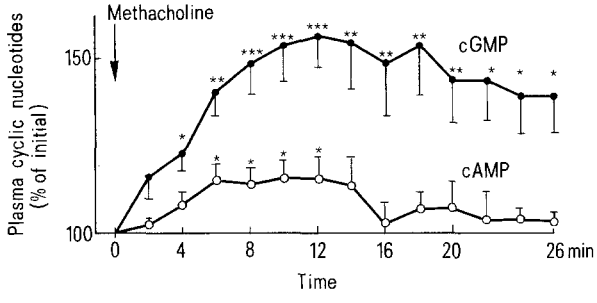
While there are previous reports of increases in tissue and plasma cyclic GMP in response to cholinergic agents in experimental animals[1-5], the present paper is the first report that methacholine administration resulted in increases in plasma cyclic nucleotides in man. Furthermore, we observed that methacholine induced an increase not only in plasma cyclic GMP but also in plasma cyclic AMP. This result in man agrees with the report of Honma and Ui for rats[5]. However, our observation is different from those of others. George et al.[1] found that in rat heart cholinergic agents left cyclic AMP concentration unchanged, or slightly decreased it, and Lee et al.[2] made similar observations. The cause of the difference between us and others is not clear.

It is impossible to clarify what mechanisms are involved in the regulation of plasma cyclic nucleotides in response to methacholine in man from our limited data, but it is likely that different mechanisms participate in the regulation of plasma cyclic GMP and cyclic AMP, because there were some differences between the patterns of increment of plasma cyclic GMP and cyclic AMP as shown in our study. It has been generally accepted that the levels of tissue and plasma cyclic GMP and cyclic AMP are under separate regulatory control. Cyclic AMP is considered to be closely related to beta-adrenergic receptors[8] and a recent observation, reported by Leveston et al.[9], that cholinergic stimulation releases endogenous cathecholamines in human plasma, may explain the observed increments in plasma cyclic AMP in our study. On the other hand, since the increases in tissue and plasma level of cyclic GMP in animals induced by cholinergic agents were antagonized by atropine, a muscarinic agent, cyclic GMP is considered to be involved in the action of cholinergic functions[1,2,5], which may explain the observed increments in plasma cyclic GMP in our study. Anyhow, further studies are required to determine what mechanisms are involved in the regulation of the response of plasma cyclic nucleotides to cholinergic agents in man.



Effect of methacholine on plasma cyclic GMP and cyclic AMP in normal subjects. 10 mg of methacholine was injected i.m. into 14 healthy adults at time-0 and increases in plasma cyclic GMP (●—●) and cyclic AMP (○—○) were plotted as a percentage of the initial value. The values shown are means ± SE. The initial values for cyclic GMP and cyclic AMP were 5.2 ± 0.3, 20.6 ± 1.1 pmoles/ml (mean ± SE) of plasma respectively. Significant increases in plasma cyclic nucleotides were observed $*p < 0.05$, $**p < 0.02$, $***p < 0.01$.

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Giant granules in adrenaline-secreting chromaffin cells of lizard adrenal glands after metyrapone administration[1]

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Summary. Secretory granules of extraordinary size, some of them bigger than the cell nucleus, abound in the adrenaline cells of lizard adrenals after metyrapone injections during 7 days. In these granules, the bounding membrane is studded with ribosomes, and the core is formed by rounded small subunits. Some granules of this type are also found in noradrenaline cells. They may represent an exceptionally increased elaboration and storage of adrenaline, induced by metyrapone probably through its action on steroidogenic tissue.

The association of chromaffin and steroidogenic tissues in the adrenal gland of vertebrates is not casual. In the lizard adrenal, which jointly contains adrenaline- and noradrenaline-producing chromaffin elements, only cells contiguous to interrenal - or cortical - tissue are of the adrenaline-secreting type[3]. So, this gland gives a morphological demonstration of the role that corticosteroids play in the formation of phenylethanolamine-N-methyl transferase (PNMT), the enzyme that allows the synthesis of adrenaline, starting from noradrenaline[4]. In turn, chromaffin tissue was shown to be able, at least in mammals, to perform various steps in steroid biosynthesis[5].

