

A SUGGESTIVE RELATIONSHIP OF NERVE CELL RNA WITH SPECIFIC SYNAPTIC SITES*

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In a previous publication we reported a chance finding of a few large masses of ergastoplasm (ribosome particles, associated with endoplasmic reticulum) in a position subjacent to large synaptic knobs on the soma and proximal dendrites of motoneurons in the monkey spinal cord.¹ Several interesting aspects of this relationship stimulated a continued watch for further examples, with the thought that the association might not have been a fortuitous one. In a large number of sections of monkey and chimpanzee spinal cords examined with the electron microscope, numerous examples were found, suggesting that the association was common and of probable functional significance.

Materials and Methods.—In connection with studies of certain pathological responses in the spinal cord of monkeys (*Macaca irus philippinensis*) and of chimpanzees (*Pan troglodytes*), material from three normal monkeys and two normal chimpanzees were systematically studied. In both materials parts of many motoneuron somata, and a correspondingly larger number of sections of dendrites, were examined in electron micrographs.

Animals were anesthetized and perfused with 4 per cent formaldehyde as previously described,² without preliminary washing out of blood. The interval between opening of the chest for cannulation of the aorta and free return of formalin solution from the right atrium was usually about 1 min. Most recently prepared animals were perfused with 4 per cent formaldehyde in Millonig's phosphate buffer at pH 6.8, and at room temperature, with better results.

After perfusion of fixative for 5 min the spinal cord was removed and briefly stored in fixative while preparations for dissection were made. The cord was invariably firm and free of visible blood, and after removal of dura mater, could be transected with a sharp razor blade without compression, so as to reveal a smooth, undistorted cut surface. Under the dissecting microscope, gray and white matter were clearly defined on the cut surface, and anterior horn could easily be removed from a transversely cut slab of about 1–2 mm thick, with sharp razor blade.

Fragments of anterior gray column were immediately postfixed in 1 per cent osmium tetroxide in Millonig's phosphate buffer at pH 7.0 for a period of 2 hr at 4°C. Tissue fragments were then dehydrated and embedded in Araldite in the usual manner.

Sections were prepared with the Porter-Blum microtome for light microscopy at 2 μ , and thin sections were mounted on grids for electron microscopy in the RCA model EMU-3F.

Characteristics of the Ribosomal Subsynaptic Association.—The regular features of the association of ergastoplasm with synaptic knobs were the uniformity of characteristics of the synaptic knobs involved, and the position of the postjunctional ergastoplasm directly subjacent to the zone of synaptic contact. The boutons were in the range of the largest to be found on motoneurons (3–6 μ in diameter),

