STUDIES ON RENAL JUXTAGLOMERULAR CELLS

III. The Effects of Experimental Renal Disease and Hypertension in the Rat*

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Following Ruyter's discovery of juxtaglomerular cells (1), Goormaghtigh's investigations led him to implicate these cells in the etiology of hypertension. In renal ischemia, whether in man or experimental animals, he observed hyperplasia and hypergranulation of juxtaglomerular cells. He believed that these changes preceded and might have been responsible for hypertension resulting from renal ischemia (2, 3). Although several independent investigators confirmed Goormaghtigh's observations (4–7), others claimed that juxtaglomerular cells bear no relation to hypertension. However, several facts deserve emphasis: in man, JG[1] granules were found to increase in malignant hypertension with acute uremia but not in essential hypertension without uremia (6, 7); in rabbits with cellophane perinephritis, the increase in granules occurred only during the early stages, before the onset of hypertension (5); and in animals in which hypertension had been produced by constriction of only one renal artery, JG granules were not increased in the contralateral kidney (3, 8) and may actually have become decreased in number (9, 10).

Furthermore, we had previously found that temporary changes in blood pressure in intact rats, induced by vasoactive drugs, altered the granulation of juxtaglomerular cells (11), although the changes were of small magnitude. An increase in blood pressure (privine) decreased granulation and a lowering of blood pressure (apresoline) increased granulation of JG cells.

From these facts, it was considered important to further elucidate the effect of hypertension *per se* on JG cells as distinct from renal ischemia. The following experiments were designed for this reason.

Unilateral Constriction of the Renal Artery.—Fifteen female rats and one male rat (Wistar strain, 6 to 9 months of age, 250 to 300 gm.) were operated on according to the method

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[1] JG, juxtaglomerular; JGI, juxtaglomerular granulation index.

of Wilson and Byrom (12). A silver clip was applied to one renal artery (left). The other kidney (right) was left intact. Seven comparable control rats were "sham-operated." All were fed Purina fox chow *ad libitum*.

Indirect measurements of blood pressure were performed by an audiometric method (13) once during the course of the experiment (1 to 7 weeks) and again when the rats were sacrificed. Direct measurements (cannulation of the femoral artery) were also performed when the rats were sacrificed.

Hypertension Produced by a "Figure-of-Eight" Ligature.—In eight rats, hypertension was produced by compression of one kidney (left) with a "figure-of-eight" ligature and removal of the opposite one, according to the method described by Grollman (14).

Unilateral Nephrectomy and Ureteral Occlusion.—Twenty-four rats (12 female, 12 male; 159 to 265 gm.) were placed in three groups, comparable with respect to weight and sex, and treated as follows. In eight rats, the left kidney was removed; in another eight, the left ureter was occluded (transected between two ligatures); the remaining eight animals were "sham-operated" to serve as a control group. Three rats with occluded ureters died 9 days postoperatively as a result of complications (infection and extensive adhesions). Three rats from each of the other two groups were sacrificed at this time. The remaining animals were sacrificed after 24 days at which time direct blood pressure measurements were performed.

Histological Procedures.—Sections of all kidneys were prepared and the degree of granulation of JG cells (JGI) was assessed by a method described previously (15, 16).

RESULTS

Unilateral Constriction of the Renal Artery.—Of the sixteen rats with one renal artery constricted, four became hypertensive early in the experiment (indicated by indirect blood pressure measurements). By the end of the experiment, a highly significant elevation in blood pressure for the group as a whole was demonstrated by both indirect and direct measurements (Table I).

Taking the average of both kidneys, the JGI[1] (index of granulation of JG cells) of the rats with partial constriction of one renal artery was five. In the "sham-operated" control rats the average JGI was eleven, giving a highly significant difference ($p < 0.01$). Therefore, degranulation of JG cells had occurred in the hypertensive rats. Considering only the left, constricted kidney of the hypertensive rats, granulation of JG cells varied from complete degranulation to persistence of granules at normal levels. This variation was not related to severity of hypertension. In the contralateral kidney, however, the degree of granulation varied inversely with the level of blood pressure ($r = -0.7$, Graph 1). In other words, the higher the rat's blood pressure, the greater was the degree of degranulation of JG cells in the right, unconstricted kidney.

