THE PRODUCTION OF FOREIGN BODY GIANT CELLS IN VITRO.*

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PLATES 71–74.

In a recent paper,[1] the effect of the addition of foreign bodies to cultures of certain tissues of the chick embryo was briefly discussed. It is the object of the present communication to describe in some detail these phenomena, which relate chiefly to the formation of foreign body giant cells, with a discussion of their possible significance.

Technique.—For the preparation of the tissue cultures, Burrows'[2] original method has been employed. This consists in suspending teased fragments of tissue, 0.5 to one millimeter in diameter, in hanging drops of plasma, cover-glasses and relatively deep hollow ground slides being used for this purpose. The method of adding the foreign bodies, upon which a successful outcome largely depends, will be given in detail.

Lycopodium spores were selected for use as the foreign objects, their diameter, forty microns, rendering them especially suitable. That is, they were found to be too large to be engulfed by phagocytic cells, and yet sufficiently small to be easily surrounded by wandering cells. Furthermore, their characteristic architecture and refractivity made them easily recognizable even when embedded in a mass of cells. In the form of the commercial "yellow powder," they do not form readily a homogeneous suspension in water or watery solutions. This difficulty may, however, be easily overcome by first moistening the powder with alcohol and then adding water. The alcohol is subsequently removed by boiling. Small drops of

* Received for publication, March 14, 1912.

[1] R. A. Lambert, *Anat. Rec.*, 1912, vi, 91.

[2] M. T. Burrows, *Jour. Exper. Zoöl.*, 1911, x, 63.

this aqueous suspension are placed on cover-glasses and allowed to dry. The thickness of the suspension and the size of the drops should be such that the spores are left widely scattered over the surface. These cover-glasses are used for the hanging drop preparations, care being taken in adding the tissue and plasma to leave, as far as possible, the spores adherent to the cover-glass.

In a majority of the experiments, spleen tissue from twenty day chick embryos and from one day chicks was used. Before discussing the phenomena associated with the addition of foreign bodies, it may be well to describe briefly the character of the outgrowth in unmodified spleen cultures.

After a few hours' incubation, small mononuclear and polynuclear leucocytes begin to wander from the piece of tissue. Larger cells with very irregular cell bodies and numerous processes follow shortly. During the two succeeding days these cells may spread to the very edge of the drop of plasma. At this time the leucocytes become rounded or very ragged in form, and in stained preparations show pyknosis and fragmentation of nuclei (figure 3). The larger cells, though early showing numerous fat droplets in their cytoplasm, may remain active from ten to twelve days.

A group of cells showing these two types is seen in figure 3, which is a drawing from a small field near the edge of the drop of plasma in a two day culture. The leucocytes show pyknotic and fragmented nuclei. The larger cells vary considerably in form, some being spindle or triangular in shape, others tending to preserve a round or oval outline. There is no striking difference in the character of the nuclei.

That this group of larger cells includes both endothelial cells and large pulp cells, seems probable, inasmuch as each possesses the power of ameboid wandering. On account of the large accumulation of fat droplets in all of these cells, the use of protoplasmic stains which might serve for finer differentiation, is not practicable.

A third type of cell is usually observed in spleen cultures on the second or third day of incubation. The cells of this type appear in the form of long sprouting outgrowths from the central masses of tissue. In their further growth, they form radiating strands of cells. In stained preparations, these are seen to be typical young connective

tissue cells. After five to six days' incubation, large multinuclear giant cells are occasionally seen spread out in a thin sheet on the cover-glass. The process of formation and significance of these giant cells will be discussed in a later paragraph.

Cultures to which lycopodium spores have been added present usually by the second day of incubation a rather striking picture, if conditions have been favorable for an active outwandering of cells. Many of the spores are seen surrounded by dense masses of wandering cells (figures 1 and 2). Further observation of such a mass shows a rather rapid fusion of the cells immediately surrounding the spore, resulting in the formation of a thick giant cell, in which the enclosed spore may be quite invisible. They contain, as a rule, numerous fat droplets and often large vacuoles also. A distinct cell membrane is seen. Protoplasmic processes may be thrown out, particularly if the spore is adherent to the cover-glass.