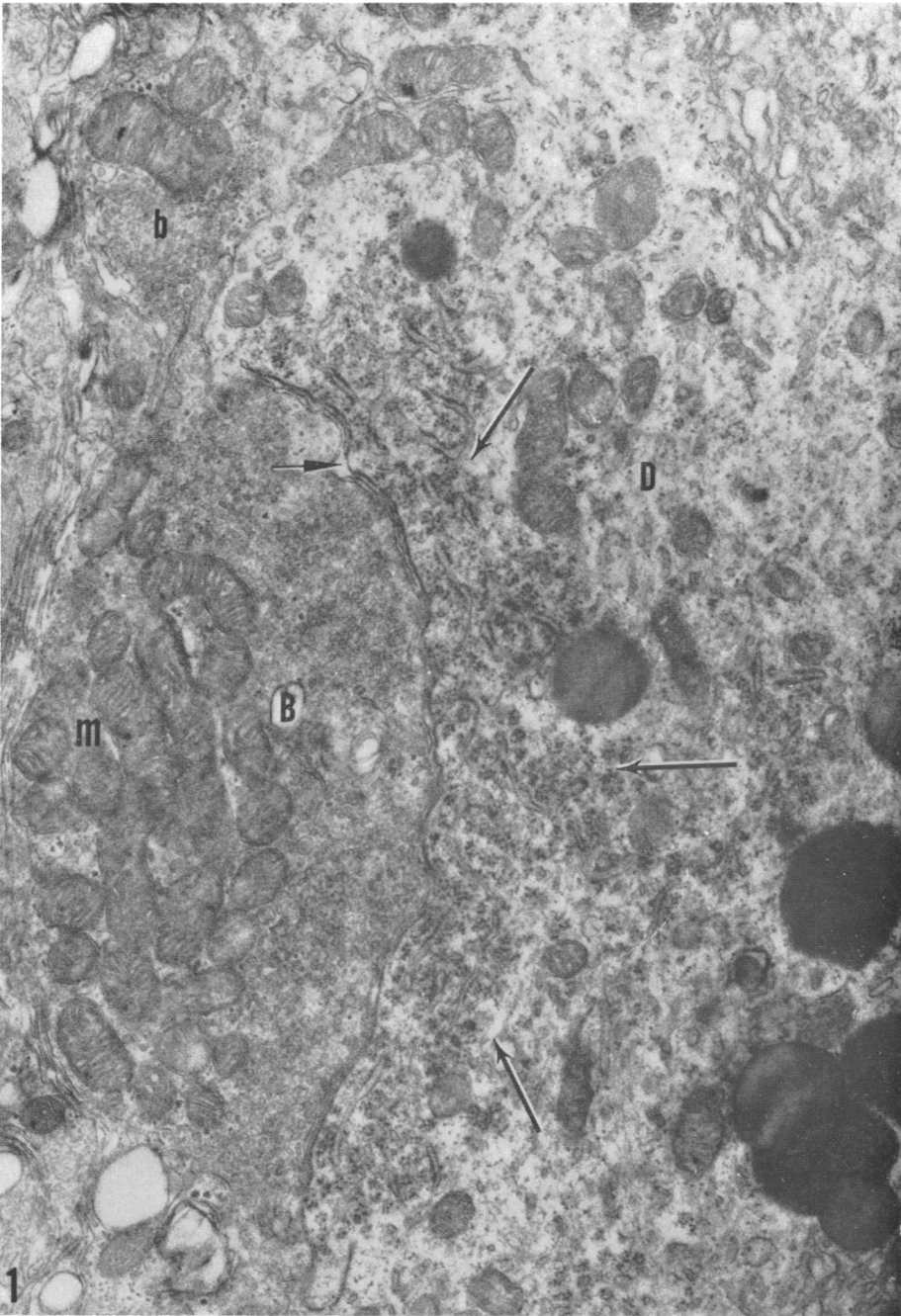


FIG. 1.—Synaptic contact in spinal motoneuron of a chimpanzee. Large dendrite (*D*) shows aggregate of ribosomes and endoplasmic reticulum (arrows) directly underlying a large synaptic knob (*B*). Adjacent small synaptic knob (*b*) does not show such an association. Next to the postsynaptic membrane is an elongated cistern (short arrow). The dark masses at the lower right are lipofuscin granules. *m* = mitochondrial cluster in large synaptic knob. $\times 28,000$.