Arteriolar changes were not observed in either kidney of the rats in this experiment.

Hypertension Produced by a "Figure-of-Eight" Ligature.—In five of the eight rats, degranulation of JG cells occurred in the ligated kidneys (average JGI = 1.4 compared to 17.5 in two intact control rats, $p < 0.01$). The histologic appearance of these compressed kidneys is illustrated in Fig. 1. In the

other three rats, severe ischemic changes developed in the poles of the kidney, where the ligature had interfered with blood supply (Fig. 2). Although de-

TABLE I

Effects of Unilateral Constriction of the Renal Artery on Juxtaglomerular Cells and Blood Pressure

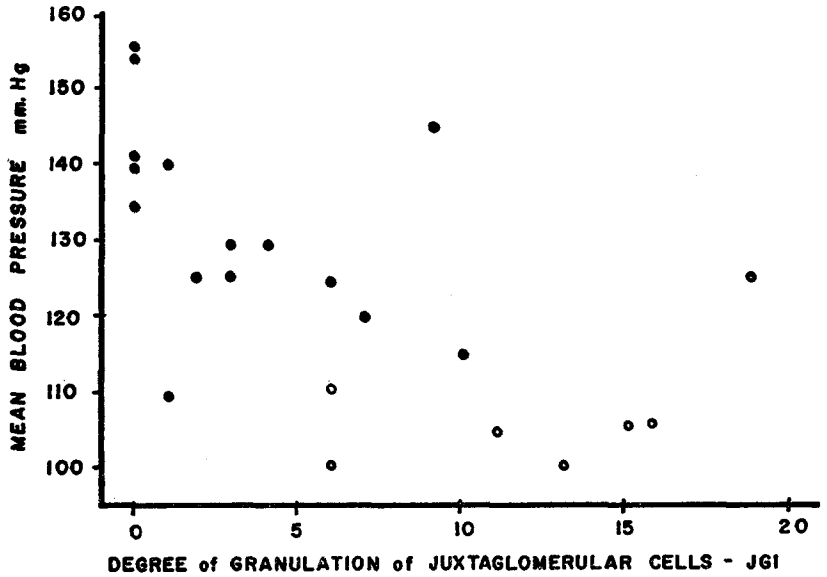
Rat No.	Indices of granulation of juxtaglomerular cells (J G I)			Blood pressure	
	Right	Left	Average	Indirect	Direct
			Sham-Operated		
				mm. Hg	*mm. Hg*
314	19	3	11.0	120	125
316	16	5	10.5	100	105
320	11	9	10.0	120	105
323	15	19	17.0	120	105
326	13	16	14.5	105	100
327	6	6	6.0	130	100
328	6	10	8.0	120	110
Mean.....	12	10	11.0	116	107
			Left Renal Artery Constricted with a Silver Clip		
305	0	12	6.0	195	140
306	9	0	4.5	120	145
307	3	1	2.0	115	130
308	2	3	2.5	190	125
309	1	0	0.5	180	140
311	0	17	8.5	150	140
312	7	7	7.0	130	120
313	6	7	6.5	140	125
315	4	6	5.0	135	130
317	3	12	7.5	135	125
318	10	10	10.0	120	115
319	1	0	0.5	125	110
321	0	11	5.5	195	135
322	0	12	6.0	190	155
324	1	7	4.0	140	—
325	0	8	4.0	185	155
Mean.....	3	7	5.0	153	133

granulation of JG cells occurred in the relatively normal areas of these kidneys, granules persisted or increased in number in the damaged poles. Hyperplasia as well as hypergranulation of JG cells was frequently seen in these ischemic areas (Fig. 3). In contrast to the hypertensive rats described above, arteriolar thickening was a prominent feature in the ligated kidneys.