Preparations of these giant cells stained *en masse* show very well their general character, and if the cells are not too thick, the character of the nuclei can also be determined. Paraffin sections, however, are necessary for more detailed studies. A section through such a giant cell is shown in figure 4. The nuclei in some of these giant cells are of two types: one small, deeply staining, and rather pyknotic; the other larger and rather vesicular. They correspond to the nuclei in the two types of wandering cells described above; that is, to the mononuclear leucocytes, and to the larger irregularly shaped cells which we are inclined to interpret as a group including both endothelial cells and large spleen pulp cells.

Giant cells are also formed about the spores attached to the central mass of tissue. As a result of the flattening and consequent diminution in thickness of this piece of tissue, gross changes taking place in it can often be readily observed in the living cultures.

Observations on the reaction about the spores is made still easier by previously staining them. The tissue may, in addition, be stained with some vital stain. An excellent contrast is obtained, for example, by treating the spores with methylene blue, and the tissue with neutral red, the spores being previously stained and washed and the vital stain added to the fluid plasma before using.

It is possible, however, without such color contrasts to observe in the compact but translucent piece of tissue the formation of dark

rings about the spores. This phenomenon is often observed about the fifth or sixth day, but it is usually more marked in cultures seven to nine days old.

To insure a period of activity of the tissue longer than six days, we have found it advisable to make transfers to fresh plasma about the fifth day. This can be readily accomplished, as we have shown in an earlier report.[3]

Figure 5 shows a section through a piece of spleen which presented in the fresh state a spore dimly visible surrounded by a dark ring. In the stained section, the ring is seen to be a giant cell which does not differ in its general character from a foreign body giant cell formed in the animal body.

From the description of spleen cultures given above, it is obvious that in the formation of the giant cells in the zone of wandering cells, only wandering cells can be concerned. In the case of those giant cells formed in the mass of tissue, however, it is not so easy to draw conclusions. The question resolves itself into a determination of the rôle played by leucocytes, connective tissue cells, pulp cells, and endothelial cells.

The fact that leucocytes remain active in cultures for only two or three days, and that these giant cells are found, as a rule, after this time, removes, we think, this group of cells from serious consideration. The character of the nuclei in the giant cell throws some light on the question. They are rather large, somewhat vesicular and resemble closely the nuclei in the adjacent spleen cells. The architecture of the organ, however, at this stage is so disturbed that fine distinctions in regard to the character of these cells cannot well be made. For this reason further experiments were resorted to which it appeared might settle definitely the question as to whether connective tissue cells played a part in the process.

These experiments consisted in the addition of lycopodium spores to cultures of the heart of chick embryos, where the outgrowth consists of diffuse connective tissue. In no instance was there observed any tendency on the part of the connective tissue either to surround the foreign bodies or to form a giant cell.[4]

[3] R. A. Lambert and F. M. Hanes, *Jour. Exper. Med.*, 1911, xiii, 505.

[4] Further experiments are being undertaken for the purpose of determining more definitely what cells take part in the formation of these foreign body giant

It should be mentioned in this connection that we have observed in spleen cultures the same phenomena associated with foreign bodies other than lycopodium spores. For example, figure 6 shows a piece of cotton fiber enclosed in a large giant cell. A distinct cell membrane and protoplasmic processes can be seen. The stages in the formation of this giant cell from a mass of wandering cells were observed.

In an earlier paragraph, reference was made to the occurrence of large flat giant cells in cultures of spleen to which foreign bodies had not been added. Giant cells of this type make their appearance from the fourth to the eighth day of incubation. They contain from ten to several hundred nuclei, and measure 200 microns to a millimeter in diameter. As a rule, they are so thinly spread out on the cover-glass that they do not exceed in thickness ordinary mononuclear cells (figure 7). Fat droplets of remarkably large size accumulate in their cytoplasm. They are found usually about the original piece of tissue, either around the border or in the zone of attachment to the cover-glass. It also happens, as we indicated above, that the giant cells which are formed from aggregations of cells about lycopodium sometimes spread themselves out in a thin sheet, if the foreign body is adherent to the cover-glass. These observations on the constant relation of giant cells of this type to the cover-glass, or to the cover-glass and some introduced foreign body have led us to suggest that we are probably dealing here also with a true foreign body giant cell, the cover-glass acting as the foreign body. The fact that such giant cells are found so frequently in the zone of attachment of the original piece of tissue to the cover-glass, that is, in an area where numerous wandering cells are massed together on one side of a foreign object, would favor this conception.

