THE NATURE OF THE NEGRI BODY

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The Negri body is characteristic of the street rabies virus infection. Although there have been a number of studies on the problem of whether the Negri body consists of a reactive product of the cell brought about by the rabies virus multiplication or contains virus particles, the final answer is still lacking. Recently, several papers concerning the morphology of the rabies virus have revealed

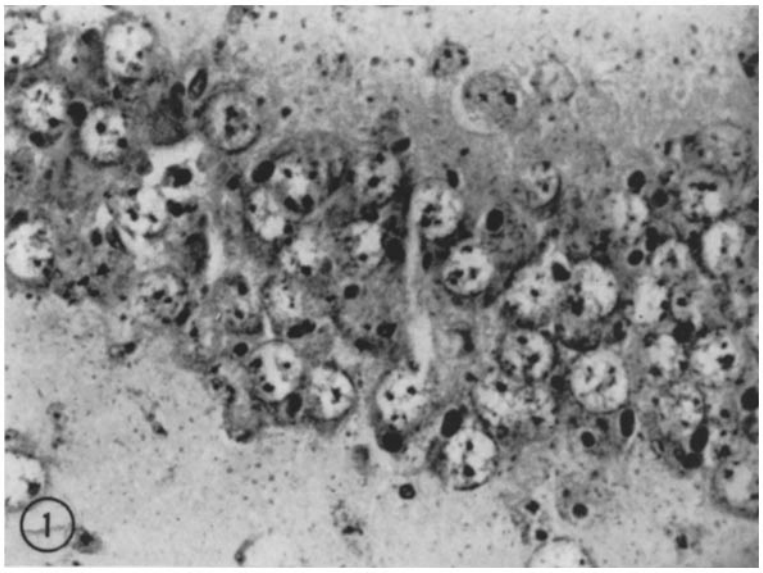

FIGURE 1 A light micrograph of Ammon's horn in the mouse hippocampus. Numerous Negri bodies varying in size are included within nearly all of the nerve cells. × 800.

that it has an elongated shape and a width of around 100 mμ. Its appearance is always associated with the same type of characteristic matrix which is composed of finely fibrillar, homogeneous material with a moderate electron opacity (1–6).

Since, according to these reports, the appearance of a characteristic matrix using fixed or street virus strains is quite common, it might appear that the matrix is not identical with the Negri body. However, in order to know definitely the nature of the Negri body seen in electron micrographs, it is necessary that it be exactly identified by the light microscope.

The Komatsukawa strain of the street rabies virus, kindly supplied by Dr. Sazawa at the National Institute of Animal Health, Tokyo, was used because of its high capacity for producing Negri bodies within neurons around Ammon's horn of the hippocampus. The specimens were obtained from brains of infant mice showing typical paralytic symptoms after intracerebral inoculation of virus suspension. Thick and thin sections of this infected nerve cell band of the hippocampus were prepared in turn so that the same inclusion bodies could be examined by light as well as by electron microscopy.

Fig. 1 is a light micrograph of Ammon's horn embedded in paraffin and stained by Massignani's procedure (7). Nearly all the neurons contain one or more bodies within their cytoplasm. The bodies are eosinophilic and sometimes encircled by a light halo. In order to compare the observations of Negri bodies by light and by electron microscopy, alternate thick (1.0 μ) and thin sections were cut from Epon-embedded blocks of the same brain.

As shown in Fig. 2, a map was constructed from light micrographs of thick sections stained with toluidine blue solution at pH 11.1 (8). The nerve cells on this map are discernible by the presence of Nissl bodies which appear in the form of dark masses and are located close to each other. Their nuclei are easily identifiable. A thin, smooth nuclear membrane surrounds moderately dark homogeneous nuclear sap. Many inclusion bodies of various sizes are found within nearly all the neurons, and some of them, especially the large ones, contain a few lumps of dark granules. Their homogeneous ground substance does not have the same affinity for the stain as does the nuclear sap. From this morphological evidence, inclusion bodies cannot be mistaken for nuclei.

Fig. 3 is an enlarged phase-contrast micrograph of the area enclosed by a rectangle in Fig. 2. In this figure the dark granules described above are most clearly demonstrated within inclusion bodies (arrows). The structure observed in Fig. 4, taken from an adjacent thin section, correlates

