 mm. Hg while on this diet. The group of rats on the 0.4 per cent sodium diet had the usual almost complete JG degranulation in the "untouched" kidney. The mean JG index of the "untouched" kidney in this group was 1 and the highest index for an individual rat was 4. On the other hand, the average JG index of the "untouched" kidney was 6 in the rats on the 0.002 per cent sodium diet, and 7 rats out of the 22 in this group had indices higher than 4. Three rats of this group had indices higher than 17 in the "untouched" kidney. This would be a very unusual finding in operated hypertensive rats on higher sodium intakes. The difference in granulation of the "untouched" kidney between these two groups is highly significant ($p = 0.005$). The diet containing very little sodium unquestionably increased the JG granulation in these "untouched" kidneys. Moreover, the slight reduction in blood pressure resulting from the low sodium intake probably does not account for the increased granulation, since all hypertensive rats on the low sodium diet had a blood pressure higher than 140 at the end of the experiment (Table I).

Varying the amount of sodium in the diet did not greatly influence the JG granulation of the "clipped" kidneys in these rats. The group on 0.4 per cent sodium in the diet had a mean JG index of 58 in the "clipped" kidney, and the group on the 0.002 per cent sodium diet had a mean JG index of 66. The "clipped" kidneys of both groups are hypergranulated compared to a normal kidney but are not significantly different from one another (Table I).

Other rats were made hypertensive by applying a clip on one renal artery and excising the contralateral kidney. All the rats studied had blood pressures over 140 mm. Hg. Sixteen weeks after the operations, they were divided into two groups evenly matched for blood pressure. One group was placed on the 0.002 per cent sodium diet and

TABLE IV

A comparison of the juxtaglomerular indices in adrenalectomized and in normal Sprague-Dawley rats

Group	No. of rats	Mean JG* index	Range of values	Standard error of the mean†
a. Normal rats on Purina chow and tap water	12	30	18 to 40	±1.9
b. Adrenalectomized rats on Purina chow and 1% saline	12	52	7 to 83	±7.2

* Juxtaglomerular.
† p value of the difference between these means: 0.004.

the other on the 0.4 per cent sodium diet for five weeks and they were then killed. The blood pressure of all the rats on either diet remained above 140 mm. Hg at the end of the experiment. The 8 rats on the 0.4 per cent sodium diet had a mean JG index of 45, while the 14 rats on the 0.002 per cent sodium diet had a mean JG index of 55. This difference was not statistically significant ($p = 0.3$) (Table III).

The effect on the juxtaglomerular apparatus of "curing" unilateral renal hypertension with a unilateral nephrectomy. Eleven rats were made hypertensive with blood pressures over 140 mm. Hg by clipping one renal artery as described above. The average blood pressure of this group was 159 mm. Hg seven weeks after the operation. From previous experience, we know that the JG apparatus of the "untouched" kidney is virtually degranulated seven weeks after the original clipping operation in hypertensive rats. At this time the "clipped" kidney was excised. Every rat had a decided fall in blood pressure following this procedure. Ten out of the 11 rats had blood pressure levels under 115 mm. Hg two weeks after the removal of the "clipped" kidney. By six weeks after removal of the "clipped" kidney, all the blood pressures were under 111 mm. Hg with an average of 96 mm. Hg. Thus all rats eventually had a blood pressure well within the normal range. When the lone kidney of these normotensive rats was examined, it had an almost complete regranulation of the JG apparatus with an average JG index of 28 and values ranging from 16 to 39 (Table III). The lone kidney of three of these rats that had become normotensive was seen two weeks after the "clipped" kidney had been removed. The JG indices in these three were 32, 23, and 24, showing that regranulation was almost complete in this short interval.

While it is obvious that regranulation occurs in the JG apparatus of these rats when they change from a hypertensive to a normotensive state, it is of interest to compare the granulation in these lone kidneys with the JG granulation of rats that have had one kidney excised without having undergone any clipping operations.