CONCLUSIONS.

1. Foreign body giant cells may be produced *in vitro* by the addition of foreign objects such as lycopodium spores and cotton fibers to cultures of chick embryo spleen.

cells. Several investigators have shown that in certain parts of the body a single type of cell may be concerned in the formation of such structures. Marchand (*Deutsch. Chir.*, 1901, xvi, 352), for example, found that the giant cells that enclosed lycopodium spores placed in the peritoneal cavity of rabbits were formed almost entirely out of the endothelial cells covering the serosa.

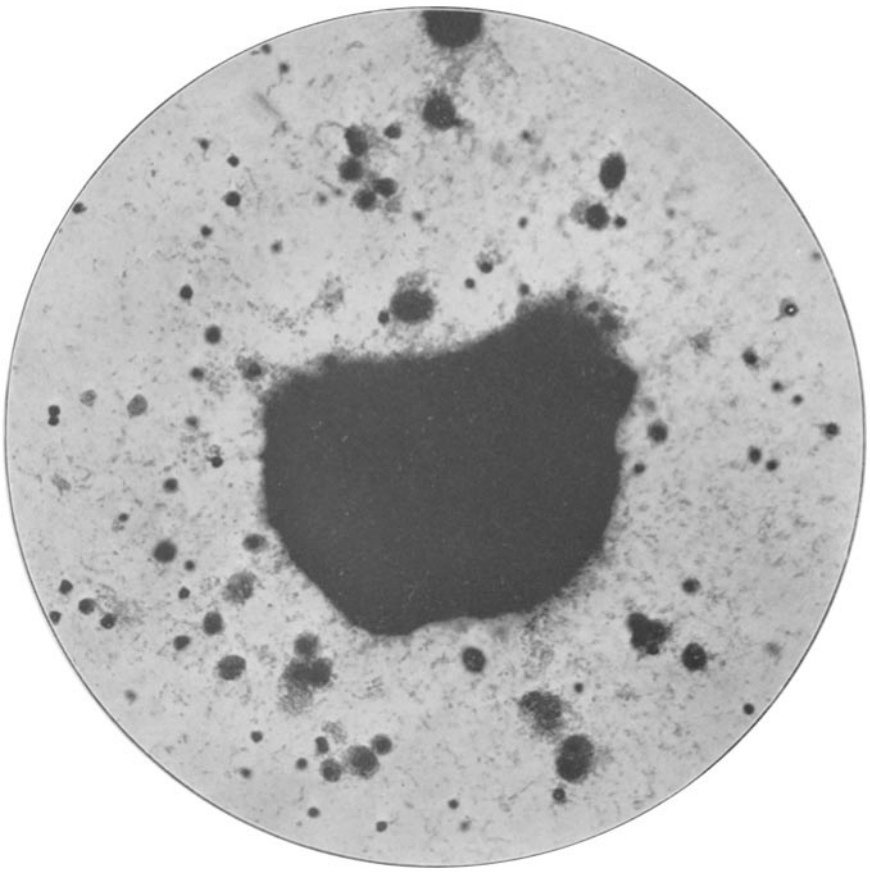

FIG. 1.

