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THE PATHOLOGY OF CERTAIN VIRUS DISEASES[1]

THE term "virus diseases," as used in this title, has reference to that group of acute infectious diseases whose etiological agents have not yet been cultivated with certainty on artificial media but have the common property of filterability through earthenware or porcelain filters.

Notwithstanding the fact that a very great number of infectious agents, both bacterial and protozoan, have been demonstrated and cultivated during the past fifty years, there remains a surprisingly large residuum of infections which may be included under the term "virus diseases."

There is a very wide distribution of the virus infections both within the animal and plant kingdoms. For example, among the diseases of man belonging to this group are such important examples as smallpox, poliomyelitis and rabies. The group may be represented among the lower animals by such instances as foot and mouth disease, rinderpest and hog cholera. In plants there is the mosaic disease of tobacco and numerous others. It is probable also that the bacteriophage of D'Herelle belongs in the same category. The biological importance of the filterable viruses may be further illustrated by the fact that certain tumor-like diseases are known to be caused by these agents, namely, Rous's chicken sarcoma and fowl leukemia.

It is of particular interest from the standpoint of pathological and cytological studies that the lesions of many virus diseases are associated with specific cellular inclusions, the nature of which has not as yet been determined with certainty. The inclusions may be situated either within the cytoplasm of the cells involved, within the nucleus or within both. As examples of intra-cytoplasmic inclusions may be mentioned those of rabies, smallpox, vaccinia, trachoma and molluscum contagiosum of man. In the fowl characteristic inclusions occur in epithelioma contagiosum. Inclusions confined within the nucleus have been demonstrated in herpes simplex, herpes zoster and varicella in man, and in the nuclei of cells of the central nervous system in the encephalitis of horses, known as Borna's disease.

These inclusions from time to time have been considered to be protozoan parasites, or the products of

[1] Read before Section N (Medical Sciences) American Association for the Advancement of Science, Nashville, Tennessee, December 28, 1927.

cellular degeneration or reaction products incorporating the virus. The last hypothesis was formulated several years ago by von Prowazek; and he designated the viruses inciting the specific cellular reactions Chlamydozoa. Lipschutz, a little later, included under the term Strongyloplasms the minute, uniform, granular components of inclusions which could be demonstrated in fresh preparations and in smears. He regarded these minute bodies as the visible form of the infectious agent. In this group he included the viruses of fowl-pox, molluscum contagiosum of man, sheep-pox, variola and vaccinia. These minute structures were demonstrated in smears from the lesions of fowl-pox and sheep-pox first by Borrel in 1904, in smears of molluscum contagiosum by Lipschutz in 1906, and about the same time in the lesions of variola and vaccinia by Paschen.

In the present study which I will report, our interest has been directed toward an investigation of the nature of the inclusions, particularly in the lesions of fowl-pox and molluscum contagiosum.

Fowl-pox is a contagious, eruptive disease which occurs often in epidemic form in chickens, pigeons, turkeys, geese, pheasants and other species of fowl. The lesions consist of a nodular exanthem which appears particularly on the unfeathered parts of the body, especially about the head. They represent essentially an epithelial hyperplasia. Only surfaces lined by squamous epithelium are affected, the lesions being strictly confined to the epidermis and to the mucosa of the mouth, nasal pharynx, the esophagus, crop and occasionally the cloaca. During the eruptive stage of the disease the virus is present in the blood and probably in all the organs of the body, although there are no demonstrable lesions in the internal organs. Within the eruptive lesions the virus is present in a very high concentration, and the disease may be induced by inoculating a susceptible fowl at any point on the skin, where epithelial cells have been injured by scarification or by plucking feathers. In early stages of the disease, while the virus is circulating in the blood, local lesions may be induced by simple scarification or the removal of feathers at any point. Notwithstanding its wide dissemination in the body it appears that this virus proliferates for the most part, if not entirely, within the eruptive lesions.

On microscopic examination the nodular lesions are found to be due essentially to an epithelial hyperplasia. Practically every epithelial cell of the lesion contains within its cytoplasm a large, discrete, compact body which possesses an eosinophilic staining reaction and has a hyalin appearance. These inclusions are larger and more numerous as the surface is approached. They may be absent in the cells of the Malpighian layer. It has been demonstrated that their formation begins near the basal layer by the condensation of an apparently amorphous material about one or more intracytoplasmic globules, or as they appear in fixed preparations, clear vacuoles. This material increases in abundance until it entirely covers or replaces the vacuole. Cells containing these inclusions are larger than normal, the mitochondria are intact, the nucleus shows no evidence of disintegration, and there is no indication that the cell thus altered is degenerating. Not infrequently one may find particles within the cytoplasm which appear to have been extruded from the nucleus. These particles do not take part in the formation of the specific inclusions. From a study of the cells in which the inclusions are developing we are convinced that the latter are not derived from preexisting material within the cell through degeneration nor through the formation of any constituent which the cell may produce during its differentiation. On the contrary, the inclusion appears to be constituted of a new and foreign material which accumulates in abundance within a vitally active cell.