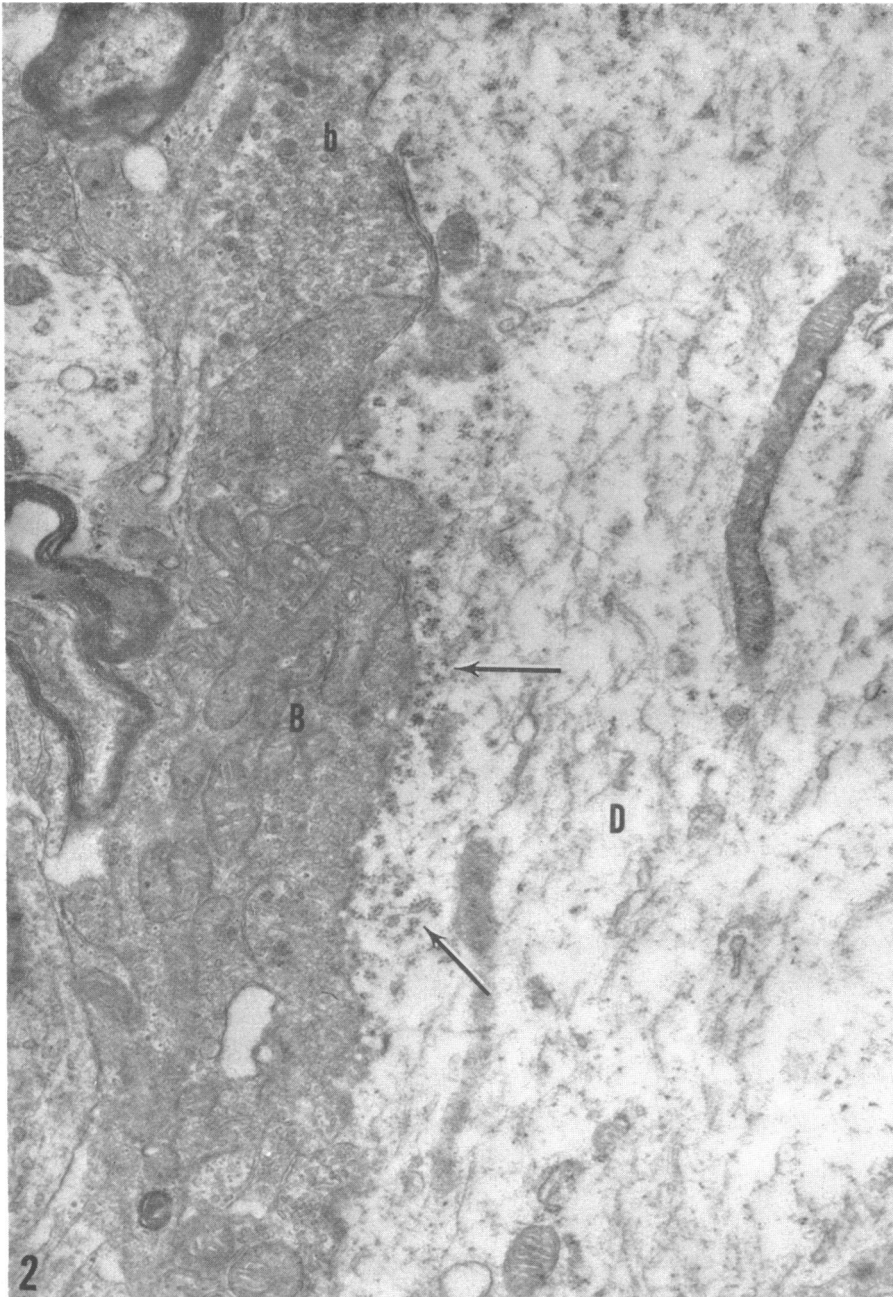


FIG. 2.—Synaptic contact in spinal motoneuron of a chimpanzee. Large dendrite (*D*) shows ribosomal aggregates (arrows) underlying a large synaptic knob (*B*). An adjacent small synaptic knob (*b*) shows much less postsynaptic ribosomal clusters, and less dense packing of small agranular vesicles and larger granular vesicles. $\times 25,000$.



FIG. 3.—Synaptic contact in spinal motoneuron of a chimpanzee. Large dendrite (*D*) shows ribosomal aggregates (arrows) underlying large synaptic knob (*B*) but not adjacent small synaptic knob (*b*). $\times 30,000$.

