STUDIES ON RENAL JUXTAGLOMERULAR CELLS

I. Variations Produced by Sodium Chloride and Desoxycorticosterone Acetate*

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Since Ruyter (1) first described epithelioid cells in the preglomerular arteriole of the kidney, many attempts have been made to elucidate the function of these apparently endocrine "juxtaglomerular cells."[1] Goormaghtigh (2), from his extensive observations in hypertension and related conditions, postulated that they were probably the source of the vasopressor substance, renin. Among others, Dunihue (3, 4) subscribed to this theory, and his more recent investigations have revealed a relationship between juxtaglomerular cells and the adrenal glands (5). In adrenalectomized animals he found an abnormal increase of secretory granules which could be prevented or reversed by administration of desoxycorticosterone acetate (DCA) (6). A similar increase in granules has also been described by McManus (7) in kidneys from cases of Addison's disease in man. Since DCA is the salt-retaining hormone, these findings suggested to us that the effect of adrenal insufficiency on juxtaglomerular cells might be due primarily to a disturbance in sodium metabolism and that this effect could perhaps be produced by dietary means alone. The current clinical use of diets low in sodium for the treatment of hypertension further stimulated our interest in this possibility.

In a preliminary experiment, we were able to demonstrate in the rat, that restriction of dietary sodium chloride for a period of 2 weeks to 1 month caused a striking increase in the numbers of granules in the juxtaglomerular cells. A further series of experiments was then designed to confirm these observations and to determine the effect of variations in the level of dietary sodium chloride with or without DCA administration.

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[1] The more common term, juxtaglomerular apparatus, is sometimes used to refer only to the arteriolar granular cells, but often includes the macula densa as well. In this report, the term, juxtaglomerular cells, will always refer *only* to the arteriolar component and will not include the macula densa of the juxtaglomerular apparatus.

Methods

Animals and Diets

Experiment 1.—20 albino rats of the Wistar strain (both sexes), weighing 90 to 110 gm., and 6 weanling rats (35 to 42 gm.) were housed in individual cages and placed in two groups, equal with respect to weight and sex. The first group was fed, *ad libitum*, a basal diet containing only traces of sodium chloride.[2] The second group was given the same basal diet supplemented with a normal amount of sodium chloride[3] and was pair-fed with rats of the first group. The animals were sacrificed at the end of 2 to 4 weeks.

Experiment 2.—80 albino rats of the Wistar strain (both sexes), weighing 90 to 100 gm., were divided into three major groups (Table I).

Group I: 30 rats comprising this group were placed in three subgroups following a preliminary period of 5 weeks, during which they had been fed the basal (salt-deficient diet). One subgroup (I *b*) was used to determine the effects of DCA administration in the presence of low sodium chloride intake; another subgroup (I *c*) was used to follow changes when the intake of salt was suddenly raised from a low to a high level. The remaining rats (subgroup I *a*) were continued on the basal diet and not subjected to any additional procedure.

Group II: 20 rats were fed the basal diet supplemented with a normal amount of sodium chloride[3] for a preliminary period of 5 weeks. They were then placed in two subgroups to determine the effect of DCA administration at this level of salt intake (subgroup II *b*); the other subgroup served as controls (subgroup II *a*), being continued on the supplemented diet.

Group III: 30 rats were fed the basal diet supplemented with sodium chloride (as in group II), but in addition, they received 2 per cent sodium chloride in their drinking water for a preliminary period of 3 weeks. They were then placed in three subgroups. One subgroup (III *b*), was used to determine the effects of DCA administration in the presence of a high intake of salt, and another (subgroup III *c*), to determine changes which might be induced in the juxtaglomerular cells when the salt intake was decreased from a high to a low level. The remaining rats (subgroup III *a*) were continued on the high salt regimen with no additional procedure.

It will be noted from Table I that the initial experimental period for group III was 3 weeks, rather than 5 weeks, as in the case of groups I and II. After 3 weeks, the rats drinking 2 per cent saline lost so much weight, and their food consumption decreased to such an extent, that in order to avoid an unduly high mortality rate, it seemed advisable to terminate the preliminary period at this time. A comparison between groups I and III, is still considered justifiable, despite this discrepancy of time, since an effect would be expected from the high salt regimen within a shorter period than that required for maximum salt depletion on the low salt regimen.

