dihydrate, A grade, Calbiochem) in concentrations ranging from $10^{-9}$ to $10^{-5}$ $M$/l, on unfed sponges, either at day 7 or at day 14, had no significant effect on gemmulation. Various combinations of repetitive addition of cAMP were also tried, without any positive result. A single application of $10^{-3}$ $M$ cAMP alters reversibly the organisation of the sponge; 3 successive applications at 2 days intervals lead to an irreversible disorganization of the aquiferous system.

To sum up this aspect of our experiments, we can say that we did not encounter one experimental situation in which a rise in overall concentration of cAMP had any effect on gemmulation.

*Discussion.* It has been abundantly substantiated that, in vertebrates, theophylline and, more generally, methylxanthines, interact with many hormonal phenomena by inhibiting the cAMP-phosphodiesterases that help to regulate the intra-cellular concentration of cAMP[16]. In other organisms, however, the situation may be different: theophylline does not inhibit the cAMP-phosphodiesterase of *Escherichia coli*[17] and inhibits only slightly one of the extra-cellular phosphodiesterases of *Dictyostelium discoideum*[18].

In the present state of our knowledge of the biochemistry of sponges, it is very difficult to interpret our finding that theophylline stimulates gemmulation whereas a general application of cAMP does not. The two facts, however, are not in contradiction to the hypothesis that theophylline acts on gemmulation by enhancing a cAMP-dependant mechanism.

Cyclic AMP might be implied in gemmulation as a first messenger[19], acting in the aggregation of sponge cells in the same way as in the aggregation of some cellular slime-molds. In such a hypothesis, the signal for the coming together of amoeboïd cells would be a local concentration gradient of cAMP. Although such a gradient could not be mimicked by a rise in overall concentration of cAMP, its building up might be accelerated by the inhibition of cAMP phosphodiesterases in or around cAMP emiting cells.

In this respect, it is worth noticing that, on many occasions, theophylline stimulated sponges start producing at the same moment, in 2 different places, 2 gemmules of equal size, though smaller than a normal gemmule. In contrast, control sponges of the same size, when they gemmulate, always produce only 1 gemmule at a time. This might be an indication that theophylline acts on gemmulation by eliciting a local process, rather than by modifying the physiological state of the whole sponge.

However, cyclic AMP might even well be involved at the intracellular level, some triggering event of the gemmulation depending on the accumulation of cAMP in a particular cell-type. In this case, our contradictory results with cAMP and theophylline might be explained by differences in the permeability of the membrane of that cell-type to both agents.

Finally, it is possible that theophylline acts on sponges in a way that has nothing to do with the metabolism of cAMP. It is therefore essential that we acquire some knowledge about the existence and the properties of cAMP phosphodiesterases in these organisms, and about a possible secretion of cAMP during gemmulation. This problem is at present being dealt with in our laboratory.

*Résumé.* La formation de gemmules dans les éponges d'eau douce est fortement stimulée par la théophylline $10^{-4}$ $M$, mais non par une augmentation globale de la concentration en adénosine monophosphate cyclique dans le milieu.

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## Lysosomal Function of Juxtaglomerular Granules

The juxtaglomerular apparatus or complex is made up of the macula densa, the lacis cells and the juxtaglomerular (JGC) or epithelioid cells of the afferent glomerular arteriole. The macula densa, a portion of the distal tubule, is in intimate contact with the vascular pole of the glomerulus. The lacis cells of OBERLING and HATT[1] are located between the afferent and efferent arterioles and the macula densa[2]. The juxtaglomerular granules (JGG) of the JGC are currently recognized as the site of renin synthesis and storage[3–8].

Microdissection studies have verified that renin activity is localized in the afferent arteriole[7]. A good correlation has been established between the renal pressor activity and the number of granules in JGC[8]. The pressor activity has been shown to be localized in JGG[9], in which renin has been identified by immunofluorescence[10]. Electron microscopic studies on the JGC of rats with unilateral renal ischemia[11], magnesium[12,13] or sodium deficiency[14] have revealed numerous, variably electrondense, homogeneous JGG enclosed by a unit membrane.

Our investigation into the possible lysosomal function[15] of JGG was prompted by their ultrastructure as well as by the presence of renin (a proteolytic enzyme) and acid

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phosphatase in these granules[16-18]. The fluorochromes neutral red and euchrysine 3R were chosen because they are known selectively to concentrate in the lysosomes of several cell types[19]. Neutral red (aminodimethylaminotoluaminozine hydrochloride), and euchrysine 3R – of which the major component is chromatographically indistinguishable from acridine orange (3,6-dimethylamino-acridine)[19] – accumulate within acid phosphatase-positive granules[19,20] and produce typical ultrastructural alterations in the lysosomal matrix[21-24]. On the basis of in vitro experiments, it would seem that the cellular uptake of the two fluorochromes is a passive phenomenon[24,25] whereas their lysosomal segregation could imply a passive[25] or active transport mechanism[24]. Their fate during passage from the cell membrane to the lysosome is not clear. Whether they are confined in endocytic-type vesicles to join with lysosomes later, or remain in free solution before their lysosomal uptake, is not yet established[19].