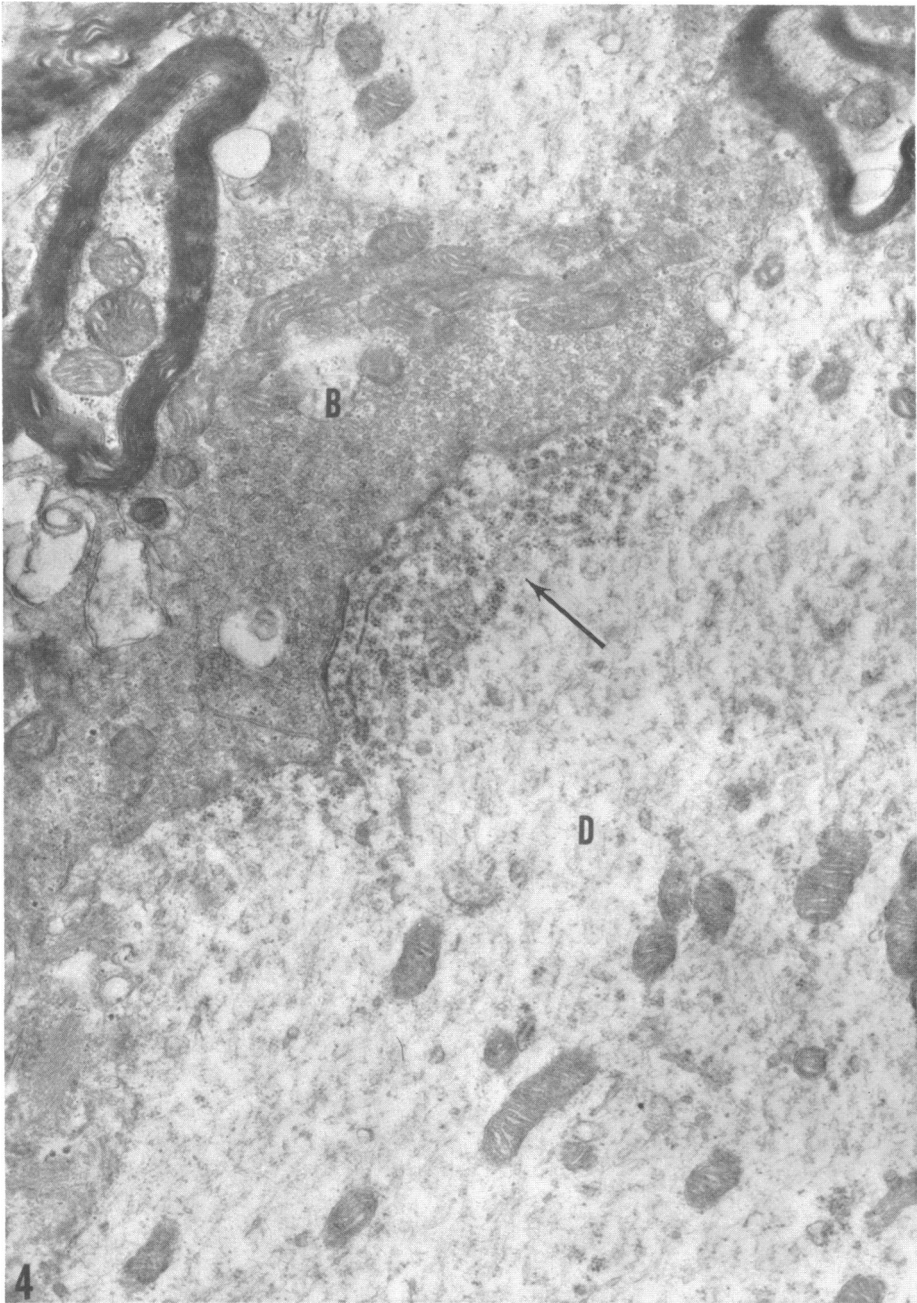


FIG. 4.—Synaptic contact in spinal motoneuron of a chimpanzee. Large dendrite (*D*) shows large aggregate of ribosomes (arrow) directly beneath a very large synaptic knob (*B*). $\times 25,000$.

