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Globoid bodies and their occurrence in cultures.

WILLIAM H. HARRIS.

[From the Department of Pathology, Tulane University,
New Orleans, La.]

The term "globoid bodies" was first used by Flexner and Noguchi¹ in 1913 as a descriptive term for the minute spherical microorganisms cultivated by them from the filterable virus of poliomyelitis. Bashford and Wilson² likewise cultivated "globoid bodies" from lethargic encephalitis. Duval and I³ have recently described a "globoid microorganism" in cultures of the blood from Dengue fever.

The "globoid bodies" described by Flexner and Noguchi consist of minute spheres or "coccoïds" measuring from 0.15 to 0.3 microns in diameter and arranged in pairs, short chains, and masses. These bodies are best demonstrated tinctorially by the Giemsa method and are readily seen in dark-field preparations. Cultivation of the "globoids" has been obtained in the Smith-Noguchi tissue media. Growth has also been successful by the employment of Noguchi's Ringer-plasma medium devised for the cultivation of the *Leptospira*. The culture of the poliomyelitis globoids appears as an opalescent haze about the tissue, increasing for a period of five days, after which there occurs a gradual sedimentation. In our cultures of Dengue Globoids, either in the Smith-Noguchi media or Noguchi plain plasma mixture, visible growth was manifested by minute clear cut spherical or oval colony formation. These colonies were seen only after 2 or 3 weeks incubation, were densest at the bottom of the culture tube, gradually appearing smaller and more sparsely scattered as the upper portion of the medium was approached. No growth was visible for the upper inch just beneath the capping layer of paraffin oil. The colonies are comparatively larger below, measuring, however, only approximately 0.5 mm. and fading into pin-point size at the upper limit of the growth. No growth has been noted upon the surface of the medium. It is evident, therefore, that

¹ Flexner, Simon, and Noguchi, N., *J. Exp. Med.*, 1913, xviii, 461.

² Bradford, Bashford, and Wilson, *Brit. Med. J.*, 1919, i, 599.

³ Duval and Harris, *J. Exp. Med.*, 1924, xl, 835.

globoids are anaerobic in their cultural nature. After several generations we were unable to perpetuate growth of the Dengue Globoids.

The various cultures of globoids have occasioned, in the lower animal, the infection similar to that from which they had been previously obtained.

There are at present nearly fifty etiological agents designated as filterable viruses that are pathogenic for man and lower animals. For some of these viruses, microorganisms which are either easily seen, such as the leptospira, or seen with difficulty, as the "globoid bodies," have been brought to view either through culture on special media or through employment of the dark-field. Very recently in the work of Barnard and Gye⁴ upon cancer, the use of the special ultra violet ray microscope has visualized what they consider to be a very minute organism even of a smaller type than the "globoid bodies." In this connection the Twort-D'Herelle bacteriophage may also be mentioned as an almost intangible entity; however its animate nature is questioned.

"Globoid bodies" are very minute forms which are regarded by some as truly animate entities and by others as artefacts formed particularly in special protein media. Thus Twort and Twort,⁵ in work upon the etiology of influenza, undertook the cultivation of an ultramicroscopic agent in this disease. They obtained colonies along the stab puncture which were later believed by them to be crystallization processes of salts in solution put in action by some small point of particulate matter acting as a nucleus. Laidlow⁶ obtained somewhat similar colonies to Twort and Twort. He found that when these "cultures" were boiled for 5 minutes or autoclaved for 30 minutes that subplants yielded similar pseudo colonization, thus apparently demonstrating their inanimate nature. The examination of these pseudo colonies, when cut out from the media, showed under the microscope sphero-crystals in masses. The chemical examination demonstrated that these masses consist of crystallization of calcium and magnesium salts of fatty acids, and were thus really calcium and magnesium soaps. Laidlow believes that these soaps may remain dispersed in a complex colloidal system such as found in certain media, but that inoculation may furnish a focus of crystallization upon which

⁴ Barnard and Gye, *Lancet*, London, 1925, ii, 117.

⁵ Twort, F. W., and Twort, D. N., *J. of Hyg.*, 1921, xx, 85.

⁶ Laidlow, P. P., *Brit. J. Exp. Path.*, 1925, vi, 36.

crystals will accumulate and form a nucleus of a pseudo-micro-organismal colony. It is noteworthy that these authors do not describe any morphology suggestive of microorganisms in the smear preparation of these pseudo-colonies. They also state that this crystallization deposit can be kept indefinitely from subplant to subplant. On the other hand, the globoid bodies obtained by us in cultures from Dengue fever, exhibit a definite microscopic morphology for the units comprising the colonies. Unlike the crystalline pseudo-colonies, their growth could not be indefinitely perpetuated. In this connection Rosenow⁷ has stated that certain aerobic bacteria, such as streptococcus, may grow anaerobically as very minute forms. However, they will assume their normal basic morphology when planted upon suitable media under aerobic conditions. The Dengue globoids grow better in the deeper areas where anaerobiosis is more marked and they do not colonize in the upper level or on the surface where aerobes would grow more prolifically. Neither did growth occur on subplants under favorable aerobic conditions.

The inoculation into guinea pigs of cultures containing these globoidal microorganisms, obtained from the blood of patients infected with Dengue Fever, reproduced the febrile and leucocytic reactions present in this disease.

There exists certain artefacts which resemble the colonization observed for globoid bodies, but these are inanimate structures and can be differentiated from the living microorganismal forms.

We believe globoid bodies are minute living microorganisms of a much smaller size than the usual very small recognized forms. They have a definite structure consistent with true microorganisms. They can be grown in culture which when inoculated into the experimental animal reproduce the disease from which they were primarily isolated.

⁷ Rosenow, *J. Inf. Dis.*, 1918, **xxii**, 281.