Female Sprague-Dawley rats fed Purina Laboratory Chow or a sodium-deficient diet[26] for 60 days were injected, through the jugular vein, with 1 ml/100 g body weight of neutral red, vital (National Aniline Division, Allied Chemical and Dye Corporation, New York), or euchrysine 3R (E. Gurr Limited, London, England) in 0.9% NaCl at a concentration of 1.5% or 0.2%, respectively. These animals, and similarly fed noninjected controls, were anesthetized with ether 1 or 3 h after administration of the fluorochromes. The right kidney was rapidly quenched in liquid nitrogen-cooled freon, and directly embedded in paraffin in vacuo for fluorescence microscopy using a Schott BG-12/OG-1 filter combination[27]. The left kidney was perfused through the lower abdominal aorta for electron microscopy[28].

In the kidneys of the injected animals, neutral red emitted a weak brick-red fluorescence, and euchrysine a bright green to yellow to red fluorescence with increasing concentration[19]. In both cases, the fluorescence was distributed in discrete granules which filled the JGC. The

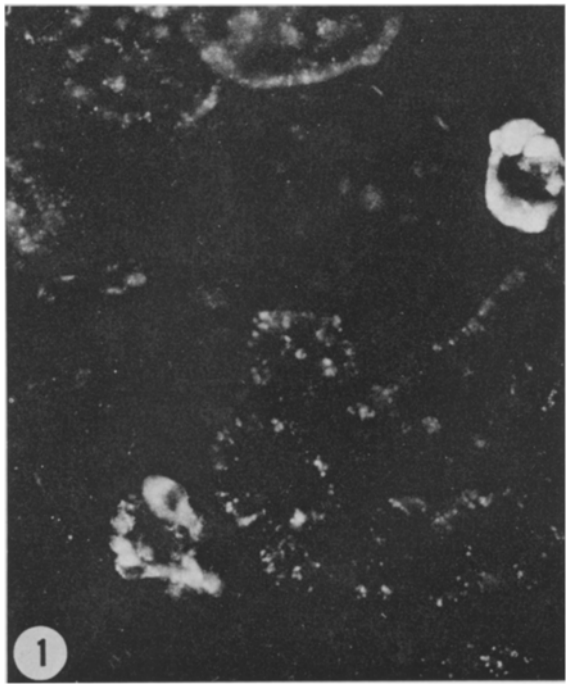


Fig. 1. Intense fluorescence of euchrysine 3R in 2 groups of juxtaglomerular cell granules (JGG), and discrete fluorescence in tubular cells, 1 h after injection. ×470.


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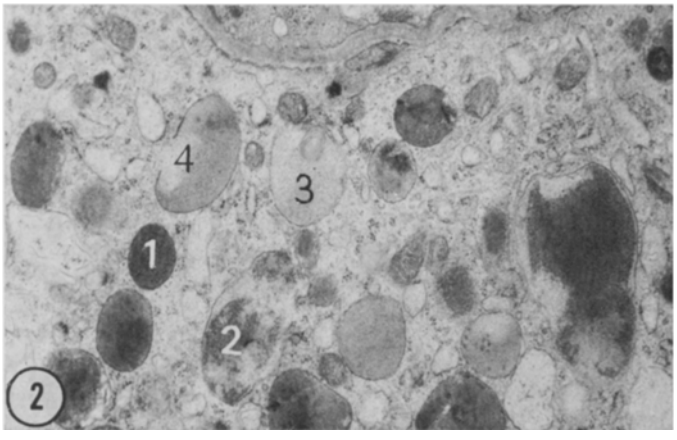


Fig. 2. Juxtaglomerular cell (JGC) of rat killed 1 h after injection of neutral red. Some granules (1) display a normal diameter, while others (2) are markedly swollen and show focal clarification of their matrix. One granule (3) contains lamellated structures; others (4) exhibit ruptured membranes. ×13,200.

latter were located in groups at the glomerular pole (Figure 1), or individually lined the walls of the juxtaglomerular arterioles. The fluorescence was much stronger 1 h than 3 h after administration of the fluorochromes. This was particularly evident with euchrysine: Several JGC fluoresced brightly in red after 1 h and mostly in yellow or green after 3 h. Administration of the other fluorochrome resulted in a faint yellow fluorescence in the nuclei and cytoplasm, indicating the presence of neutral red in nonionized form[29]. After injection of euchrysine or neutral red, the proximal and distal tubules contained fewer and less fluorescent granules than the JGC. Here again, the fluorescence was more marked after 1 than after 3 h. JGC filled with fluorescent granules were more numerous in the sodium-deficient than in the Purina-fed animals. This was due to the increase in the granularity and number of JGC induced by sodium-deficiency[14, 26]. In noninjected rats, the JGC were not autofluorescent though autofluorescent granules were present in cortical tubules.