Eleven Wistar rats had one kidney excised and two months were allowed to elapse for a new equilibrium to be established. The lone hypertrophied kidney of these rats was then studied. The average JG index in this lone kidney was 34 (S.D., ± 11). Thus the regranulated JG complexes of the previously hypertensive rats are not greatly different from those of simple one kidney rats. Moreover, these simple one kidney rats have a JG granulation quite similar to that seen in completely normal rats (Table III).

B. *Adrenal insufficiency*

A number of male Sprague-Dawley rats were randomly divided into two groups with 12 rats in each group. The control rats in Group A with intact adrenals ate Purina laboratory chow and drank tap water *ad libitum*. The rats in Group B underwent a complete bilateral adrenalectomy and subsequently were allowed to drink only a 1 per cent saline solution. They were also eating Purina laboratory chow. All of these rats were killed 18 days after the adrenalectomy of the rats in Group B. The mean JG index in the normal rats of Group A was 30 (S.D., ± 6). The mean JG index in the adrenalectomized rats of Group B was 52 (S.D., ± 24). This difference was highly significant with a p value of 0.004. These results are

similar to those found by Dunihue in adrenalectomized cats (10) (Table IV).

C. *Rats treated with desoxycorticosterone in conjunction with either high or low sodium diets*

Twenty-nine rats were given a subcutaneous injection of 125 mg. of desoxycorticosterone trimethylacetate (DCTMA).[4] This injection was repeated in four weeks. One group with 10 of these rats was fed a "synthetic" diet containing only 0.002 per cent sodium (described above), and water *ad libitum*. Another group with 19 of these rats was fed this same diet with 0.4 per cent sodium added (described above) and was allowed to drink nothing but 1 per cent sodium chloride. A control group that was fed the sodium-deficient diet but did not receive DCTMA can also be compared with the above groups.

All rats were killed two and a half weeks after the second injection of DCTMA. The group on DCTMA and a high sodium intake had a virtual disappearance of the JG granulation. Their average JG index was 0.3 with a range of 0 to 3. Sixteen out of the 19 rats had JG indices of 0. All of these rats had some elevation of blood pressure at the end of the experiment with an average final blood pressure of 142 mm. Hg. On the other hand, the group of rats that received DCTMA and a diet very low in sodium did not show this extreme JG degranulation. The mean JG index was 17 for the group. None of these rats on the low sodium diet became hypertensive during the course of the experiment, and the average blood pressure for the group was 99 mm. Hg at the end of the experiment. These results confirm the previous observations of the Hartrofts (11) (Table V).

The rats receiving DCTMA in conjunction with a diet low in sodium had a much lower JG index than the control group receiving the diet only. This was true in spite of the fact that their blood pressure was lower than that of the sodium-deficient control group. This would seem to indicate that DCTMA alone has a significant ability to cause degranulation of the JG cells independent of hypertension (Table V).

D. *Postdesoxycorticosterone hypertension*

It is well known that desoxycorticosterone in conjunction with a generous intake of sodium will produce hypertension in rats. An example of this effect is demonstrated above. It has also been shown that in certain rats this hypertension may persist long after completion of desoxycorticosterone absorption and while the rats are taking in the usual amounts of sodium (12). This type of hypertension has been called "postdesoxycorticosterone" hypertension and is a prime example of the principle that a hypertension may persist long after its original cause has been removed.

To produce this type of hypertension a 25 mg. desoxycorticosterone acetate pellet was implanted subcutaneously in each of 150 male weanling rats. Another similar pellet was implanted five weeks

[4] We wish to thank Dr. Robert Gaunt of the Ciba Pharmaceutical Company for generously supplying the desoxycorticosterone.

TABLE V

A comparison of the juxtaglomerular indices in Wistar rats receiving desoxycorticosterone and either a high or low sodium intake

Group	No. of rats	Mean blood pressure at end of experiment (*mm. Hg*)	Data concerning JG* indices		
			Mean	Range of values	Standard error of the mean
a. Rats receiving DCTMA† and 1% NaCl in food and water	19	142	0.3	0 to 3	±0.1‡
b. Rats receiving DCTMA and a very low sodium diet	10	99	17	0 to 51	±6.2§
c. Control rats receiving a very low sodium diet	8	110	48	29 to 62	±4.0

* Juxtaglomerular.
† Desoxycorticosterone trimethylacetate.
‡ p value of the difference between groups a and b: 0.009.
§ p value of the difference between groups b and c: 0.00006.