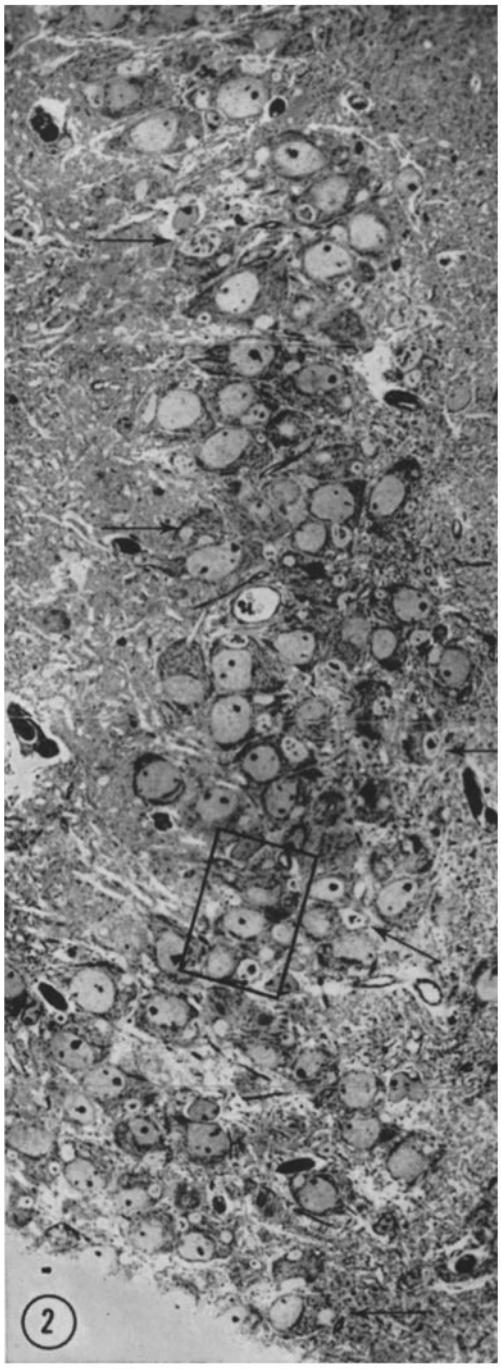

FIGURE 2 A light micrograph of a section of Epon-embedded hippocampus, after staining by toluidine blue. Nerve cells, most of which contain lightly stained inclusion bodies, are closely packed. Several representative inclusion bodies are indicated by arrows. Phase-contrast and electron micrographs of the rectangular area are shown in Figs. 3 and 4, respectively. × 350.

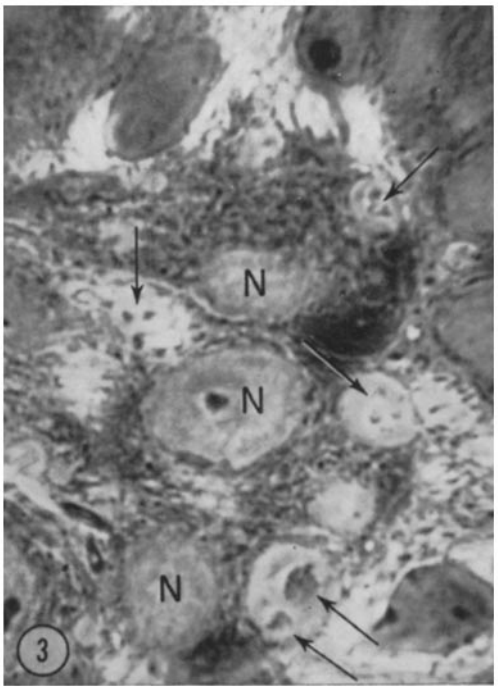

FIGURE 3 Several inclusion bodies contain a few lumps of dark granules (arrows). *N*, nucleus of nerve cell. × 1800.

well with that of Fig. 3. In Fig. 4 inclusion bodies can be readily identified as homogeneous aggregates which replace the cytoplasmic components at the periphery of nerve cells. Within or contiguous to these masses of inclusion bodies are found clumps of rabies virus particles within swollen vesicles of the endoplasmic reticulum. Dark granules shown in Fig. 3 (arrows) are identified in this electron micrograph (Fig. 4) as a clump of rabies virus particles in association with cytoplasmic constituents.

The area enclosed by a rectangle in Fig. 4 is shown at higher magnification in Fig. 5. Viruses projecting like rods into the lumen of the endoplasmic reticulum are seen intermingled with ribosomal aggregates. The ground substance of the homogeneous masses in Fig. 4 is composed of finely fibrillar, moderately electron-opaque material; this morphological feature corresponds exactly to that of the characteristic matrix described previously (1–6). Hence, the inclusion bodies seen in Figs. 2 and 3 may be regarded as identical with the matrix, and we may therefore use only the expression "matrix" in sections embedded in Epon.