Unilateral Nephrectomy and Ureteral Occlusion (Table II).—In the sham-

operated rats, the JGI of the right kidney was the same as that of the left kidney (JGI = 21). Blood pressure levels in these rats, measured just before they were sacrificed, averaged 99 mm. Hg.

In the unilaterally nephrectomized rats, the average JGI of the remaining (right) kidney was not significantly different from that of either kidney in control rats (above). Blood pressure levels were normal (104 mm. Hg). The



GRAPH 1. Relationship between blood pressure and degree of granulation of juxtaglomerular cells in the contralateral kidney of rats made hypertensive by constriction of one renal artery ($r = -0.7$). This relationship did not hold for the clamped kidney, but the correlation represented by the graph demonstrates that in the unclamped kidney, JG cells became degranulated with an increase in blood pressure.

Black dots, contralateral, unclamped, kidney of rats with one renal artery constricted. Open dots, corresponding kidney of the sham-operated rats.

remaining kidneys weighed 30 per cent more than kidneys of the control rats. No other differences in organ weights were found.

In the rats that survived the entire experimental period with one ureter occluded, severe unilateral hydronephrosis developed. Thickening of arterial and arteriolar walls was a prominent feature in these hydronephrotic kidneys. Despite tubular atrophy and fibrosis, JG granules persisted in the thin shell of atrophic renal cortex (Fig. 4). The average JGI of the hydronephrotic kidneys (JGI = 21) was the same as that of the control kidneys, but the JG cells in the contralateral kidneys were degranulated (JGI = 4). Mean blood pressure levels were significantly elevated (118 mm. Hg) in these rats as compared to control rats (B.P. = 99 mm. Hg). The contralateral kidneys were hyper-

trophied, though to a lesser degree than were the remaining kidneys in the unilaterally nephrectomized rats.

TABLE II

Effects of Unilateral Nephrectomy and Ureter Ligation on Juxtaglomerular Cells and Blood Pressure

Rat No.	Indices of granulation of JG cells—JGI			Blood pressure (direct)
	Right kidney	Left kidney	Average	
		Left Unilateral Nephrectomy		
				mm. Hg
551*	25			105
552	5			105
553	25			105
554	10			
555*	17			
556*	18			
557	16			100
558	21			
Mean	17			104
		Left Ureter Ligation		
559	2	15	8.5	125
560	0	17	8.5	115
562	3	18	10.5	110
564	13	39	26.0	105
566	1	15	8.0	135
Mean	4	21	12.5	118
		Sham-Operated Controls		
567*	27	24	25.5	
568	22	19	20.5	105
569	23	17	20.0	
570	18	29	23.5	100
571*	14	10	12.0	
572*	23	31	27.0	
573	18	16	17.0	95
574	19	21	20.0	95
Mean	21	21	20.7	99

* Sacrificed 9 days postoperatively; blood pressure measurements not obtained.

DISCUSSION

By the three different methods used in this investigation for producing hypertension in rats, juxtaglomerular cells were altered consistently. Granulation of juxtaglomerular cells was diminished in hypertensive rats when renal blood supply was unrestricted. Furthermore, the degree of granulation in the contralateral kidney of rats with one renal artery constricted was inversely

correlated with the level of blood pressure. Since JG cells were not altered in the remaining kidney of comparable, unilaterally nephrectomized rats, degranulation in the hypertensive rats could not be explained on the basis of compensatory renal hypertrophy.[2]

It is possible that the changes in JG cells produced in these experiments could be explained on the basis of electrolyte and adrenal changes, in view of our previous observations (15, 16). However, since neither measurements of electrolytes nor adrenal studies were done in these experiments, this problem must await further investigation.