TABLE VI

A comparison of the juxtaglomerular indices in "postdesoxycorticosterone" rats with either elevated or normal blood pressure

Group	No. of rats	Mean blood pressure at end of experiment (*mm. Hg*)	Data concerning JG* indices		
			Mean	Range of values	Standard error of the mean†
a. Post-DCA‡ rats with hypertension	17	160	7	1 to 15	±1.0
b. Post-DCA rats with normal blood pressures	16	104	25	8 to 50	±3.1

* Juxtaglomerular.
† p value of the difference between these means: 0.0000001.
‡ Desoxycorticosterone acetate.

later. The rats were all drinking a 1 per cent sodium chloride solution for six months after the first pellet implantation. Most of them became hypertensive during this period. They were then offered tap water for the next four and a half months, at which time the experiment was terminated. The blood pressure of each rat was determined twice during the last week of the experiment, and the average of these readings was considered the blood pressure for each rat. When the rat was killed, the entire subcutaneous area of the back was examined to be certain that both pellets had been completely absorbed. In two rats the remnant of a pellet was seen and these rats were excluded from the study. This was done to insure that all the rats were indeed in the "postdesoxycorticosterone" phase. Seventeen rats remained moderately to severely hypertensive after the pellets had completely disappeared. The blood pressure was above 140 in every rat of this group with a mean of 160 mm. Hg. Many more rats had very mild hypertension with blood pressures between 120 and 140 mm. Hg. Sixteen rats had blood pressures in the normal range (below 118 mm. Hg) in the postdesoxycorticosterone period. The mean blood pressure of this group was 104 mm. Hg. The JG granulation of the hypertensive group and the normotensive group was compared. In the normotensive group the mean JG index was 25 (S.D., ±12). In the hypertensive group the mean JG index was 7 (S.D., ±4). This difference was quite significant ($p = 10^{-7}$) (Table VI).

E. *Experimental hypertension in rats associated with adrenal cortical regeneration*

Skelton has recently demonstrated a form of experimental hypertension in rats which appears during the regeneration of adrenal cortical tissue following adrenal enucleation (13). In creating this experimental situation, we removed one kidney and one adrenal in male weanling Holtzmann rats. The contralateral adrenal capsule was nicked, and the medullary and cortical tissue was gently pressed out through the nick leaving only the capsule and a thin layer of adrenal cortical cells subjacent to it. These rats were then given a 1 per cent saline solution to drink. Many of them subsequently developed hypertension. A control group of weanling rats with intact adrenals was also included in the experiment. The rats in this control group had one kidney excised and were drinking only a 1 per cent saline solution. Half of the rats with the single regenerating adrenal had a blood pressure over 140 mm. Hg six weeks after their initial operation. The average blood pressure of these nine rats at this time was 171 mm. Hg. Their average JG index was 7. The control group had a mean blood pressure of 121 mm. Hg (S.D., ±7), and an average JG index of 20. The rats with the hypertension of adrenal regeneration had a significantly lower JG index than the operated control rats ($p = 0.0004$) (Table VII). In many ways the hypertension of adrenal regeneration resembles the hypertension associated with desoxycorticosterone. However, the JG granules in desoxycorticosterone hypertension practically disappear, whereas they are definitely present, even if in diminished numbers, in the hypertension of adrenal regeneration.

DISCUSSION

Unfortunately these studies do not delineate the exact function of the JG apparatus. The granular cells in it have all the appearances of secretory cells, even when seen with the electron microscope

TABLE VII

The juxtaglomerular indices in rats with hypertension of adrenal regeneration and in control rats with intact adrenals*

Group	No. of rats	Mean blood pressure at end of experiment (*mm. Hg*)	Data concerning JG† indices		
			Mean	Range of values	Standard error of the mean‡
a. Rats with adrenal regeneration hypertension	9	171	7	0 to 16	±1.7
b. Control rats with intact adrenals	9	121	20	10 to 33	±3.0

* All rats in this study had one kidney excised and were drinking a 1 per cent saline solution throughout the experiment.
† Juxtaglomerular.
‡ p value of the difference between these means: 0.0004.