The present observations refer to ultrastructural changes produced in the chromaffin tissue of lizard adrenal by the effect of metyrapone, a substance that specifically interferes steroidogenesis, inhibiting 11β-hydroxylation[6] and, in certain conditions, the cleavage of cholesterol to pregnenolone[7,8]. The action of metyrapone on the structure of interrenal tissue and several other endocrine glands of the same species of lizard has been already studied[9-12].

Material and methods. 8 adult male specimens of the teiid lizard *Cnemidophorus lemniscatus* (L.), with snout-vent lengths between 7 and 8 cm, were captured in July and maintained in natural photoperiod and temperature. 4 animals received daily i.p. injections of 2.5 mg of metyrapone (metopirone bitartrate CIBA), 1% in aqueous solution, during 7 days. The remaining lizards were given similar injections, but of saline solution, and served as controls.

On the 8th day, after anesthesia of the animals with ether, the adrenal glands were quickly removed, and fixed in cold 3% glutaraldehyde, buffered at pH 7.2 with sodium phosphate 0.1 M. The material was post-fixed in 1% osmium tetroxide equally buffered, dehydrated in acetone, and embedded in an epon-araldite mixture. Thin sections, stained with uranyl acetate and lead citrate, were examined through a JEM-7A electron microscope, with an accelerating voltage of 80 kV.

Observations and discussion. Ultrastructural features of adrenaline and noradrenaline cells in control lizards correspond to those previously described for the same species[3] and, in general, for squamate reptiles[13–17]. Glutaraldehyde fixation allows a distinction between adrenaline- and noradrenaline-storing granules[18,19]. As adrenaline diffuses in the fixative, only the protein basis of the granules remains; these then appear finely granular, with moderate electron-density. On the other hand, noradrenaline precipitates in situ, affording a great opacity to the granules, often with a patchy pattern. Moreover, adrenaline granules are evenly rounded; their diameter is in general lesser than 300 nm, and rarely exceeds 400 nm (figure 1, a). Noradrenaline granules are more densely packed and frequently exhibit an ovoid or irregular shape; large granules are more numerous (figure 2, a).

In metyrapone-treated lizards, many granules of diverse size, but much larger than the usual ones, appear in adrenaline cells (figure 1, b–d). Some of them are bigger than the cell nucleus, the bounding membrane measuring more than 8 µm in diameter, and the core 4 µm. These granules have uniform moderate density, like those of the adrenaline type, but clearly show a macromolecular organ-