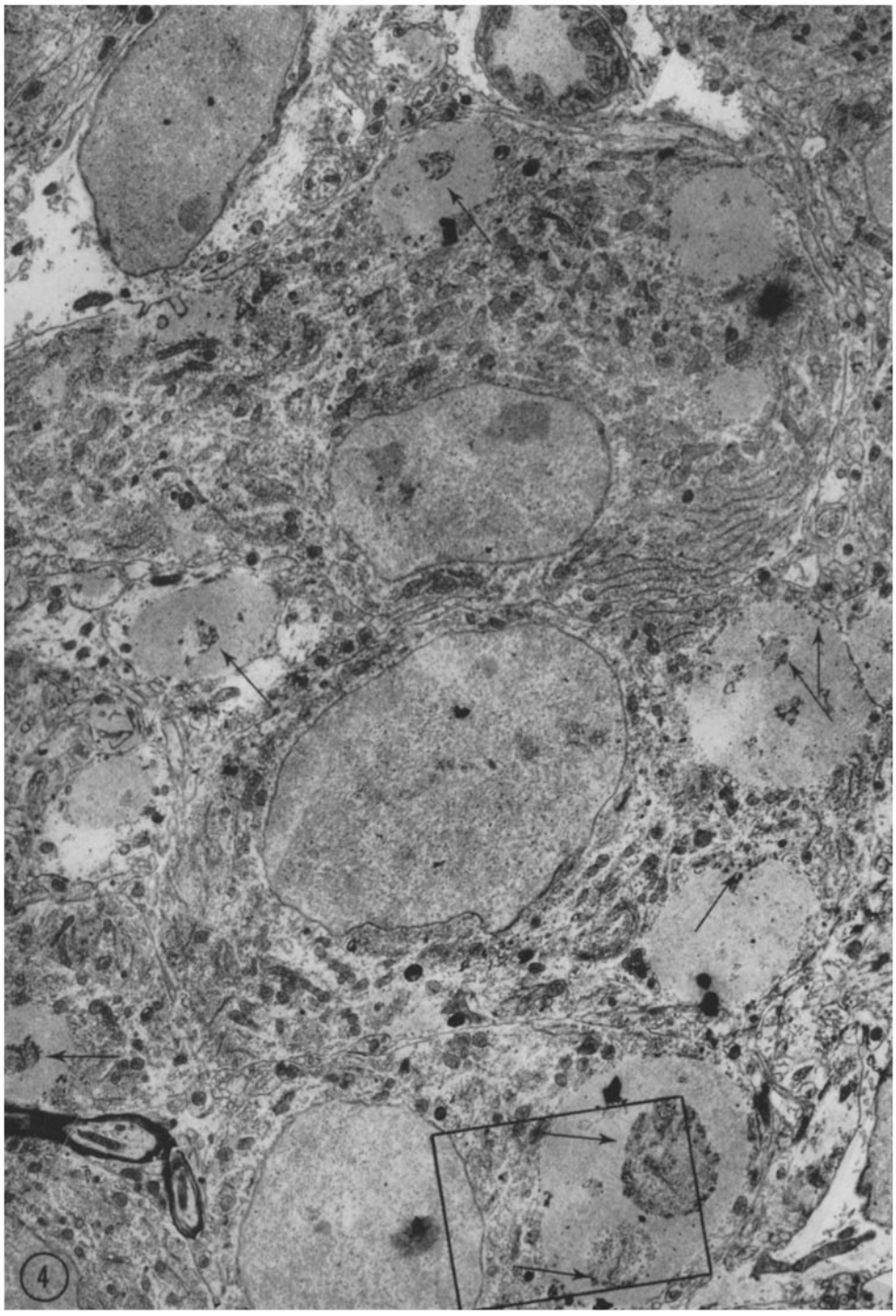

FIGURE 4 A considerable number of matrices are visible in the same relation to inclusion bodies as in Fig. 3. Elongated virus particles are found within and contiguous to the matrices (arrows). × 4500.