The final experimental period in groups I and II was 1 to 10 days, rather than an unbroken period of 10 days for all animals. In these cases, one animal from each group was

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[2] The basal, sodium chloride–deficient diet consisted of the following: casein, 18 per cent; fibrin, 1 per cent; zein, 1 per cent; cellu flour, 2 per cent; salt mixture, 4 per cent; vitamin mixture, 1 per cent; sucrose, 60.65 per cent; beef fat, 10 per cent; corn oil, 2 per cent; choline chloride, 0.35 per cent; cod liver oil concentrate, 0.015 per cent; alpha tocopherol, 0.01 per cent. The salt mixture contained: $CaCO_3$, 11 per cent; $CaHPO_4 \cdot 2H_2O$, 32.5 per cent; K_2HPO_4, 27.5 per cent; $MgSO_4 \cdot 3\frac{1}{2}H_2O$, 10 per cent; cellu flour, 15 per cent; $FeC_6H_5O_7$, 3.0 per cent; trace elements, 1 per cent.

[3] The sodium chloride supplement replaced the cellu flour in the salt mixture of the basal diet, thus supplying 0.6 per cent NaCl.

TABLE I

Experiment 2

Group	Subgroup	No. of rats	Experimental procedure				Indices of granulation of juxtaglomerular cells				
			Initial period		Final period						
			Procedure	Duration	Procedure	Duration	+	++	+++	++++	Weighted total
				wks.		*days*					
I	I *a*	10	Fed basal salt-deficient diet	5	As for the initial period	1–10	8.6 (1–16)	6.7 (3–12)	2.6 (0–9)	0.4 (0–3)	35.6 (15–79)
	I *b*	10			Continued on same diet. Injected with 2.5 mg. DCA* per day	1–10	9.9 (3–19)	6.8 (0–12)	2.3 (0–8)	0.3 (0–1)	35.1 (7–66)
	I *c*	10			Transferred to diet with normal amount NaCl. 2 per cent saline in drinking water	1–10	8.0 (2–11)	5.3 (3–8)	2.3 (0–9)	0.1 (0–1)	28.7 (7–65)
II	II *a*	9	Basal diet plus normal supplement NaCl	5	As for initial period	1–9	6.1 (2–9)	2.0 (0–4)	0.4 (0–2)	0.0 (0)	11.5 (2–16)
	II *b*	10			Continued on same diet. 2.5 mg. DCA per day	1–9	5.2 (1–9)	1.7 (0–6)	0.1 (0–1)	0.0 (0)	9.4 (3–16)
III	III *a*	9	Basal diet plus normal supplement NaCl. 2 per cent saline in drinking water	3	As for initial period	10	4.5 (1–10)	1.0 (1–3)	0.0 (0)	0.0 (0)	6.0 (1–14)
	III *b*	10			Continued on same diet. 2.5 mg. DCA per day	10	1.1 (0–4)	0.0 (0)	0.0 (0)	0.0 (0)	1.1 (0–4)
	III *c*	9			Transferred to basal diet	10	5.5 (1–8)	2.0 (0–4)	0.3 (0–1)	0.0 (0)	11.0 (3–18)

* Percorten. Ciba Pharmaceutical Products Inc., Ltd., Montreal.

sacrificed daily. This was done in order that any series of changes, which might have developed in the juxtaglomerular cells, could be traced at every stage.

The food consumption of each animal was recorded daily, and those in the experimental groups were pair-fed with rats fed only the basal diet (group I during the preliminary period; subgroup I *a* thereafter). Rats in group III were an exception to this since their food intake voluntarily decreased while on the high salt regimen. Two rats in group III and one rat in group II died during the initial period. These were not included in the final data.

Histological

Slices of renal tissue (3 mm. in thickness) were excised from the midportion of each kidney in the transverse plane at right angles to the superior-inferior longitudinal axis. These were fixed by immersion in Zenker-formol (Helly's) fluid, and embedded in paraffin in the usual manner. The sections were stained according to the method described by Wilson (8).

Indices of Granulation of the Juxtaglomerular Cells

The kidney sections to be examined were labelled in such a way that the observers had no way of knowing the experimental history of the specimens until the investigation was completed. The objectivity of this method was facilitated by the fact that the experimental procedures employed had not produced pathological changes or other stigmata sufficiently characteristic to betray the history of the animal. The entire cortical area of one section from each kidney was covered systematically (using a mechanical stage) under the "high dry" magnification of the microscope.[4] All glomeruli so encountered were counted, regardless of plane of section. Units of juxtaglomerular cells[5] were recorded at the same time, and each unit was assessed for degree of granulation as follows:—

One plus. Cells with only a few granules clustered around the nuclei.