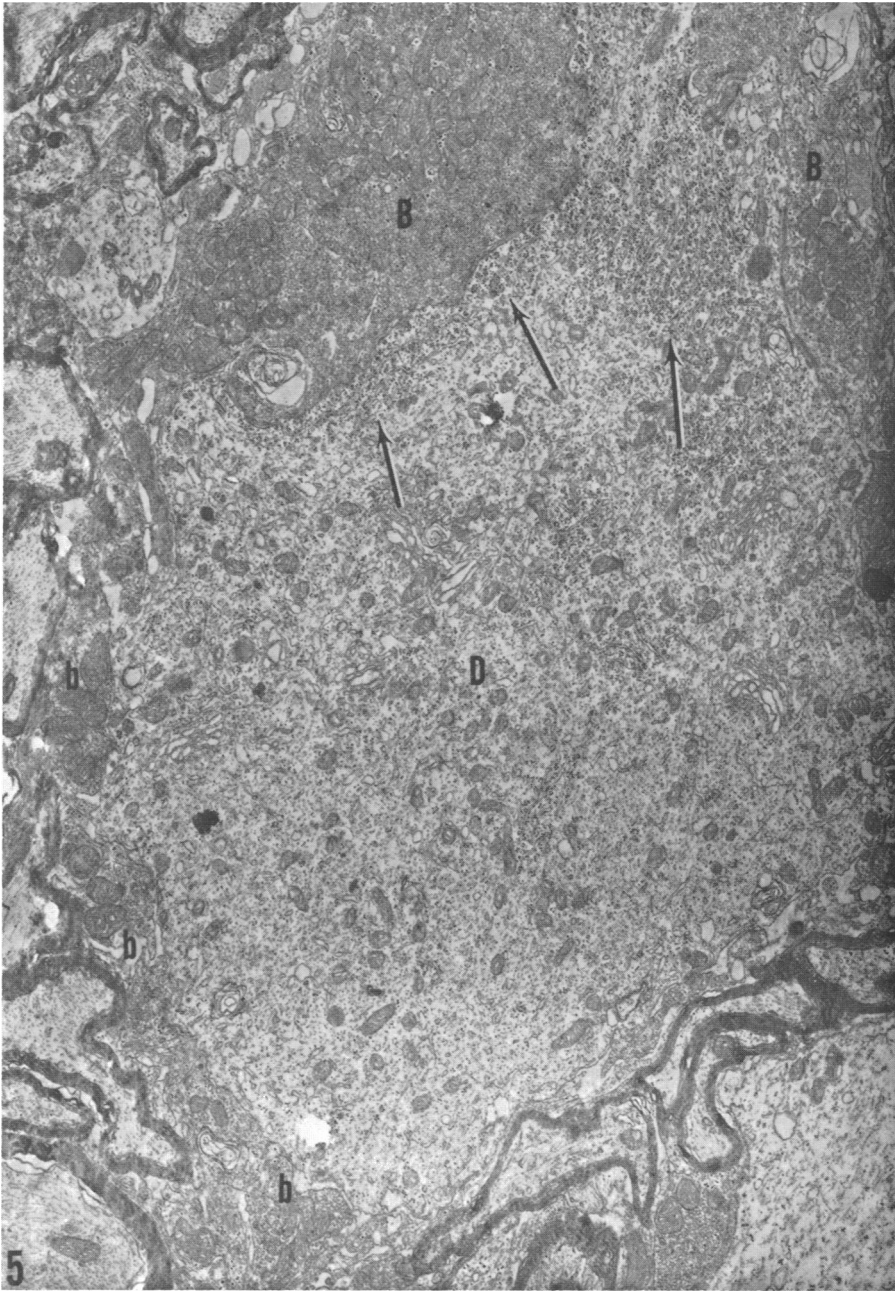


FIG. 5.—Synaptic contacts on large dendrite (*D*) of spinal motoneuron of a Philippine macaque. Large dendrite (*D*) bears several large synaptic knobs (*B*) in a region of postsynaptic concentration of ribosomal aggregate (arrows). Elsewhere on the dendrite surface, many small synaptic knobs (*b*) are seen, unassociated with postsynaptic ergastoplasm. $\times 11,000$.

were on proximal dendrites and on somata but not on distal dendrites, and invariably showed a dense packing of synaptic vesicles and of mitochondria. Each of these two components, however, was often sharply segregated in different portions of the knob. Adjacent small synaptic knobs usually showed a contrasting picture of more loosely arranged agranular vesicles, with or without a number of granular vesicles, and with fewer mitochondria in evidence (Figs. 1-5).

Junctional membranes were not characterized by membrane densities or cleft filaments. On the postjunctional side, the postjunctional membrane is usually separated from a cistern which marks the boundary of the ribosomal-ER aggregate, with an interval equal to that of the synaptic cleft (Figs. 1, 3). The subsynaptic cistern has obvious resemblance to that described by Rosenblueth.³

Discussion.—The occasional close apposition of Nissl bodies to the neuronal plasma membrane is a familiar light-microscopic observation. This apposition, however, is not general but is usually restricted to the region of proximal dendrites of motoneurons and of certain other types. In fact, Nissl bodies tend to avoid the nuclear and plasma membranes of mature motoneurons. Our electron microscopic findings would therefore arouse no interest except for the fact that the position of the marginal Nissl bodies tends to coincide with the junctional territory of a single large bouton, and such large boutons are most often found on soma and proximal dendrites. Thus, the association seems to be of functional significance, rather than of chance occurrence.