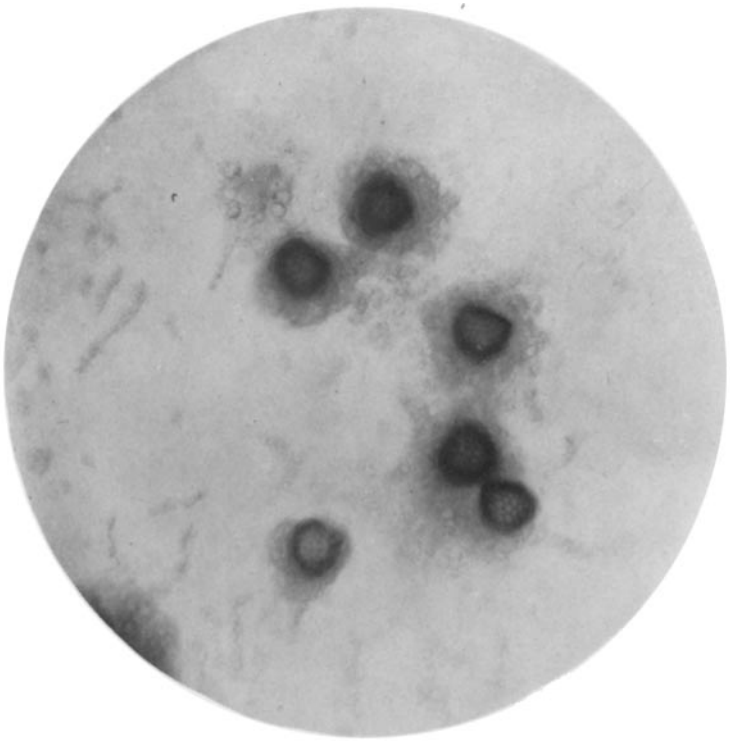

FIG. 2.

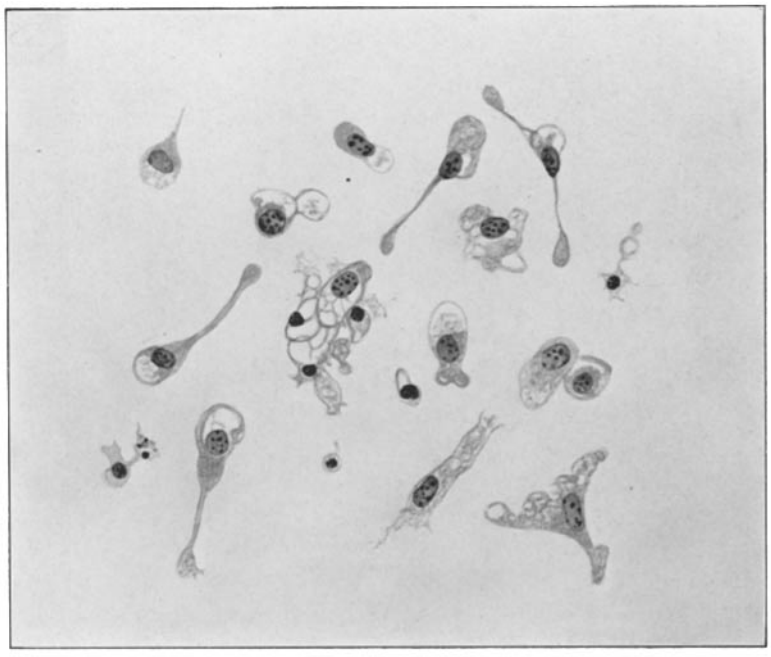

FIG. 3.

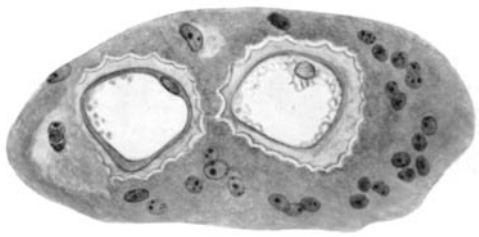

FIG. 4.

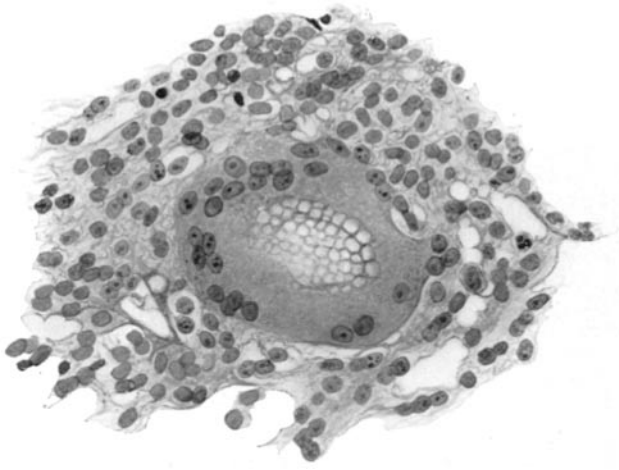

FIG. 5.

FIG. 6.