Two plus. A few cells (1 to 3) with a greater number of granules than the preceding classification, which are scattered throughout the cytoplasm, but do not completely fill the cells.

Three plus. A few cells (1 to 3) so densely packed with granules that the ground substance of the cytoplasm is obscured and the cells appear swollen.

Or a unit composed of many cells with degree of granulation corresponding to that described for "two plus."

Four plus. More than three cells distended with densely packed granules.

The above classifications are artificial to the extent that transition stages were, of course, encountered, but the definitions served as a practical guide in classifying large numbers of units. Typical examples of each of the 4 types are illustrated in Figs. 1 to 4.

The totals recorded under one-, two-, three-, and four-plus, were multiplied by the factors 1, 2, 4, and 8 respectively, since, at a very conservative estimate, at least 8 times as many granules were present in groups of cells classified as four-plus, as were present in those classified as one-plus, etc. (Figs. 1 to 4). The weighted totals were then expressed per 100 glomeruli to obtain the indices of granulation of juxtaglomerular cells. In Experiment 2, over 20,000 glomeruli were counted.

Reliability of Indices of Granulation of Juxtaglomerular Cells

To test the reliability of the method used for assessing indices of granulation, the following test was carried out several months after the original data were obtained. Two sets of

[4] Leitz binocular research microscope; 42 × ocular (N.A. 0.85); 6 × ocular; approximate magnification of 200 diameters.

[5] A "unit" of juxtaglomerular cells refers to a group of granular cells found together in the same arteriole (*i.e.*, at the pole of the same glomerulus).

sections of renal tissues were prepared from 9 of the salt-deficient rats (subgroup I *a*) and 9 of the control rats (subgroup II *a*). The staining procedure in this case was modified slightly in that Bowie's stain was substituted for crystal violet.[6] The method for assessing granulation was the same as described above, the two sets of sections being counted independently by two observers. The results of this test will be discussed in a later section of the paper.

Blood Pressure Measurements and Organ Weights

When each animal was sacrificed, the level of blood pressure (systolic-diastolic average) was measured directly by cannulation of the femoral artery with a needle or small plastic tubing which was attached to a mercury manometer by a system filled with Ringer's solution. Ether anesthesia was used and 2 per cent novocain was applied locally to prevent spasm of the exposed artery.

The wet weights of the kidneys, hearts, and adrenal glands were also recorded.

RESULTS

Indices of Granulation of the Juxtaglomerular Cells

Experiment 1.—The average of the indices for animals which received the basal diet supplemented with a normal amount of sodium chloride was 14, while that of the salt-deficient rats was 26.

Experiment 2.—The results of this experiment are summarized in Table I and Graph 1.

The indices of granulation varied inversely with sodium chloride intake. The average normal index (subgroup II *a*) was 12, while that of the salt-deficient rats (subgroup I *a*) was 36, and in contrast, that of the rats subjected to the high salt regimen (subgroup III *a*) was only 6.

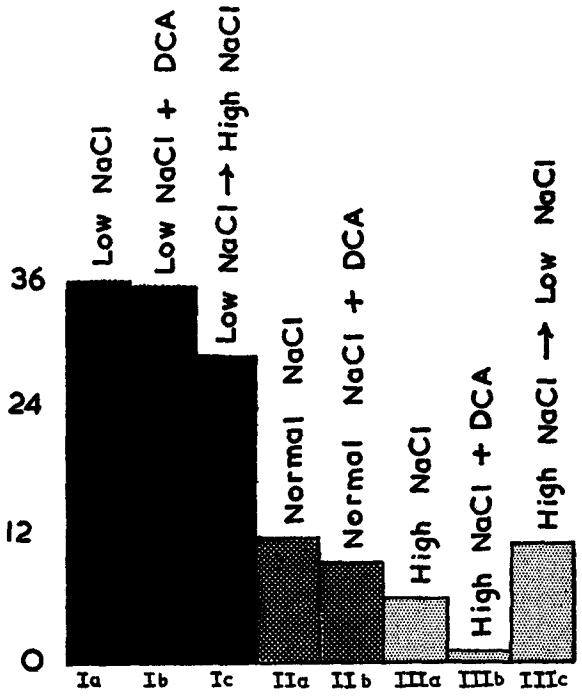
When salt-deficient animals were transferred to a high salt regimen (subgroup I *c*), they neither ate well, nor drank more than small amounts of 2 per cent saline during the final experimental period. The average index of this group was 29, lower than that of the other salt-deficient rats, but still appreciably higher than normal (index 11.5). When rats which had received

