

CONTRIBUTION TO THE CULTIVATION OF THE  
PARASITE OF RABIES.\*

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PLATE 19.

Galtier<sup>1</sup> and Pasteur<sup>2</sup> demonstrated the infectiousness of rabies. Negri<sup>3</sup> next described the characteristic inclusions in the ganglion cells, and Babes,<sup>4</sup> J. Koch,<sup>5</sup> Volpino,<sup>6</sup> Proescher,<sup>7</sup> and others found minute granular or pleomorphic particles in the central nervous system of animals infected with rabies. Remlinger,<sup>8</sup> Poor and Steinhardt,<sup>9</sup> Bertarelli and Volpino<sup>10</sup> demonstrated the filterability of the rabic virus; but the nature of the virus itself had remained unknown.

In the early part of 1912 I undertook to cultivate the rabic virus and I have now obtained certain results regarding which a brief report is appended. About fifty series of cultivations were made with the brain or medulla removed aseptically from rabbits, guinea pigs, and dogs infected with "street" virus, "passage" virus, or "fixed" virus. Usually the animals were etherized just before spontaneous death occurred.

The method that has yielded the result to be reported is similar to that employed successfully for the cultivation of the spirochætæ

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<sup>1</sup> Galtier, *Compt. rend. Acad. d. sc.*, 1879, lxxxix, 444.

<sup>2</sup> Pasteur, L., Chamberland, and Roux, *Compt. rend. Acad. d. sc.*, 1881, xcii, 159.

<sup>3</sup> Negri, A., *Ztschr. f. Hyg.*, 1903, xliii, 507; xliv, 520; 1909, lxiii, 421.

<sup>4</sup> Babes, V., *Traité de la rage*, Paris, 1912; *Ztschr. f. Hyg.*, 1907, lvi, 435.

<sup>5</sup> Koch, J., *Ztschr. f. Hyg.*, 1910, lxvi, 443.

<sup>6</sup> Volpino, G., *Arch. per le sc. med.*, 1904, xxviii, 153.

<sup>7</sup> Proescher, F., *Berl. klin. Wchnschr.*, 1913, l, 633.

<sup>8</sup> Remlinger, M. P., *Ann. de l'Inst. Pasteur*, 1903, xvii, 834; 1904, xviii, 150.

<sup>9</sup> Poor, D. W., and Steinhardt, E., *Jour. Infect. Dis.*, 1913, xii, 202.

<sup>10</sup> Bertarelli, E., and Volpino, G., *Centrabl. f. Bakteriol., 1te Abt., Orig.*, 1904, xxxvii, 51; Bertarelli, E., *idem*, 1905, xxxix, 399.

of relapsing fever.<sup>11</sup> In the cultures very minute granular and somewhat coarser pleomorphic chromatoid bodies arise which on subsequent transplantation reappear in the new cultures through many generations. The same bodies, so far as appearances are concerned, are obtained from "street," "passage," and "fixed" virus. The smallest of these bodies are just on the limit of visibility with Zeiss apochromatic 2 millimeter lens. On four different occasions I observed in the cultures from "passage" and "fixed" virus nucleated round or oval bodies surrounded with membranes totally different from the minuter granular bodies although arising in the cultures in which the latter occurred. Their appearance was sudden and their duration four to five days, when they diminished coincident with an increase in granular bodies. In one of the four instances the larger bodies appeared in a culture prepared from the brain of a rabbit previously inoculated with a culture of "passage" virus which had given rise to the symptoms of rabies. In two of the instances the larger nucleated bodies arose in cultures prepared from "fixed" virus in which Negri bodies were difficult to demonstrate either in sections or in films.

The general morphological characters of the nucleated bodies are shown, magnified 1,100 times in the plate (figures 1 to 13). Along with them are also shown, for comparison, Negri bodies taken from the brain of dogs succumbing to "street" virus (figures 14 to 20). The cultivated nucleated bodies are actively multiplying, by division or budding, and exhibit the appearance not of bacteria but of protozoa. In size they range from about one micron to twelve micra. Under the dark-field microscope, as in the stained preparations, they show definite characters. The center is nuclear and the membrane is distinct and highly refractive. The united individuals in a multiplying mass may be enclosed for a time in a common capsule (?). By inoculating cultures containing the granular, pleomorphic, or nucleated bodies, rabies has been reproduced in dogs, rabbits, and guinea pigs, as shown by the typical symptoms and positive animal inoculations; while the film preparations from the brain of the animals contained always the granular and sometimes the nucleated bodies in large numbers.

<sup>11</sup> Noguchi, H., *Jour. Exper. Med.*, 1912, xvi, 199.

A fuller report on this subject is reserved for a later publication.