The importance of the present results lies in the distinction between the effects of renal ischemia and hypertension on JG cells. Juxtaglomerular cells present in renal tissue with unrestricted blood supply became degranulated with an increase in blood pressure. This effect was compatible with results obtained previously by injections of a pressor substance (11). Restricted blood supply to the kidney, in many cases, prevented this change and interfered with a possible correlation of granulation of juxtaglomerular cells to the level of blood pressure. As has been shown by others, renal ischemia is associated with hypergranulation and therefore has the opposite effect on JG cells as does an elevated blood pressure. Both Goormaghtigh (3) and Dunihue (5) observed that hypergranulation of JG cells occurred early in experimental renal ischemia. Dunihue further showed that after the onset of hypertension in such animals, granulation of JG cells decreased to within normal limits, a phenomenon easily explained by the degranulating effect of an elevation in blood pressure.

It is not always possible, particularly in human disease, to study the separate effects of the two variables, renal ischemia and blood pressure, as they were in these experiments. Failure to distinguish these two factors may explain some of the conflicting reports in the literature concerning the relationship of JG cells and hypertension. The present results emphasize the importance of considering the state of renal parenchymal preservation (areas of scarring, degree of atrophy, etc.) in attempts to correlate blood pressure, degree of renal ischemia, and changes in juxtaglomerular cells.

SUMMARY

Hypertension in rats produced by constriction of one renal artery was associated with degranulation of juxtaglomerular cells in the contralateral,

[2] The conclusion that uncomplicated compensatory renal hypertrophy does not affect JG cells must be qualified, however. In this investigation, adult rats were used and the index of granulation in the remaining kidney was compared to the corresponding kidney in the control and hypertensive rats rather than to the removed kidney. Tverdy (17), working in Goormaghtigh's laboratory, found that granulation of JG cells was greater in the remaining kidney in unilaterally nephrectomized mice than in the removed one. His results, although different from our own, are still compatible with our conclusion that degranulation of JG cells in hypertensive rats cannot be explained on the basis of compensatory renal hypertrophy.

unclamped, kidney. These findings are consistent with those of other investigators. Furthermore, the degree of granulation (JGI) in the unclamped kidney was inversely correlated with the level of blood pressure ($r = -0.7$).

Degranulation of JG cells also occurred in rats made hypertensive by application of a "figure-of-eight" ligature to one kidney and removal of the other one, except when the interference in blood supply was so severe that scarring resulted. In these damaged areas, granules persisted or increased in number even though they were decreased in adjacent relatively normal areas.

Occlusion of one ureter in rats produced severe hydronephrosis in the homolateral kidney and an elevation in blood pressure. Juxtaglomerular cell granules persisted in the hydronephrotic kidney but were decreased in the contralateral one. This finding confirmed the results of the above experiments.

Unilateral nephrectomy in comparable rats had no effect on the degree of granulation of JG cells in the remaining kidney or on the level of blood pressure under the conditions of these experiments. The possibility that degranulation of JG cells in the contralateral kidney in the rats described above was due to compensatory hypertrophy was thereby excluded. An elevation in blood pressure was therefore implicated as an important factor in causing degranulation of juxtaglomerular cells.