[6] Bowie's stain was used because a more intense color of the granules could be obtained than with crystal violet. The staining procedure is as follows:—

1. Decerate and hydrate sections in the usual way.
2. Leave overnight in 20 per cent ethanol to which has been added 10 drops of Bowie's stock solution (Cowdry, E. V., Microscopic Technique in Biology and Medicine, Baltimore, The Williams & Wilkins Co., 1943, 37) per 100 cc.
3. Wipe slides around the edges and blot dry with bibulous paper.
4. Dip into acetone to remove excess stain, and then briefly into absolute ethanol.
5. Differentiate and counterstain in 0.1 per cent alcoholic acid green C.I. or light green, with the aid of a microscope, until the red blood cells lose their purple color and become bright red. This is a very critical end-point for Zenker-formol-fixed sections. The amount of green in the section can be varied by varying the concentration of acid green or light green.
6. Wash in two changes of benzene and mount in benzene balsam. The granules of the juxtaglomerular cells should be an intense purple color.

2 per cent saline were transferred to the basal diet (subgroup III *c*), the resulting index was normal (11.0) as compared with 6.0 for rats which remained on the high salt regimen (subgroup III *a*).

Injections of DCA did not reduce the number of juxtaglomerular granules which had accumulated in the salt-deficient animals (subgroup 1 *b*), but did so to a remarkable degree in rats which received 2 per cent saline (subgroup III *b*), the index of the latter being only one-tenth as great as normal. DCA



GRAPH 1. Indices of granulation of juxtaglomerular cells.

also lowered the index, but to a lesser degree, in the rats which had consumed a normal amount of sodium chloride (subgroup II *b*).

Reliability of the Indices of Granulation

The results of this test are shown in Table II. The mean indices for the salt-deficient and control rats respectively obtained by one observer, were 36 and 15, as compared with the values 39 and 19 obtained by the other observer. In both cases the difference between the salt-deficient and control animals was highly significant ($P < 0.01$) as indicated by the t test (the respective values for t were 5.1 and 4.5).

Variations in Cytology of the Juxtaglomerular Cells

These will be described under three headings:—

Normal.—The typical appearance of the juxtaglomerular cells observed in the kidneys of control rats is illustrated in Fig. 1. The cells are almost always found in the media of the afferent arteriole at the pole of the glomerulus. They are only slightly larger than the adjacent smooth muscle cells, but may be distinguished by their round or oval nuclei and absence of myofibrils. Only a few granules are present, usually clustered around the nucleus in a "halo" formation, and in size they are somewhat smaller than pancreatic zymogen granules, but larger than those in beta cells of islets of Langerhans.

TABLE II

Indices of Granulation

Rat No.	Salt-deficient rats*		Rat No.	Control rats*	
901	35	29	946	13	8
902	29	21	947	23	18
903	30	34	949	14	14
904	34	36	950	27	20
905	33	29	955	24	19
920	69	57	957	20	17
922	37	53	958	18	14
924	37	33	959	18	15
925	43	31	960	12	9
Mean.....	39	36	Mean.....	19	15

* Values determined by two observers; hence two sets of values for each group of rats.

Juxtaglomerular cells of this type (one plus, above) were found in varying numbers in the kidneys of all the experimental animals, but were most characteristic of those in the control group (II *a*) and of others with indices which approached normal (subgroup III *c*).

Hypergranulation.—The transition stages in the accumulation of excess granules in juxtaglomerular cells have been briefly described in defining the classifications used to assess indices of granulation (Figs. 2 to 4). Cells in the initial phase of hypergranulation differ from those described above in that the granules, increased in number, are no longer so characteristically confined to the perinuclear position but have become scattered more diffusely throughout the cytoplasm (Fig. 2). In extreme cases (Fig. 4), the cells are sometimes enlarged to three or four times their normal diameter, presumably as the result of distension by the excess number of granules. Like the first type, these swollen cells are always found in the media of the afferent arteriole in a single layer. But unlike the normal, such cells are no longer so typically restricted to the

juxtaglomerular position. Rather, hypergranulated cells have replaced the smooth muscle cells and occupied the media for a considerable distance proximal to the granulated pole. Granules appear larger in hypergranulated cells than in sparsely granulated ones (compare Figs. 3 and 4 with Figs. 1 and 2).