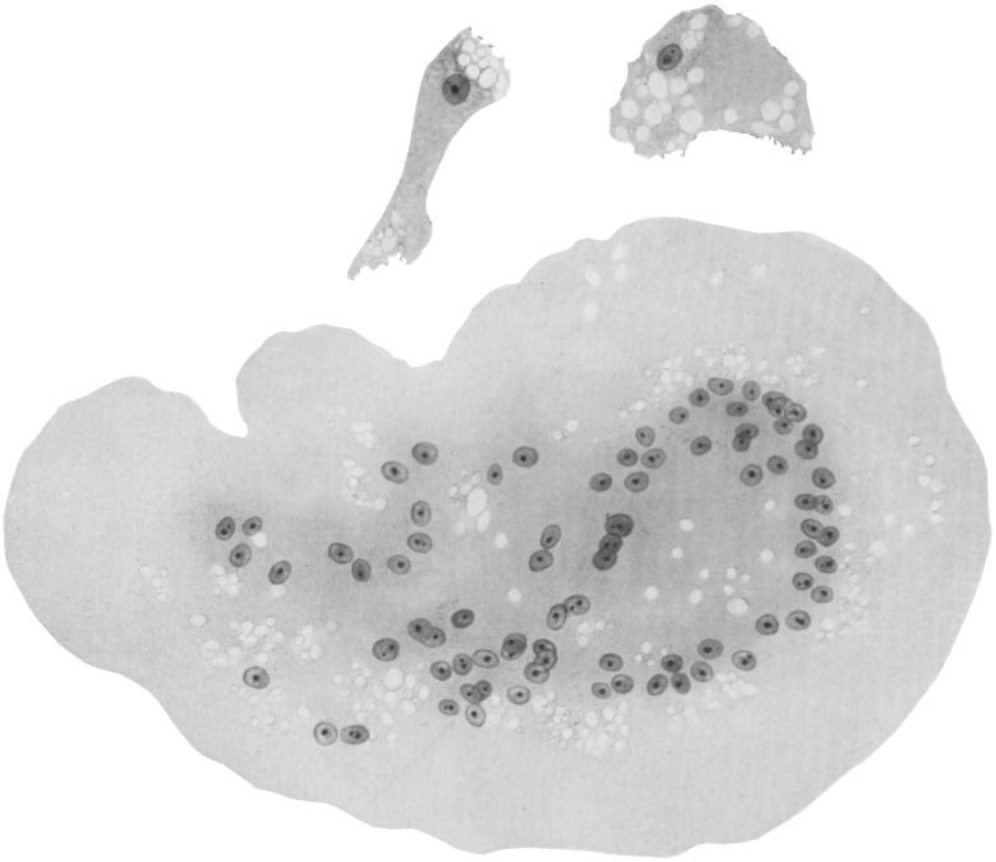

FIG. 7.

2. These giant cells are formed by the fusion of large mononuclear wandering cells, probably endothelial cells and pulp cells. Connective tissue cells do not take part in their formation.

3. The large giant cells sometimes seen spread out over the cover-glass in cultures of chick embryo spleen are probably foreign body giant cells, the cover-glass acting as the foreign body.

EXPLANATION OF PLATES.

PLATE 71.

FIG. 1. Photomicrograph of a two day culture of chick embryo spleen containing lycopodium spores. In the zone of wandering cells, cell masses are seen enclosing spores. The spores are stained with methylene blue; the tissue is unstained. Low power.

PLATE 72.

FIG. 2. Photomicrograph of a field in the preparation shown in figure 1. The spores and surrounding cells appear in greater detail. The characteristic architecture of the lycopodium is shown. High power.

FIG. 3. A group of wandering cells near the edge of the drop of plasma in a two day spleen culture. Leucocytes show pyknotic nuclei and ragged cytoplasm. The larger cells vary somewhat in their morphology.

PLATE 73.

FIG. 4. Section through a giant cell enclosing two lycopodium spores. The cell is from an eight day culture of chick embryo spleen. The spores are cut in median section.

FIG. 5. Section through the original piece of tissue containing a foreign body giant cell from a six day culture of chick embryo spleen. The lycopodium spore is cut tangentially.

FIG. 6. Giant cell formed about a piece of cotton fiber from a five day culture of chick embryo spleen.

PLATE 74.

FIG. 7. Large cover-glass giant cell and two mononuclear wandering cells, from an eight day spleen culture. The vacuoles in the cytoplasm represent fat droplets. The nuclei of the giant cell and of the small cells are identical in appearance.