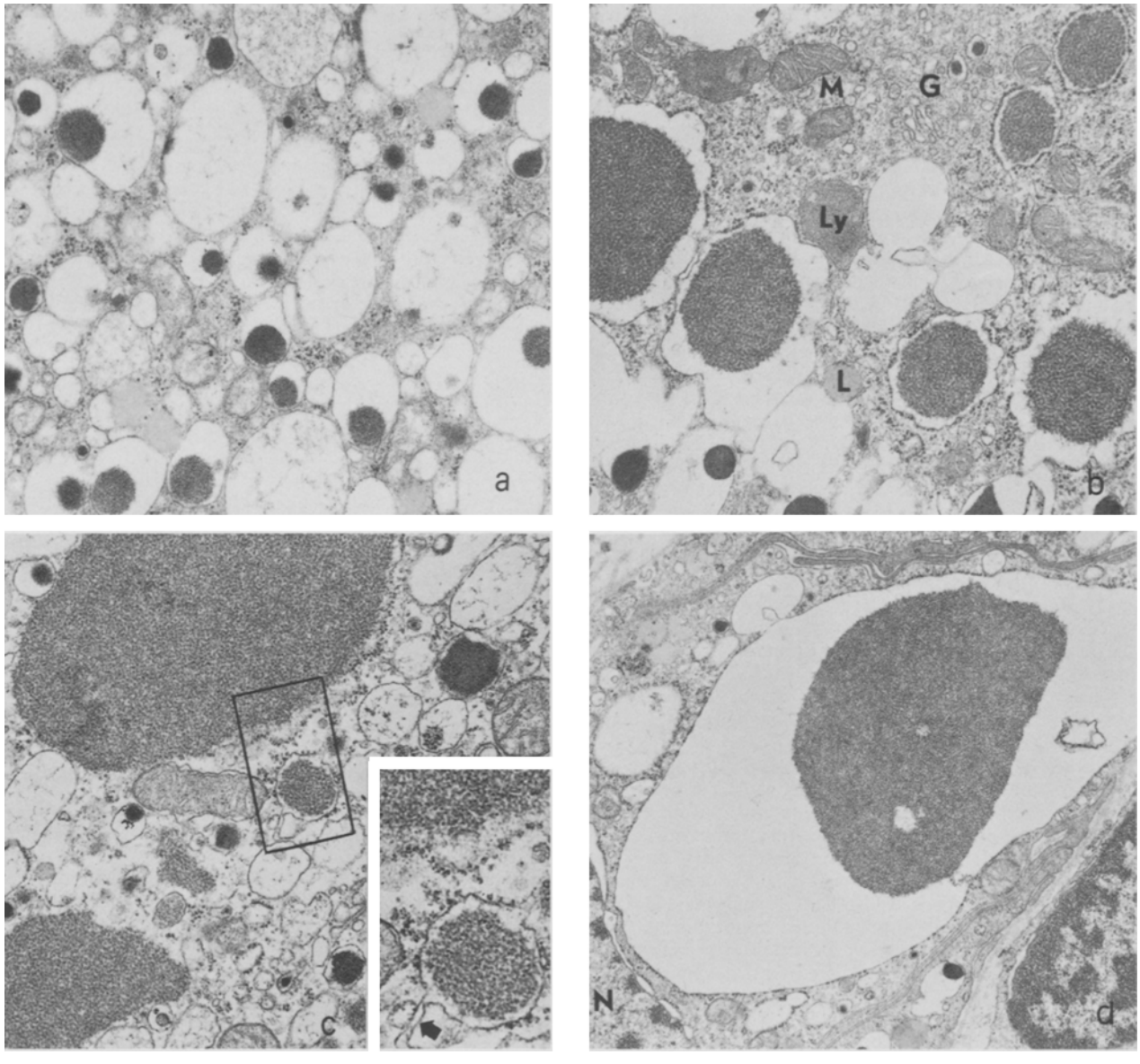

Fig. 1. *a* Adrenaline cell of a control lizard with normal secretory granules. × 27,000. *b–d* Adrenaline cells of metyrapone-treated lizards containing numerous giant granules of diverse size. *b* 6 giant granules appear together with other, normal ones. G, Golgi apparatus with new formed granules;.L, lipid droplet; Ly, lysosome; M, mitochondria. × 27,000. *c* The boxed area is more magnified in the inset, showing that the cores of giant granules are formed by small subunits, and that the membrane, studded with ribosomes, is continuous with tubules of endoplasmic reticulum (arrow). × 27,000; inset × 54,000. *d* A giant granule, exceeding the cell nucleus in size, occupies a great part of the cytoplasm. × 11,750.