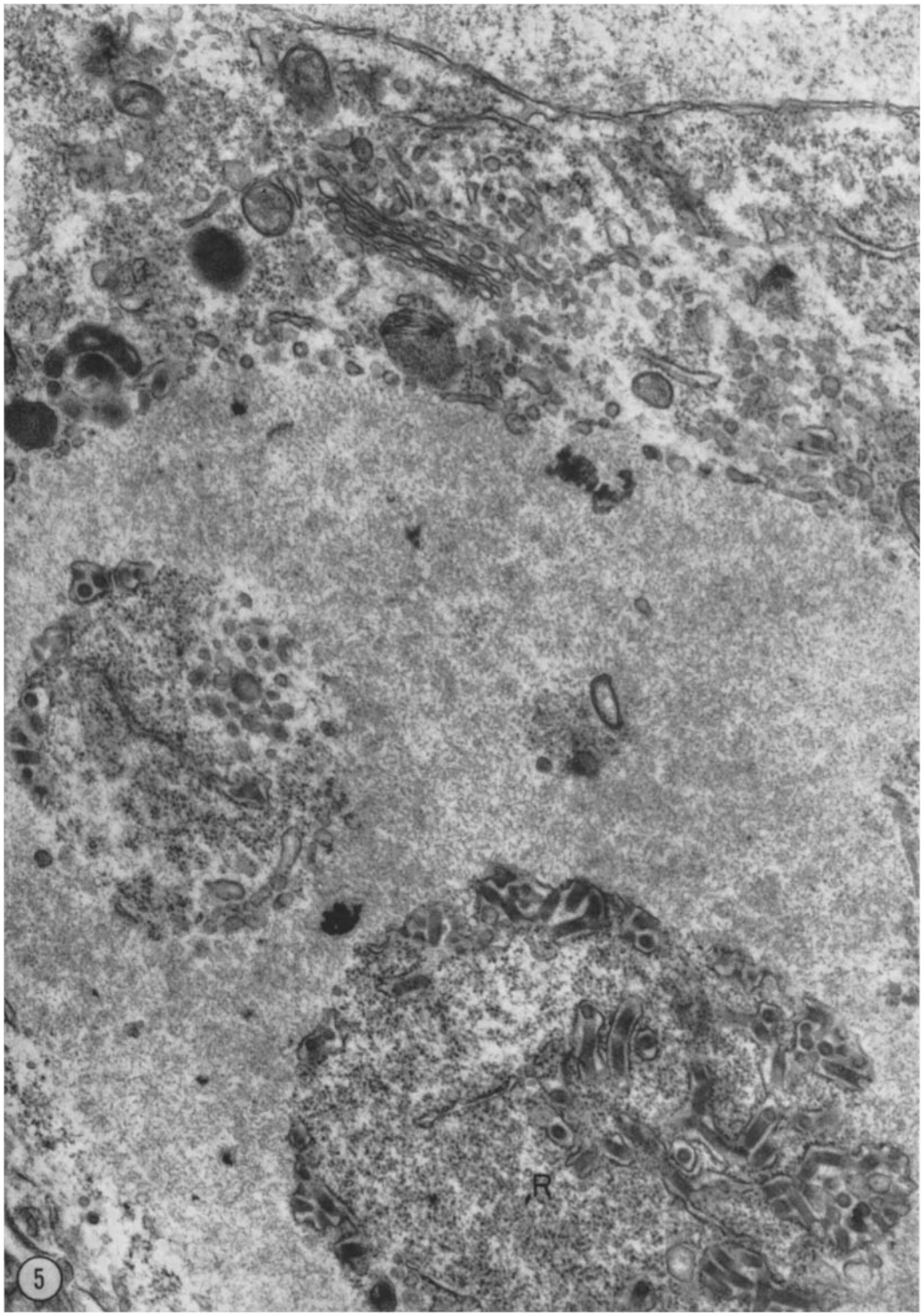

FIGURE 5 A higher magnification electron micrograph of two inner bodies enclosed within the rectangular area shown in Fig. 4. These inner bodies are composed of numerous elongated virus particles and cytoplasmic components. *R*, ribosomes. × 25,000.

Comparison of Epon- and paraffin-embedded tissues reveals that, apart from a large number of matrices in sections embedded in Epon (Fig. 2) and about an equal number of Negri bodies in sections embedded in paraffin (Fig. 1), no other pathological formation can be found within nerve cells in the hippocampus. Therefore, it becomes obvious that the cytoplasmic matrix seen in Epon-embedded tissue is morphologically identical with the Negri body. The present observations indicate that the Negri body is not a degenerative structure of infected cells, but is characteristically related to virus replication. Translucent halos covering Negri bodies are not seen in sections of Epon-embedded material, either by light or by electron microscopy. The halo, however, should be regarded only as an artifact which is formed during the course of fixation, especially by alcohol, because this halo is not usually demonstrated in paraffin-embedded tissues fixed by Zenker's solution. The matrices seen in Epon-embedded tissues cannot take eosinophilic stain by any of several conventional methods used for the Negri body. However, eosinophilic staining alone is not the most important criterion for identifying Negri bodies.

If the Negri body corresponds to the matrix, it might be expected that all rabies virus strains are to some extent capable of producing Negri bodies, because the matrix is consistently observed in cells undergoing infection by all rabies viruses, regardless of the presence of identifiable Negri bodies (1, 3, 5, 6). However, it is well known that Negri bodies almost always appear only after infection with street viruses and that even street viruses of certain strains do not produce the Negri body. With this evidence at hand we may conclude that the degree of "negrigenesis" depends upon the formation of numerous and extensive foci of virus synthesis. Furthermore, observations of consecutive thick and thin sections indicate that the so called inner bodies which are the important constituents of the Negri body are composed of virus particles associated with some amount of cellular constituents.

Oval and dense bodies which occasionally appeared in a mouse brain infected with a street rabies virus were presumed, in our previous paper, to be Negri bodies (5). A comparison between profiles of these bodies and those of the Negri body herein described makes this presumption unlikely, because no morphological relationship is seen between them. The bodies previously described are probably non-specific products from cellular components.

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