The extreme of this type of cell (four plus, above) has been encountered *only* in the kidneys of the salt-deficient rats (group 1).

Degranulation.—As only juxtaglomerular cells with visible granules enter into the counts, degranulation is reflected by a low index. This was the case for rats treated with DCA or high salt and especially with a combination of these factors (subgroups II *b*, III *a*, and III *b*). The degranulated cells sometimes had

TABLE III

Blood Pressures and Organ Weights

Group*	Initial body weight	Final body weight	Blood pressure‡	Kidney weight	Heart weight	Adrenal weight
	gm.	*gm.*	*mm. of Hg*	*gm.*	*gm.*	*mg.*
I *a*	104	163	95	1.24	0.47	35
I *b*	98	153	90	1.17	0.44	31
I *c*	100	161	88	1.28	0.49	35
II *a*	107	198	104	1.39	0.55	27
II *b*	95	188	99	1.51	0.57	29
III *a*	100	149	107	1.30	0.49	32
III *b*	101	127	82	1.17	0.47	33
III *c*	104	165	114	1.29	0.50	28

* See Table I.

‡ Average of systolic and diastolic levels, measured by direct cannulation of femoral artery.

a vacuolated appearance. Granules which have persisted often vary greatly in size, but most appear smaller than normal.

Although not frequently found, evidence of hyperplasia of the juxtaglomerular cells has been encountered occasionally in the kidneys of rats with low indices of granulation. In these instances, the sparsely granulated cells form several concentric layers in the arteriolar media at the juxtaglomerular pole.

Blood Pressure and Organ Weights.—Table III has been included to show the lack of positive correlations of blood pressure and organ weights (kidneys, heart, and adrenals) with changes in juxtaglomerular granulation index.

DISCUSSION

Juxtaglomerular Granulation Index (JGI).—The method developed for assessing degrees of juxtaglomerular granulation in these experiments was not intended to be a quantitative measure. Its scope is only that of any *comparative* index. By the method, results have been obtained which proved reproducible

by two observers working independently from separate sets of sections cut from the same paraffin blocks of renal tissue. As elucidated below, the JGI is considered a useful and practical method of estimating relative differences in numbers of juxtaglomerular granules among groups of experimental animals.

The JGI possesses inherent limitations, in that it depends on histology alone (without bioassay or biochemical confirmation) and in that it cannot but be influenced, to some extent, by subjective impressions of the investigator. In devising the method, efforts were made to minimize variables from these sources. Fixation, sectioning, and staining procedures (8) were such, that although oil immersion objective lenses were not employed, even small aggregates of granules were rendered so prominent that they did not readily escape notice. The section labels were masked during the appraisal. Finally, the factors chosen to weigh the juxtaglomerular units according to their intensity of granulation were conservative even though this doubtless minimizes actual differences. For example, a unit classed as four plus (Fig. 4) was multiplied by a factor of only 8, although a glance at the photomicrographs was sufficient to indicate that, in reality, there were clearly present many more than eightfold the number of granules to be found in a unit classified as one plus (Fig. 1). Thus, differences in JGI's among groups were always the very least of probable real values which might have been revealed by a quantitative method of assay, if such had been practicable. Thus, a threefold difference between two JGI's means that at least three times as many granules were present in the one group as in the other, but in terms of actual numbers of granules, this might be of the order of 30 times, or even 300 times.

The Endocrine Nature of Juxtaglomerular Cells.—Until the substance represented by the granules in the juxtaglomerular cells can be isolated and its physiological properties determined, only indirect evidence (such as the JGI) can be used to postulate the function of these cells. Goormaghtigh (9) described cytological characteristics of the juxtaglomerular cells to be as undoubtedly endocrine as those of the cells of anterior pituitary. The presence of secretory-like granules and a secretory cycle (10), as well as the proximity of the cells to the circulating blood, favor the endocrine cell theory; but the unique position of the juxtaglomerular cells in relation to the organ in which they are situated does not resemble that of any other endocrine gland in the body. Even the cells in the islands of Langerhans are surrounded by capillaries and form units distinct from the exocrine portion of the pancreas. The juxtaglomerular cells, however, being in the wall of the afferent arteriole, form part of the structure of the nephron itself which is the functioning unit of the kidney. In addition, as pointed out by McManus (11), a specialized portion of the distal tubule, the macula densa, and the juxtaglomerular cells are always in intimate contact and this might favor the passage of substances from one to the other. On the other hand, only the intima of the arteriole separates the

granules from the blood stream. It is well to keep in mind therefore that if a hormone-like or enzymatic substance is produced by juxtaglomerular cells, it could conceivably act either locally within the nephron or as a true "chemical messenger" which may circulate throughout the body. In any case, the evidence at the present time strongly indicates that the juxtaglomerular cells perform a secretory function.