Remarkable ultrastructural changes were observed in the JGG upon uptake of neutral red or euchrysine. The most striking alterations appeared at 1 h, which coincided with the maximum staining seen by fluorescence microscopy. One h after neutral red injection, practically all the granules showed a 2-to 5-fold increase in diameter. This was equivalent to a 8- to 125-fold increase in volume. The matrix displayed diffuse attenuation of density, and structures such as multiple concentric lamellas as well as irregular osmiophilic masses of near normal density. The limiting membrane was often intact; occasionally it was ruptured (Figure 2). 3 h after the injection, the changes were more general and uniform but slightly less intense. Analogous alterations were noted after administration of euchrysine: The granules were more uniformly swollen, and lamellar material was found less frequently in the matrix (Figure 3). The lysosomes of the proximal and distal tubules, as well as those of the cortical collecting ducts, were also involved. They were swollen, vacuolated and showed lamellar material in the matrix with irregular areas of matricial condensation.

It has been hypothesized that, because of their acid phosphatase content, JGG could have lysosomal properties[16]. The localization of neutral red in JGG, after parenteral injection, has already been described[30] and confirmed[7] at the light microscopic level. However, to the best of our knowledge, this finding has never been correlatde with the fact that neutral red, among others, selectively concentrates in the lysosomes of several cell types. JGG are not quite comparable to other renal lysosomes since they do not display autofluorescence. The nonoccurrence of such a phenomenon probably indicates that, contrary to lysosomes in several tissues, the JGG of rats do not possess significant amounts of vitamin A and lipofuscin pigment[31]. Even if further studies should reveal other biochemical dissimilarities between JGG and lysosomes, the present results suggest that they are functionally identical.

The possibilities raised by our findings may extend not only to obvious problems of physiology, pathology and therapeutics, related to the function of the juxtaglomerular complex, but also to other cellular structures. The presence of acid phosphatase has been demonstrated in mature and immature secretory granules of exocrine cells[32–34], in melanocytes[35] and in a number of endocrine structures such as the adrenal medulla[36], B-cells of the pancreas[37], hypothalamic neurosecretory cells[38], and the anterior lobe of the hypophysis[39]. The role of acid phosphatase in the specific granules of these cells remains a mystery. It has been suggested: 1. that acid phosphatase may be involved in secretory granule-condensation[32]; 2. that it may be 'carried over' as membrane-bound sites of activity in the secretory granules[38, 39]; and 3. that activation of acid phosphatase could constitute a mecha-

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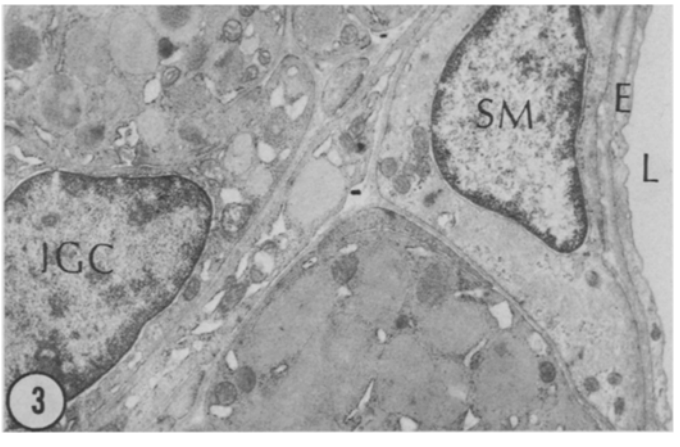


Fig. 3. Rat sacrificed 3 h after injection of euchrysine shows JGC separated from the arteriolar lumen (L) by endothelium (E) and a smooth muscle cell (SM). All the granules are uniformly swollen. Attenuation of the matrical density is evident. ×5,200.

nism for controlling and triggering the destruction of secretory material[39,40]. Extrapolation of the present results to other endocrine glands would mean that their specific granules share with lysosomes not only structural features but also functional properties.

*Résumé.* Le rouge neutre ou l'euchrysine 3R, deux substances fluorescentes qui se concentrent sélectivement dans les lysosomes de divers types de cellules, a été administré à des rats normaux ou à des rats en carence sodée. Comme l'ont démontré la microscopie de fluorescence et la microscopie électronique, les deux substances se sont accumulées dans les granules des cellules juxtaglomérulaires du rein.