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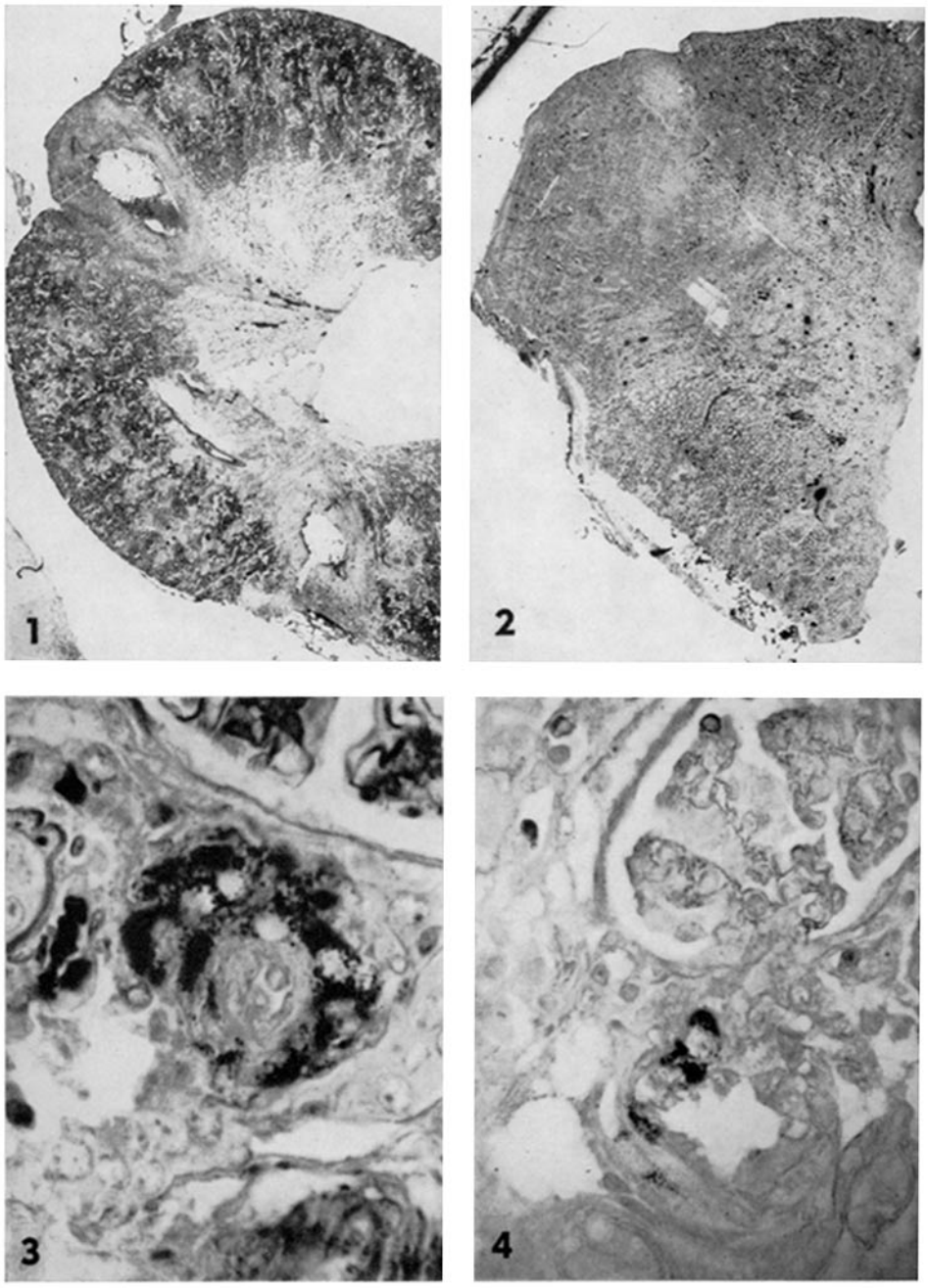
EXPLANATION OF PLATE 43

FIG. 1. Very low power photomicrograph of a section from a kidney that had been ligated with a "figure-of-eight" ligature several months previously by the method of Grollman. The ligature sites are conspicuous. Only minimal parenchymal damage occurred in this kidney. JG cells were degranulated throughout the section. Bowie–light green stain.

FIG. 2. Renal section from a similar rat. In this case the poles of the kidney became scarred (central region between the two depressed areas). The JG granules were markedly increased in the scarred areas but complete degranulation occurred in the adjacent relatively normal regions. Bowie–light green stain.

FIG. 3. Hyperplasia and hypergranulation of juxtaglomerular cells in the scarred region of the section shown in Fig. 2. The arteriole in which the JG cells are located is cut in cross-section. A portion of the glomerulus is at the upper right. Bowie–light green stain.

FIG. 4. Oil immersion photomicrograph of a section from a hydronephrotic kidney (see text). The afferent arteriole is cut in cross-section. Persistent granules can be seen in the juxtaglomerular cells in the arteriolar wall. Juxtaglomerular cells in the contralateral normal kidney were degranulated. Bowie–light green stain.



(Hartroft: Renal juxtaglomerular cells. III)