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Lysosomal Nature of Juxtaglomerular Granules

Abstract. *Demonstration by a combined histochemical and electron-microscopic technique of the ultrastructure of and acid phosphatase within the juxtaglomerular granules in kidneys of normal man and rat and in those from members of both species with renal hypertension indicate the identity of juxtaglomerular granules as lysosomes.*

Hypertension produced by decreased or altered renal blood flow is associated with an increase in pressor substance in the kidneys and blood (*1*). Correlation of renal pressor activity with the number of granules in renal juxtaglomerular cells (JGC) (*2*), identification of renin by immunofluorescence (*3*), and the location of pressor activity in juxtaglomerular granules (JGG) (*4*) suggest that renin is associated with JGG. Although the function of the JGC is influenced by blood volume, by electrolyte concentrations, and perhaps by cells of the macula densa of the distal nephron and unmyelinated nerve fibers within this cellular complex, little is known concerning the synthesis or release of renin.

Study of the ultrastructure of JGC of ischemic renal tissue obtained by needle biopsy from six patients with renal hypertension, and from a similar number of rats after unilateral renal artery constriction (*5*), revealed abundant electron-opaque JGG enclosed within solitary, smooth limiting membranes, as well as within saccules of the Golgi apparatus. Paracrystalline forms of JGG and lipofuscin droplets were prominent in JGC of man, but not in those of the rat. In both species, the endoplasmic reticulum, principally of the smooth variety, was frequently dilated. Its matrix was often comprised of flocculent, slightly electron-opaque material. The ultrastructural appearance of JGG, the relationship of these granules to Golgi structures, the presence of lipofuscin within the cells, and the proteolytic nature of renin prompted inquiry into the lysosomal nature of the granules.

Renal biopsies were performed on two patients: (i) a 50-year-old man with renal hypertension and (ii) a 45-year-old normotensive man. In addition, ischemic kidneys from five rats with hypertension (160 mm-Hg) induced by unilateral renal artery constriction and a similar number of normotensive rats were examined. A combined histochemical and electron-microscopic method for identification of acid phosphatase activity was used (*6*), except that tissue was first fixed in cold 8-percent glutaraldehyde in sodium cacodylate, *p*H 7.4, for 3 hours at 4°C.

The reaction product for acid phosphatase appeared as electron-opaque deposits within many limiting membranes as well as within the matrix of mature JGG, within occasional Golgi lacunae, and around lipofuscin droplets in JGC of all tissues examined; the deposit was most conspicuous in JGC of kidneys from the man and rats with hypertension (Figs. 1 and 2). No precipitate was noted in control sections incubated in buffer and lead nitrate in the absence of glycerophosphate substrate.

These observations appear unrelated to those of Finegan (*7*), who, using light-microscopic techniques, observed alkaline phosphatase in some cells of the juxtaglomerular apparatus lying between JGC and the macula densa. Of the many species that were examined, only the rat had demonstrable alkaline phosphatase in its kidney, and the functional significance of the enzyme was uncertain.

Although caution is theoretically warranted in regarding as lysozymes all particles which contain acid phosphatase, the presence of the enzyme within granules or vacuoles that are limited by a solitary, smooth membrane conforms with the biochemical and morphological definition of these subcellular particles. Accumulating evidence indicates the lysosomal nature of secretory vacuoles in a variety of tissues (*8*). The demonstration of acid phosphatase within Golgi lacunae of JGC indicates a source for the acid phosphatase (and perhaps other lysosomal enzymes) in JGG. This interpretation is consistent with the general hypothesis which identifies the

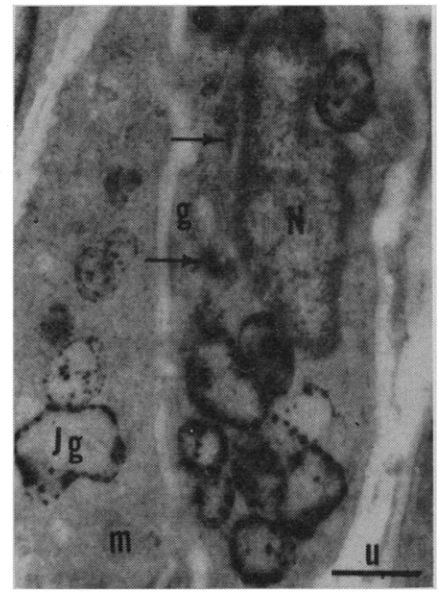

Fig. 1. Reaction product of acid phosphatase in limiting membrane and matrix of juxtaglomerular granules (*Jg*) of portions of two juxtaglomerular cells of ischemic kidney from hypertensive rat. Reaction is also evident in portions of Golgi (*g*), indicated by arrows, but not in the nucleus (*N*) or mitochondria (*m*).

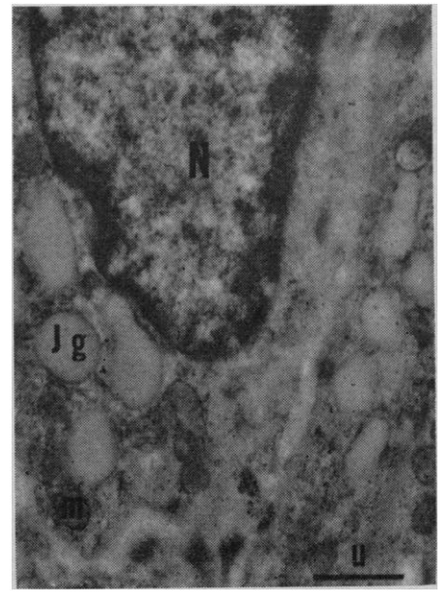

Fig. 2. Portions of juxtaglomerular cells from a control section of the kidney depicted in Fig. 1, incubated in buffer and lead nitrate without substrate. No reaction product is evident within juxtaglomerular granules (*Jg*). The nucleus (*N*) appears larger than in Fig. 1 due to plane of section.