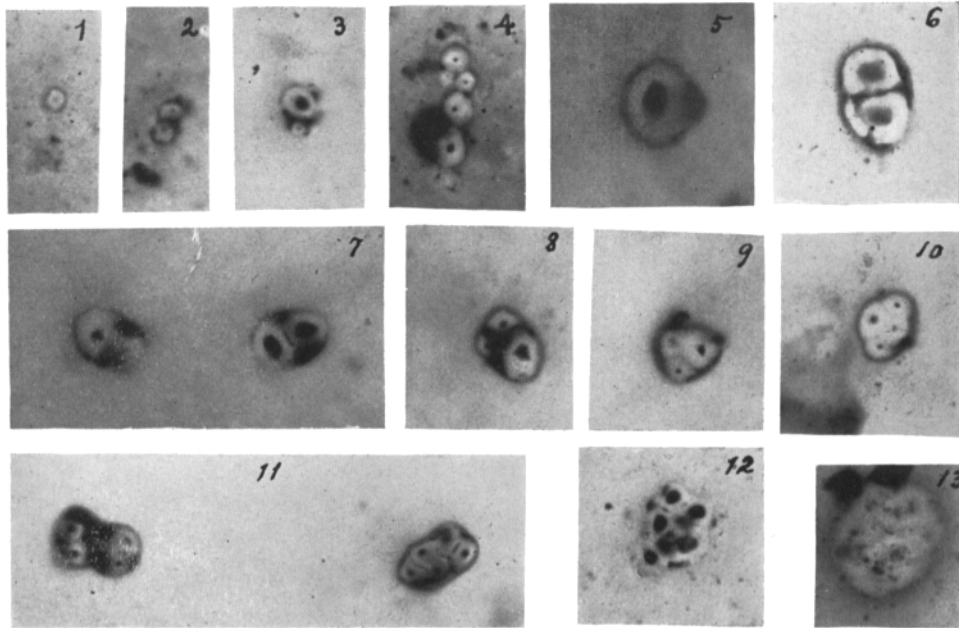
EXPLANATION OF PLATE 19.

All microphotographs were taken from the film preparation stained with Giemsa solution. Magnification uniformly  $\times 1,100$ .

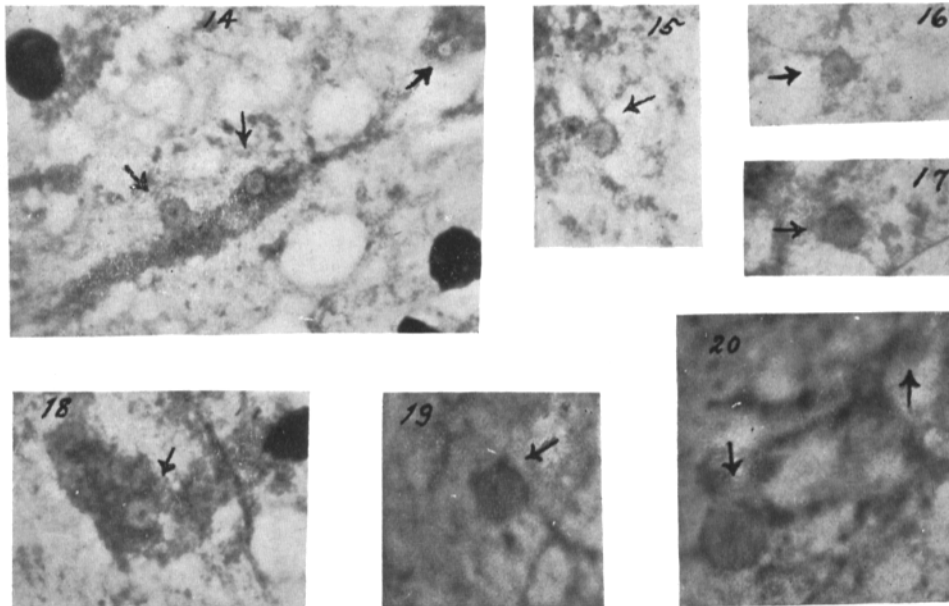
FIGS. 1 to 13. These represent the nucleated bodies of different stages of development in a culture (second and fourth generations, five days old) prepared from the brain of a rabbit experimentally infected with "fixed" virus. In the original material there were no such forms to be seen either in the films or in sections.

FIGS. 14 to 20. These show Negri bodies found in the films prepared from the brain of dogs dying of natural infection with "street" virus. The bodies possess central nuclear substances and distinct cell outlines (membranes). They are usually distributed singly.

A. Nucleated bodies in culture (Giemsa stain).



B. Negri bodies in the brain films (Giemsa stain).



(Noguchi: Cultivation of the Parasite of Rabies.)