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## Lung Inclusion Bodies: Different Ultrastructure in Simian and Non-Simian Mammals

The lamellated osmiophilic inclusion bodies (LOPBs) in the Type II cells of the lung are the source of pulmonary surfactant[1]. We report differences in structure and organellar origin between bodies from man and monkeys, and those from non-simian mammals.

We have studied the bodies in Bennett's Wallaby (*Protomnodon rufogrisea*), mouse, rat, hamster, guinea-pig, rabbit, cat, tree shrew (*Tupaia belangeri*), mouse lemur (*Microcebus murinus rufus*), squirrel monkey (*Saimiri sciurea*, a New World species), and rhesus monkey. We have also studied 2 specimens of human lung of normal appearance, 1 from a lobectomy and 1 from an accident case, and 4 specimens from infants dying of the respiratory distress syndrome of the newborn (RDS). We use glutaraldehyde and osmium fixation, post-fixation with lead ferricyanide solution with the anion 5% in

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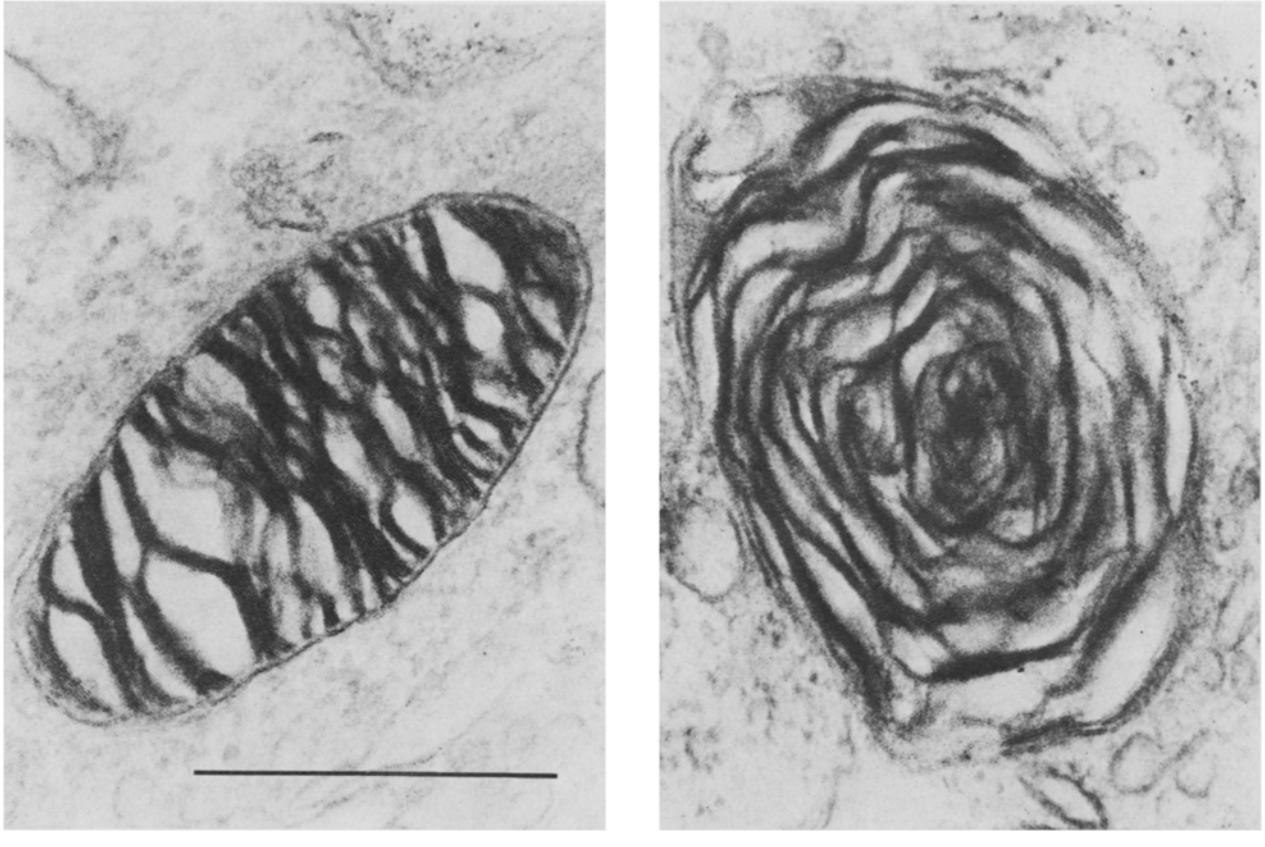

Left. Cross-barred lamellated osmiophilic body from mouse lung. Glutaraldehyde – osmium – lead ferricyanide fixation, acetone-Araldite embedding. Scale bar 500 nm. Right. Concentric LOPB from lung of squirrel monkey. Same preparation method and scale.