(14). It is reasonable to assume that the secretory state associated with hypergranulation is the opposite of that associated with degranulation. It is not even certain whether the degranulation of the JG apparatus is associated with rapid secretion or with a cessation of secretion. However, the state of granulation varies greatly in certain hypertensive states as well as with variations in sodium intake and adrenal function. It has been thought by some that the JG granules may contain renin. No direct proof of this exists, but Peart, Gordon, Cook, and Pickering have shown in the rabbit that renin exists only in or around glomeruli and not in purely tubular areas (15). Hence it is possible that the hypothesis is correct.

These juxtaglomerular secretory cells are abundant in all mammalian species, including man (Figure 1). They have also been seen in lower vertebrates such as the frog (16). These cells in the wall of the afferent arteriole are next to the macula densa, a specialized area of cells in the wall of the distal convoluted tubule which is always near the vascular pole of the glomerulus (17). There is also a rich network of nerves surrounding all the specialized juxtaglomerular structures (18). It is well known that the mammalian kidney has an uncannily accurate ability to keep renal blood flow and glomerular filtration constant in the face of widely varying perfusion pressures (19). Renal vascular resistance can make tremendous adjustments to maintain this autoregulation of blood flow and filtration, even when all vasomotor nerves leading to the kidney have been severed and under conditions of local adrenergic blockade with Regitine® (20). These changes in renal resistance are much more responsive to changes in perfusion pressure rather than to changes in blood flow (20). The whole fabric of evidence indicates that the main changes in resistance which accomplish renal autoregulation can be reasonably assigned only to the preglomerular arteriole. Recent experiments indicate that the cell separation theory cannot account for more than a small part of the changes in resistance occurring during autoregulation (20). As mentioned above, the granular JG cells are in a perfect anatomic situation to sense changes in perfusion pressure. Being in the wall of the arteriole, they could easily sense changes in the stretch of the wall and could then secrete more or less of their hormone in response to this stimulus. Changes in perfusion pressure would also tend to alter the glomerular filtration rate. Such a change in filtration rate might somehow be sensed by the specialized cells of the macula densa in the distal tubule. Since the cells of the macula densa are quite close to the JG secretory cells, without even reticular fibers separating them (21), the JG cells might change their rate of secretion in response to a change in glomerular filtration. In either case, this substance might act primarily on the sphincter of small smooth muscle cells located in the afferent arteriole in the region between the JG secretory cells and the glomerular capillary tuft. Both Clara (22) and Appelt (23) have described this sphincter situated at the entrance into the glomerulus. The state of contraction of the cells in this micromuscular sphincter could provide the fine

adjustment of resistance to blood flow that would have to be present on the upstream side of the glomerular tuft in order to have autoregulation of both renal blood flow and filtration.

The data obtained in our study partly support some of this speculation. In situations where the renal arterial bed is fully exposed to hypertensive blood pressures, the granulation of the JG cells tends to be decreased. Examples of this can be found in the "untouched" kidney of unilateral renal hypertension, in post-DCA hypertension, in acute DCTMA hypertension, and in the hypertension of adrenal regeneration. When the blood pressure in the renal arterial bed is lowered, the JG cells tend to increase their granulation. Examples of this can be found in the kidneys of completely adrenalectomized rats, in the various "clipped" kidneys of rats where a clip on the renal artery of either a normal or an "untouched" kidney decreases the pressure distal to the clip, and especially in the "untouched" kidney of rats in whom renal hypertension has been "cured" by excising a "clipped" kidney. Wide variations of sodium intake could also conceivably operate in this manner. High intakes of sodium tend to increase blood pressure and blood volume; exceedingly low intakes tend toward a low blood pressure and blood volume. Either extreme would be likely to produce some change in the stretch of the JG cells.