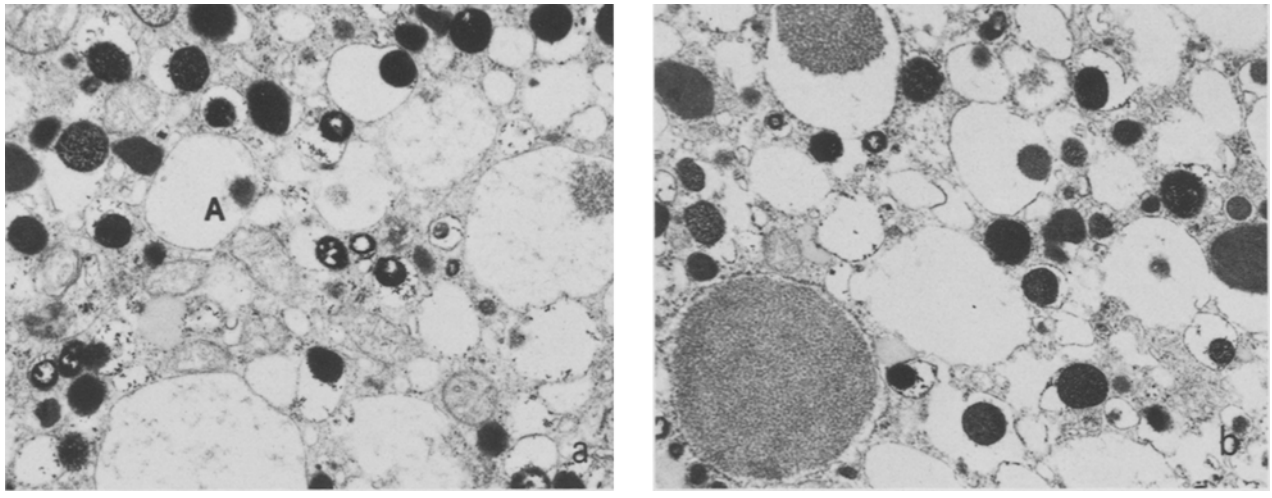

Fig. 2. *a* Noradrenaline cell of a control lizard, with densely packed secretory granules of great opacity, often exhibiting a patchy pattern. Among them, one granule seems to be of the adrenaline type (A). × 23,500. *b* Noradrenaline cell of a metyrapone-treated lizard. 2 giant granules, similar to those of adrenaline cells, appear among its normal ones. × 23,500.

ization: rounded subunits which seem to be linked by bridges, with diameters varying between 7 and 10 nm, form the cores. Such subunits are frequently observed in noradrenaline granules, but only in certain conditions can they be recognised in those storing adrenaline[20]. Another difference from normal is that the membranes of the giant granules are studded with numerous ribosomes. Connections between these membranes and tubules of endoplasmic reticulum occur (figure 1, c), which are not exclusive of these probably intracisternal granules, since they also exist in normal ones[24]. Ribosomes showing an unusual adherence to the membranes of secretory granules have been observed in other cells subjected to extraordinary stimuli, such as pituitary corticotrophs in metyrapone-treated lizards[21], in which subunits also become visible in the core. The presence of ribosomes may be an expedient for the quicker elaboration of substances that is required. If it is dubious that ribosomes participate in the synthesis of catecholamines, they are certainly needed in the formation of the protein basis of adrenaline granules.

Some abnormal granules of this kind are also found in noradrenaline cells of metyrapone-treated lizards, though with lesser frequency (figure 2, b). Probably they are the equivalent of the few normal granules of the adrenaline type that usually occur in noradrenaline cells of untreated lizards[3] (figure 2, a).

The cores of giant granules, like those of adrenaline granules with the method employed, only show a structure that corresponds to a mainly proteinaceous substratum. So, the presence therein of the precursor amines, either noradrenaline or dopamine, which produce dense precipitates[17] can be precluded. It is probable that giant granules contain adrenaline, but there is no direct evidence of it. Though large granules with ribosome-studded membranes are frequent in cells with enhanced production of secretory substances, they might also result from a disturbance in the processes of synthesis, transport or release. The assumption that giant granules contain adrenaline, and are the morphological expression of an exceptionally increased elaboration and storage of this hormone, is supported by the finding, in rats treated with metyrapone[22], of a highly augmented blood concentration and urinary excretion of adrenaline, accompanied by a decline in noradrenaline excretion.

Whether metyrapone is able to act directly upon chromaffin cells is not known. If the effect is exerted through the steroidogenic tissue, the mediators cannot be glucocorticoids, whose production is blocked by the drug. Though 11-deoxycorticosterone has been found to be without effect on PNMT[23], it is possible that other 11-deoxy compounds, or even diverse precursors which are secreted in much greater amounts as a consequence of corticotrophic feed-back reaction[6,8], may be responsible for an increased activity of that enzyme in chromaffin cells.

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