Significance of the Variations in JGI Encountered.—To interpret the significance of the cytological variations in the juxtaglomerular cells described in this report, it must be recalled that a secretory cell may function both to manufacture and to store its product. The number of granules within the cell, seen histologically, therefore is determined by a ratio of the rate of production to the rate of liberation. In sodium chloride deficiency this ratio was increased in the case of the juxtaglomerular cells as indicated by the accumulation of granules (high JGI). Two possible reasons for this are evident. The granules either were being formed at a rate greater than normal, with their liberation from the cell inadequate to prevent their accumulation or, more likely, were being liberated at a rate slower than normal with the level of production remaining the same. Because of the absence of features associated with active secretion—such as vacuolation, hyperplasia, etc.,—it is felt that the latter explanation is the more probable. Conversely, degranulation of the juxtaglomerular cells observed in rats which had consumed large amounts of sodium chloride, with or without DCA, was probably the result of an increased rate of liberation with exhaustion of the granules within the cell, rather than a decrease in granule manufacture. The evidence of vacuolation and hyperplasia in some of the animals so treated favors this concept. Thus, sodium chloride deficiency apparently reduced the demand for the "juxtaglomerular substance," causing the granules to accumulate within the cells, while a high sodium chloride regimen increased the demand for the "substance" resulting in greater liberation of the granules from the cells.

If the foregoing interpretation is accepted, the results of the present investigation are compatible with the findings of Goormaghtigh and others concerning hypertension, as well as with the previously mentioned effects of adrenal insufficiency. In the following development of the arguments supporting this statement, two theories are suggested to explain the function of the juxtaglomerular cells:—

Possible Functions of Juxtaglomerular Cells.—Dunihue (6) demonstrated that a striking accumulation of granules occurred in the juxtaglomerular cells of bilaterally adrenalectomized animals, and that this phenomenon could be prevented or reversed by injections of DCA. Although this observation suggested that the level of adrenal cortical hormones might have been the primary factor in his experiments, it is obvious that profound alterations in sodium metabolism had been produced; but the etiological importance of the latter was

not known. However, the evidence provided by the present experiments clearly demonstrates the close relationship between sodium chloride and juxtaglomerular cells. Even in the presence of the intact adrenal glands, restriction of dietary salt produced storage of juxtaglomerular granules, and, although this was reversed to some extent by restoring NaCl to the diet, it was not altered by increasing the level of the adrenal corticoid DCA. DCA was effective only when the level of dietary sodium chloride was high. Furthermore, excessive NaCl intake alone produced degranulation of the juxtaglomerular cells, effectively reversed by subsequent restriction of salt. These findings indicate that the results obtained by Dunihue were probably secondary to changes in salt metabolism produced by adrenalectomy and/or DCA administration, rather than direct effects of these procedures. One might even postulate that the function of the juxtaglomerular cell hormone is concerned with the regulation of sodium in a manner counteractive to that of DCA. In this connection the intimate structural relation between the juxtaglomerular cells and the macula densa of the distal tubule, mentioned above, might be of importance, since the distal tubule is considered the mechanism for the "fine adjustment" of the level of sodium excretion (12). In such a situation a local action of the "juxtaglomerular hormone" on the renal nephron itself to alter reabsorption by the distal tubule is a reasonable possibility. But the pathological effects of hypertension must be included in this picture.