Golgi apparatus as the site of "packaging" or modification of secretory products synthesized within the endoplasmic reticulum. Recognition of the lysosomal nature of JGG provides information relative to the mechanism of release of renin. Possibly the proteolytic enzyme renin represents another enzyme of lysosomes in JGC or, conversely, certain lysosomal enzymes in JGC have activities similar to that of renin capable of initiating angiotensin formation and hypertension.

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Lipids of the Living Coelacanth, Latimeria chalumnae

Abstract. *The muscle of* Latimeria chalumnae *contains 30 to 71 percent (dry weight) of lipid deposited extracellularly. Wax esters constituted 90 percent or more of the lipids from muscle and fat storage tissues. These esters, by gas-chromatographic analysis, consisted of* C_{30} *to* C_{40} *homologs with one or two double bonds.*

The living coelacanth fish, *Latimeria chalumnae*, has excited the interest of biologists concerned with vertebrate phylogeny ever since its discovery off eastern South Africa in 1938 (*1*). In the intervening years over 30 specimens have been captured, all but the first in the vicinity of the islands of Anjouan and Great Comoro in the Comoro Archipelago between Madagascar and Mozambique. Several preserved specimens have been carefully dissected, and aspects of their gross and microscopic anatomy and gross chemical composition have been described (*2*). The rarity of the species has combined with the difficulty of access of the Comoros to prevent all but the sketchiest studies of behavior and ecology (*3*). Nothing is known of the physiology and very little of the biochemistry (*4*) of this form.

We recently obtained a newly caught male coelacanth, perfectly preserved by formalin and deep-freezing (*5*). The freshness and excellent condition of this specimen have permitted us to make a detailed study of the pattern of lipid composition of the various tissues. This pattern, while exotic, resembles that found in several species of bathypelagic fishes. We have examined trunk muscle, liver, spleen, the fat-filled presumptive swim bladder, and fat storage tissues in several locations.

The techniques employed (*6*) were: (i) extraction of the lipid from the tissue with chloroform-methanol as solvent; (ii) separation of the various lipid classes by adsorption column chromatography on silicic acid, monitored by thin-layer chromatography (TLC); (iii) saponification of the chromatographically pure wax esters—the principal lipid type present in the muscle and adipose tissues—and separation of the constituent long-chain alcohols and fatty acids on florisil columns; and (iv) gas-liquid partition chromatography (GLC) of the intact wax esters and of the alcohol and acid moieties (as the trifluoroacetate and methyl ester derivatives, respectively) (Tables 1, 2, and 3).

The liver lipid fraction consisted of triglycerides, 78.4 percent; wax esters plus sterol esters, 8.2 percent; and polar lipids (including phospholipids), 9.1 percent. The composition of the triglyceride fatty acids is given in Table 3. The pattern of the spleen lipids was unusual: hydrocarbons, 3.6 percent; wax esters plus sterol esters, 14.7 percent; triglycerides, 6.2 percent; free fatty acids, 16.1 percent; cholesterol, 12.8 percent; and polar lipids, 46.7 percent. The hydrocarbons were identified by GLC as the C_{20} to C_{32} normal alkanes plus squalene (about one-third of the total). No unesterified long-chain alcohols were detected, and the only phospholipid in the polar lipids was lecithin (by TLC; *6*). Lecithin could not have been the principal constituent of this fraction, since 57.8 mg of material on methanolysis gave only 6.0 mg of chromatographically pure methyl esters. In Table 3, therefore, the analysis of the largest homogeneous fraction of spleen lipids is given, namely the free fatty acids. The total fatty acids of the polar lipid fraction were similar, but had lower values for palmitic and palmitoleic acids and correspondingly higher values for stearic acid.

We have not yet compared the fatty acid patterns of wax esters, cholesteryl esters, and triglycerides from a single tissue of the coelacanth since the wax esters so predominate in the lipid from muscle and adipose tissues that isolation of either cholesteryl esters or triglycerides is difficult; indeed, the presence of glycerides in these tissues has not been established. With the organ lipids the problem is the separation of the cholesteryl esters from the wax esters quantitatively.

The low percentages of C_{20} and C_{22} polyunsaturated acids in the organ lipids were unexpected. There was no evidence that hydrolysis or autoxidation occurred during the shipping, storage, and extraction of the tissues. The formalin probably would have destroyed any phosphatidyl ethanolamine present (*7*), but it would not be expected to react with the polyunsaturated fatty acids. However, in view of the year which intervened between capture of the fish and completion of the GLC analyses of the methyl esters in the organs, the values in Table 3 for the polyunsaturated acids of the liver

Table 1. Composition of the lipids of *Latimeria chalumnae* tissues.

Total lipid (%)			
Wet weight	Dry weight		Wax ester content
	This work	Millot (*8*)	
	Ventral muscle		
34.1	71.4		94.3
	Dorsal muscle		
7.7	29.7	23.9	92.7
	"Swim bladder"		
61.9	98.9	95	97.2
	Pericardial		
81.0	98.7		92.7
	Pericerebral		
76.2	95.5	92.3	92.9
	Postocular		
60.8	92.7		90.0
	Liver		
24.1	67.7	32.3	8.2*
	Spleen		
3.5	18.6		14.7*

* Includes sterol esters.