Since cytoplasmic RNA is ordinarily associated with trophic rather than conducting properties of neurons, one's first impulse is to consider the possibility that the large boutons or their subjunctional territory require material of a proteid nature, synthesized by the underlying ergastoplasm. Since this possibility has not been considered previously, the nature of this material must be speculative until histochemical or subcellular fractionation studies can be directed at the question.

The variation in amount of subjunctional "Nissl" material shown in the examples offered in Figures 1-5 further suggests that variable functional demands are involved, and it seems reasonable to suppose that the subsurface cistern of Rosenblueth³ represents one extreme of a functional spectrum, with a large "Nissl body" representing the other. The metabolic requirement which leads to this association must also be placed in perspective with the curious but unexplained fact that the specific ribosome-ER aggregates of large neurons (Nissl bodies) are not only found within a limited distance from the cell nucleus, but are sharply excluded from axon hillock regions, and most subsynaptic areas, as well as from the distal reaches of axons and dendrites. Yet, although the Nissl body as a cytoplasmic organelle may be restricted in location, the need for its product at all synaptic sites could, of course, be satisfied by transport over intervening cytoplasmic territory. If this were true, the instances of a special degree of association of Nissl material and synaptic junctions would still represent a special degree of need for products of protein synthesis, and would thus indicate a specific functional type of synapse.

Moreover, the suggestion is implicit that postjunctional synthetic processes involving RNA may be required for the maintenance or function of impinging boutons in general. It is interesting, for example, that in embryonic motoneurons of monkeys (80 mm CR length) the ergastoplasm is almost wholly concentrated at the plasma membrane of the cell body, at a time when impinging boutons are be-

ginning to establish functional contact. In view of the fact that the only known nerve growth factor is of protein nature and is highly specific,⁴ one may speculate that selective establishment of synaptic contacts may be determined by specific proteins synthesized at the synaptic membrane of the receptive neuron. Following this line of reasoning, one can further visualize the possibility that the existence of subsynaptic ergastoplasm in the adult indicates a continued role of the adult neuron in adaptive adjustments of synapses, such as the formation of new contacts.

Summary.—In electron micrographs of the anterior gray column of the spinal cord of monkeys and of two juvenile chimpanzees, aggregates of ribosomes and endoplasmic reticulum, corresponding to ergastoplasm, were frequently noted to be in especially close apposition to the cell membrane in the region of proximal dendrites. This apposition did not appear to be random, but rather associated with overlying synaptic boutons of definable characteristics, namely, large size (3–6 μ), and densely packed and segregated masses of mitochondria and of “synaptic vesicles.” Variation in amount of ribosomal material to be seen in subsynaptic relation to large boutons extended from large “Nissl bodies” to a subsynaptic cistern associated with a small scatter of ribosomes. It is possible that the subsurface cistern of Rosenblueth³ represents one extreme of a functional series of stages. Since ribosomal aggregates tend to avoid the cell and nuclear membranes in normal circumstances, the possibility is suggested that the large boutons on soma or proximal dendrites either possess special metabolic requirements associated with post-junctional protein synthesis, or perhaps a quantitatively greater need than other junctional regions on the adult motoneuron receptive surface. The possible role of subsynaptic ergastoplasm in the developing neuron is also discussed.

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¹ Bodian, D., *Bull. Johns Hopkins Hosp.*, 114, 13–119 (1964).

² Bodian, D., and N. Taylor, *Science*, 139, 330–332 (1963).

³ Rosenblueth, J., *J. Cell Biol.*, 13, 405–421 (1962).

⁴ Cohen, S., these PROCEEDINGS, 46, 302–311 (1960).

STAINING PROPERTIES OF LANTHANUM ON CELL MEMBRANES*

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Baker¹ and Palay² have suggested that Ca^{++} helps to stabilize the structure of membranes during fixation. Afzelius³ also has suggested that the replacement of K^+ by Ca^{++} in the permanganate salt of Luft's fixative improves membrane fixation.

Recent experiments by Lettvin, Pickard, Moore, and Takata have shown that La^{+++} acts in the peripheral nervous system like a “super- Ca^{++} .”⁴ Furthermore, La^{+++} has an electron scattering power high enough to produce contrast in electron microscope images.⁵

The introduction of La^{+++} either prior to fixation (e.g., incubation in $\text{La}(\text{NO}_3)_3$)