Goormaghtigh's illustrated descriptions of hyperactive juxtaglomerular cells in hypertension (2) show not only an increase in granules and hyperplasia but also the presence of vacuolated, clear cells. With reference to the previous discussion of manufacture and liberation by a secretory cell, this indicates that both production and secretion of the granules have been accelerated. Thus in hypertension the effect on juxtaglomerular cells is probably the opposite from that of sodium deficiency, in which the juxtaglomerular cells are underactive, and is more akin to the effect produced by a high salt regimen, except that in the latter only an increased liberation of the granules is evident. Salt, the adrenal glands, and blood pressure are closely associated (13). For example, salt restriction or adrenalectomy alleviates hypertension while DCA accentuates it, indicating a disturbance in sodium metabolism in this condition, which, in some instances, has been detected as an excess level of plasma sodium (14). Similarly, salt administration in combination with certain procedures involving renal manipulation produces hypertension (15). In a general way therefore it can be stated that in conditions in which there is a tendency to high sodium levels (high salt regimen, DCA administration, and hypertension) the juxtaglomerular cells are overactive, while they are underactive in sodium deficiency (dietary restriction of salt and adrenal insufficiency). This statement could of course be modified by substituting "hypertension" and "hypotension" for "high sodium" and "sodium deficiency," thus relating the level of blood

pressure to changes in juxtaglomerular cells. The fact that significant changes in blood pressure were not detected in the course of this investigation does not necessarily contradict the latter assumption since it would be quite possible for a vasoactive compensatory mechanism to be in play, which, if effective, would not be reflected by changes in blood pressure. Goormaghtigh's hypothesis can be considered in this light.

If, as Goormaghtigh suggests (2), the juxtaglomerular cells secrete the vasopressor substance, renin, then hypertension is the direct result of the hyperactivity of these cells which in turn must have been initiated by some other mechanism. On the other hand, if these cells are responsible for elaboration of a *de*pressor substance then their hyperactivity must have been stimulated by the high blood pressure itself, thus representing a compensatory mechanism. Such a concept would equally well explain the observed cytological changes of the juxtaglomerular cells in hypertension and is more compatible with the findings of Grollman *et al.* (16). These workers, with the theory that the kidney normally produces a depressor substance, succeeded in producing a severe and sustained hypertension in bilaterally nephrectomized dogs. Such animals of course do not have juxtaglomerular cells. Our findings can also be explained on the basis of the renal depressor theory in that, hypothetically, there was a diminished demand (a tendency to low blood pressure) for the normally secreted depressor substance during sodium deficiency, causing the granules to pile up in the juxtaglomerular cells. A converse situation would exist in the case of the high salt regimen and DCA administration.

These two theories—that the juxtaglomerular cells are involved either in the regulation of sodium metabolism or in the regulation of blood pressure and the development of hypertension—are in effect complementary to each other. As a working hypothesis, it might be postulated at this time that the juxtaglomerular cells may be responsible, through the elaboration of a vasoactive substance or through the regulation of sodium metabolism, for maintaining normal blood pressure.

SUMMARY

Accumulation of granules in the juxtaglomerular cells occurred in rats which were maintained for 5 to 6 weeks on a diet low in sodium chloride. Cytological evidence suggests that this was probably a storage phase of secretion following a decrease in the rate of liberation of the granules. Administration of DCA (desoxycorticosterone acetate) to salt-deficient rats did not alter this appearance of the juxtaglomerular cells.

Two per cent sodium chloride taken in the drinking water consumed for 4 weeks by similar animals caused degranulation of the juxtaglomerular cells. This effect was enhanced by DCA. DCA administered to animals on a normal salt intake produced a lesser degree of degranulation. Cytological changes in

degranulated cells suggested that these represent a stage of hyperactivity in the secretory cycle produced by an increase in the rate of liberation of granules.

A hypothesis is suggested that the juxtaglomerular cells are involved in the hormonal regulation of sodium metabolism and/or blood pressure.