Whether or not the substance in the granules of the JG cells influences local renal circulation, the question also arises as to whether it influences the caliber of arterioles throughout the systemic circulation. If it did, it could be very important in producing the increased generalized peripheral resistance of arterial hypertension. In hypertension it could act either by producing an overabundance of a pressor substance or by failing to secrete the normal amount of a depressor substance. Since hypertension can be produced by removing both kidneys (24, 25), the latter concept is possibly more attractive. If the substance of the JG cells had a role in renal autoregulation as well as an effect on systemic arterioles, its effect in the hypertension of renal "ischemia" would have to be the opposite for the two different sets of arteriolar smooth muscle. In partial constriction of a renal artery, autoregulation would demand a relaxation of the preglomerular sphincter; while at the same time a generalized constriction of arteriolar lumina would be occurring throughout the systemic circulation.

It is difficult to explain the partial degranulation of the JG cells in the "untouched" kidney of rats whose opposite kidney has been clipped without the development of hypertension. Ninety-five per cent of rats with unilateral clipping develop at least a slight elevation of blood pressure, and these particular rats represent the exceptional 5 per cent. Since one kidney has been clipped, these rats probably have a tendency to become hypertensive. It may be that the JG cells of the "untouched" kidney in this group of rats became degranulated in response to this tendency to hypertension; and their process of degranulation, if it represented the release of a depressor substance or the failure to release the usual amount of a pressor substance (see above), might actually be opposing this hypertensive tendency and thereby helping to maintain the normotensive state. This would imply that the JG cells of the "untouched" kidney were exerting some sort of antihypertensive activity. If this antihypertensive mechanism became exhausted, similar to exhaustion of the pancreatic β-cells, the tendency to hypertension would be converted to actual hypertension. We need much more evidence before this idea can be anything more than pure speculation.

With our present lack of information, we could be completely overlooking even more important determinants of juxtaglomerular granulation. In any case, the JG cells do undergo marked changes in granulation in various forms of experimental hypertension. Their exact role in these experimental models deserves further study.

SUMMARY

The juxtaglomerular index, which indicates the degree of granulation of cells in the juxtaglomerular aparatus, averages 35 in normal Wistar rats. When hypertension is produced by partially constricting one renal artery, the juxtaglomerular granules double in the ischemic kidney and virtually disappear in the "untouched" contralateral kidney. If the ischemic kidney is then excised seven weeks later, the hypertension disappears

and juxtaglomerular granules reappear in normal abundance in the previously completely degranulated contralateral kidney. After partial constriction of one renal artery, the juxtaglomerular granules in the opposite kidney disappear; but if a constriction is then placed on the artery of this degranulated contralateral kidney, the juxtaglomerular granules reappear. Another group of rats with one ischemic kidney and a contralateral degranulated kidney was placed on a sodium deficient diet. In half the rats, this diet caused the juxtaglomerular granules to reappear in the previously degranulated kidney. This diet also increased granulation in normal rats. Partially constricting the renal artery in a rat with only one kidney will produce hypertension, and the granulation of the lone ischemic kidney remains slightly above normal.

Adrenalectomy doubles the juxtaglomerular index; desoxycorticosterone and salt cause the granules to disappear while concomitantly producing hypertension.

Some rats that had received desoxycorticosterone pellets remained hypertensive long after all pellets had been absorbed. The mean juxtaglomerular index for this group was 7, compared to a value of 25 in a similarly treated group that had become normotensive following the absorption of the desoxycorticosterone pellets.

Hypertension was produced in other rats by removing one adrenal and enucleating the other. Both the hypertensive and the control groups had one kidney removed and were drinking saline solution. The hypertensive group with adrenal regeneration folowing enucleation had a juxtaglomerular index of 7, while the control group with intact adrenals had an index of 20.