In fresh preparations it is easily possible to tease out from fragments of a nodule numerous inclusion bodies, so that they are free from the cell. Such liberated bodies suspended in physiologic saline solution have the appearance of round, elliptical or lobulated, refractive, homogeneous, hyaline masses which are plastic and can be pressed or squeezed easily into various forms. When these masses are suspended in distilled water they swell rapidly, increasing greatly in size within a period of one half to one hour. As they imbibe water they assume a grossly granular appearance, later becoming transformed into a mulberry-like mass of globules suspended within a hyalin matrix. When the globules have reached a considerable size it can be seen by ordinary illumination under high magnification that they contain innumerable minute particles exhibiting an extremely rapid molecular motion. Sometimes the rapidly moving particles will stick to the periphery of a globule so that they may be photographed. The inclusions do not swell to the point of rupture, but remain definitely circumscribed. By compression, however, the minute bodies may be liberated and can be seen with ordinary illumination or in a dark-field preparation, as minute, spherical bodies having a diameter of about .25 micron. If physiological saline be added to the distilled water preparation the unruptured bodies will rapidly contract through the loss of water, becoming hyaline, wrinkled and apparently homogeneous.

By compressing an inclusion and staining it by a suitable method in a dry preparation it can be seen that it is composed essentially of two constituents; the one consisting of great numbers of minute,

spherical bodies uniform in size and staining reaction, the other a homogeneous, faintly staining, amorphous material in which the minute bodies are suspended. The staining method which we have devised for this purpose is as follows:

A smear preparation dried over a flame is mordanted one minute with a .25 per cent. aqueous solution of potassium permanganate. It is then rinsed in running water and stained one minute with a few drops of the following solution: Alcohol (30 per cent.), 100 cc.; basic fuchsin, 1 gram; phenol (crystals), 1 cc.; anilin oil, 1 cc.

The stain is washed off in running water, and the preparation, blotted and dried, is ready for examination. This method of staining is not attended by the formation of any precipitate and stains the minute bodies quite sharply.

Thus from a morphological standpoint it is possible to demonstrate that the specific inclusions of fowl-pox are formed within the cells by the accumulation of a foreign material within the cytoplasm unattended by evidences of degeneration, and that these specific inclusions are constituted of minute, uniform bodies in great numbers, suspended and surrounded by a hyaline matrix. Morphologically, therefore, it is possible to demonstrate structures which resemble a minute micro-organism as the important constituent of the inclusion. These minute structures are in numbers sufficient to account for an infectiousness of material from the lesion in high dilutions. The minute bodies are small enough apparently to pass through the pores of a Berkefeld filter. It would appear from the fact that minute bodies are surrounded by a homogeneous matrix that filtration experiments might be attended with difficulties, and this experimentally is found to be the case. The incorporation of the minute structures within a hyaline matrix, which seems to be of a semipermeable nature, and appears to have a lipoid and proteid structure, might protect an enclosed micro-organism against such physical changes as dehydration by drying or glycerination.

We have demonstrated that the minute bodies are preserved in dried or glycerinated preparations after periods of many months (six) by soaking such material for twenty-four hours in distilled water, making smears and staining as above indicated. This material was infectious.

It has further been possible to show, we believe, that active virus is associated with the inclusions. By bacterial putrefaction it has been possible to soften and disintegrate the epithelial cells of lesions, leaving the inclusions apparently intact. By repeated washings and centrifuging at low speed the inclusions have been freed in large part from adherent cellular material and bacteria. A suspension of these inclusions was centrifuged at high speed. The supernatant fluid inoculated upon the skin of a susceptible fowl proved to be non-infectious, while the sediment, consisting of inclusions, proved to be highly infectious when similarly inoculated upon the skin.

From these observations we are convinced that in fowl-pox we have to do with a specific infectious disease due to a living microorganism which is visible under the microscope. This microorganism, we believe, invades the epithelial cells of the lesion; and the daughter cells, resulting from the division of an infected cell, will carry the virus by direct transmission. The microorganism proliferates within the cytoplasm of infected cells resulting in the formation of minute colonies suspended in a hyaline lipo-protein material which constitutes the specific inclusion.

The epithelial inclusions of molluscum contagiosum of man are composed essentially of the same type of minute bodies which can be demonstrated in suitably stained smear preparations and in fresh preparations with ordinary illumination. In fresh preparations the minute bodies can be seen in rapid molecular motion in every way similar to that exhibited by the bodies within the globules of swollen fowl-pox inclusions. In molluscum, however, there is not so dense a matrix; consequently, the inclusions are more diffuse and, because of their corpuscular content, appear finely granular in stained sections of the lesion. In molluscum contagiosum it is believed that we have to do with an acute infectious disease similar to fowl-pox, and caused by a visible microorganism which penetrates and proliferates within the affected cells of the lesion. This conception has been advanced by Lipschutz and by da Rocha-Lima.

Through the studies of Borrel, Paschen and others it seems possible that a similar microorganism may be the etiological factor in the diseases sheep-pox, variola and vaccinia.

This group of virus diseases then offers, we believe, a particularly favorable material for further etiological investigations.

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ANALOGIES BETWEEN PHYSIOLOGICAL RHYTHMS AND THE RHYTHMICAL REACTIONS IN INORGANIC SYSTEMS

THE periodic or rhythmical reactions of inorganic chemistry are surface reactions, characteristic of poly-