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EXPLANATION OF PLATE 20

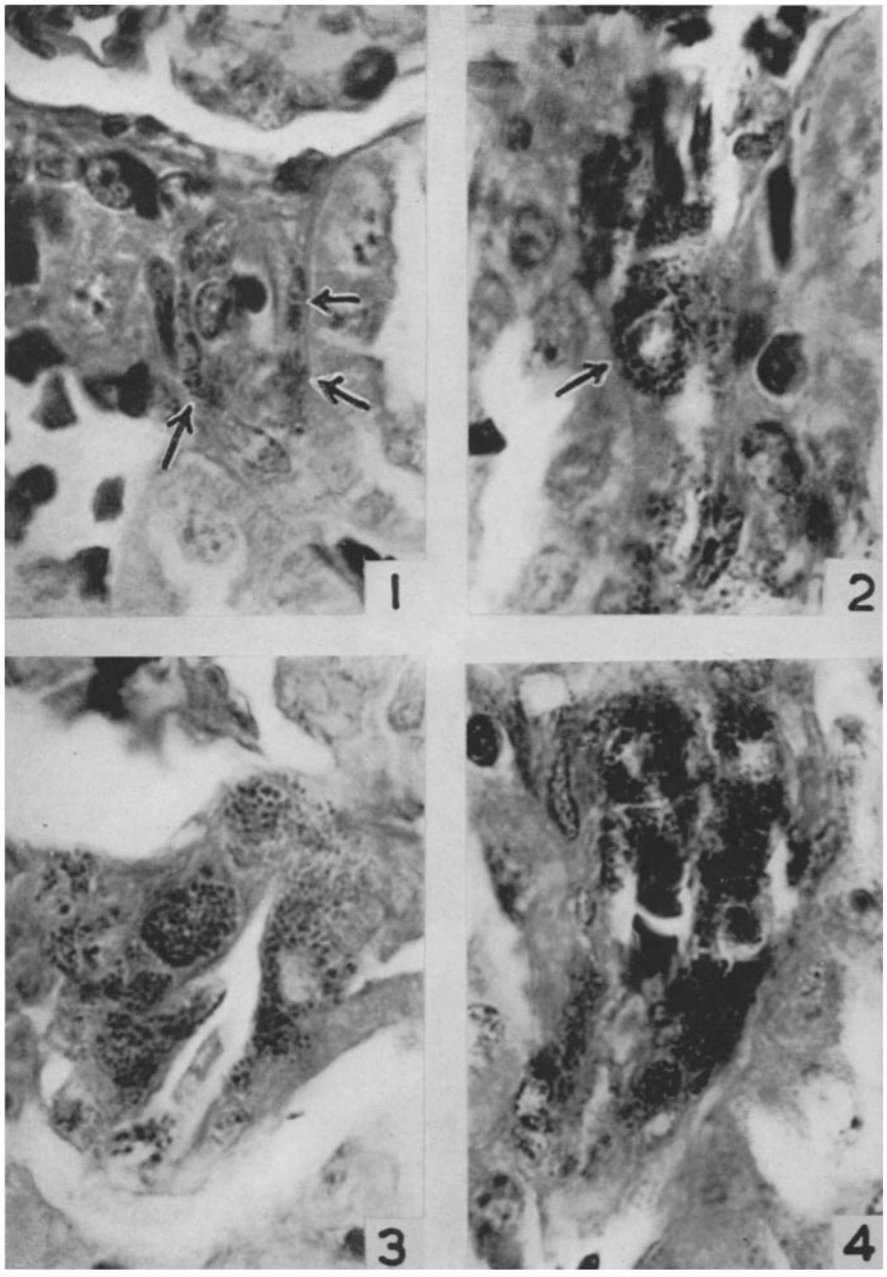
Figs. 1 to 4 illustrate juxtaglomerular cells in the afferent arterioles of rats. In all cases the glomerulus lies just above the field shown. Technical data are the same for all: paraffin sections; Zenker-formol fixation; Wilson juxtaglomerular cell stain; Wratten E filter; × 1500.

FIG. 1. Kidney from a control rat. The arrows point to juxtaglomerular granules in the media of the afferent arteriole (cut obliquely). Notice the position of the granules in a "halo" formation surrounding the nucleus. This unit of juxtaglomerular cells was classified as one plus (see text) and was typical of those encountered in the kidneys of control rats.

FIG. 2. Kidney from a control rat. Juxtaglomerular cells of this type (arrow) were classified as two plus (text). These were not as typical of the control group as those in Fig. 1, but were found more frequently in salt-deficient rats. Notice that the granules are increased in number (compare with Fig. 1) and scattered throughout the cytoplasm.

FIG. 3. Kidney of a rat which received a low salt diet for 5 weeks. Several juxtaglomerular cells well filled with granules are present in the afferent arteriole. The granules have increased in size (compare with Figs. 1 and 2) as well as in number. Units of this type were classified as three plus and were encountered most frequently in salt-deficient rats, being absent in rats given 2 per cent saline and only rarely present in control rats.

FIG. 4. Kidney from a rat which received a low salt diet for 5 weeks and was injected with DCA for 6 days. The juxtaglomerular cells seen here are distended and tightly packed with granules. Units of this type were classified as four plus and could be found only in the kidneys of salt-deficient rats. (DCA did not affect the amount of granules in salt-deficient rats but markedly reduced their numbers in rats which received 2 per cent saline.)



(Hartroft and Hartroft: Renal juxtaglomerular cells. I)