REFERENCES

1. Tobian, L., Thompson, J., and Twedt, R. The degree of granulation of the renal juxtaglomerular apparatus in relation to hypertension and sodium intake. Clin. Res. Proc. 1957, **5**, 234.
2. Goormaghtigh, N. Existence of an endocrine gland in the media of the renal arterioles. Proc. Soc. exp. Biol. (N. Y.) 1939, **42**, 688.
3. Hartroft, P. M., and Hartroft, W. S. Studies on renal juxtaglomerular cells. I. Variations produced by sodium chloride and desoxycorticosterone acetate. J. exp. Med. 1953, **97**, 415.
4. Wilson, C., and Byrom, F. B. Renal changes in malignant hypertension. Lancet 1939, **1**, 136.
5. Floyer, M. A. The effect of nephrectomy and adrenalectomy upon the blood pressure in hypertensive and normotensive rats. Clin. Sci. 1951, **10**, 405.
6. Friedman, M., and Freed, S. C. Microphonic manometer for indirect determination of systolic blood pressure in the rat. Proc. Soc. exp. Biol. (N. Y.) 1949, **70**, 670.
7. Dunihue, F. W., and Candon, B. H. Histologic changes in the renal arterioles of hypertensive rabbits. Arch. Path. (Chicago) 1940, **29**, 777.
8. Schloss, G. Der Regulationsapparat am Gefässpol des Nierenkörperchens beim experimentellen renalen Drosselungshochdruck der Ratte. Helv. med. Acta 1947, **14**, 22.
9. Hartroft, P. M. Studies on renal juxtaglomerular cells. III. The effects of experimental renal disease and hypertension in the rat. J. exp. Med. 1957, **105**, 501.
10. Dunihue, F. W., and Robertson, W. Van B. The effect of desoxycorticosterone acetate and of sodium on the juxtaglomerular apparatus. Endocrinology 1957, **61**, 293.
11. Hartroft, P. M., and Hartroft, W. S. Studies of renal juxtaglomerular cells. II. Correlation of the degree of granulation of juxtaglomerular cells with width of the zona glomerulosa of the adrenal cortex. J. exp. Med. 1955, **102**, 205.
12. Friedman, S. M., Friedman, C. L., and Nakashima, M. Sustained hypertension following the administration of desoxycorticosterone acetate. J. exp. Med. 1951, **93**, 361.
13. Skelton, F. R. Development of hypertension and cardiovascular-renal lesions during adrenal regeneration in the rat. Proc. Soc. exp. Biol. (N. Y.) 1955, **90**, 342.
14. Hartroft, P. M. A preliminary study of the electron microscopy of renal juxtaglomerular cells; correlation with light microscopy. Anat. Rec. 1956, **124**, 458.
15. Peart, W. S., Gordon, D. B., Cook, W. F., and Pickering, G. W. Distribution of renin in the rabbit kidney. Circulation 1956, **14**, 981.
16. Okkels, H. Morphologie particulière du pôle vasculaire du glomérule rénal chez la grenouille. Bull. Histol. Techn. micr. 1929, **6**, 113.
17. McManus, J. F. A. The juxtaglomerular complex. Lancet 1942, **2**, 394.
18. De Muylder, C. G. The "Neurility" of The Kidney. Oxford, Blackwell Scientific Publications, 1952, pp. 1–41.
19. Winton, F. R. Pressures and flows of blood and urine within the kidney *in* his Modern Views on the Secretion of Urine. Boston, Little, Brown, and Co., 1956, pp. 61–77.

20. Haddy, F., Scott, J., Fleishman, M., and Emanuel, D. Effect of change in blood flow rate upon renal vascular resistance. J. Lab. clin. Med. 1957, **50**, 822.
21. McManus, J. F. A. Further observations on the glomerular root of the vertebrate kidney. Quart. J. Micr. Sci. 1947, **88**, 39.
22. Clara, M. Anatomie und Biologie des Blutkreislaufes in der Niere. Arch. Kreisl. Forsch. 1938, **3**, 42.
23. Appelt, H. Untersuchungen über die Arteriolae afferentes und über die Gefässkörperchen (Goormaghtigh-Bechersche Zellgruppen) in der Niere des Menschen und der Maus. Z. Mikr.-anat. Forsch. 1939, **45**, 179.
24. Braun-Menendez, E., and Von Euler, U. S. Hypertension after bilateral nephrectomy in the rat. Nature (London) 1947, **160**, 905.
25. Tobian, L., Jr. Hypertension following bilateral nephrectomy. J. clin. Invest. 1950, **29**, 849.