Cytogenesis of the Multinucleated Foreign Body Giant Cell

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CYTOGENESIS OF THE MULTINUCLEATED
FOREIGN BODY GIANT CELL

by
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A Thesis Submitted to the Faculty of the Graduate
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for the Degree of
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# TABLE OF CONTENTS

ACKNOWLEDGMENTS ........................................... 1

PART I. INTRODUCTION ...................................... 1

PART II. REVIEW OF LITERATURE ........................... 3

PART III. MATERIALS AND METHODS ..................... 13

PART IV. RESULTS ............................................ 16

PART V. DISCUSSION ......................................... 22

PART VI. SUMMARY AND CONCLUSIONS ................... 25

APPENDIX ...................................................... 27

ILLUSTRATIONS ............................................... 30

BIBLIOGRAPHY ................................................. 40
PART I
INTRODUCTION

Since Johannes Muller (1838) described the multinucleated foreign body giant cell, the pathologist and cytologist have been vitally interested in the origin and development of this cell.

The development of the multinucleated foreign body giant cell has been reported to originate from:

- Fixed connective tissue cells
- Tubular epithelium
- Surface epithelium
- Alveolar epithelium
- Serosal epithelium
- Fixed endothelium
- Exudative cells
- Wandering cells of the tissues.

The most widely accepted theories propounded are as follows:

(a) Mononuclear cells fuse to form multinucleated cells, (b) repeated mitotic division without cytoplasmic division, (c) repeated amitotic division of the nucleus without cytoplasmic division.

Some question exists as to the nature of formation of the foreign body giant cell, either by incomplete division of single cells or fusion of mononuclear cells.

1.
The present study was undertaken to collect further evidence whether the cytogenesis of the multinucleated foreign body giant cell is by fusion of tissue histiocytes.
PART II
REVIEW OF LITERATURE

Faber (1893) credited Johannes Muller with the first description of the foreign body giant cell in 1838.

Further, from the literature we can conclude that the multinucleated giant cell can be found in tissues in both physiologic and pathologic processes.

Foreign body giant cells may be found in a great variety of conditions. The commonest is tuberculosis, and various inflammatory reactions and specific granulomas, such as syphilis, leprosy, actinomycosis, blastomycosis, and sarcoidosis.

Robin (1849) observed their presence in bone tissues. Virchow (1858) described the multinucleated foreign body giant cell. At first he was of the opinion that the histiogenesis of these cells was exclusively from the cells of connective tissue, but later admitted the possibility of their origin from epithelium, from endothelium, and from the cells of the muscles and nerves.

Langhans (1868) appears to have been the first to devote an entire paper to the discussion of giant cells, much of his work was done on fresh tissues either teased out or crushed and examined in salt solution, serum or glycerin, or in chromic or acetic acids. He usually made his preparations from fine miliary tubercles of the
pleura or peritoneum. He demonstrated the cells, however, in practically every tissue of the body. He found that they varied greatly in shape, size and the number of nuclei. He described their shape as being oval, elongated or sausage like, elliptical, irregular and more or less stellate. Some were sharply defined, some had attached mantles of adherent spindle cells and others were less well demarcated.

He reported that they varied in size, from small cells with from two to four nuclei, to large cells measuring from 0.2 to 0.3 mm. in diameter and having from thirty to one hundred or more nuclei. The nuclei were characteristic, usually round or oval with sharp outlines and vacuolated centers, and generally contained nucleoli. The arrangement of the nuclei was for the most part characteristic; the nuclei were peripheral with the long axis of the nucleus at right angles to the cell wall. But he found it did not hold throughout. Some of the cells had the nuclei grouped in a bipolar arrangement, and some had them diffusely distributed throughout the cell. The protoplasm of the giant cell was pale, homogenous or finely granular, with the center usually clear. Sometimes, the outlines of a fine fibrillar net-work could be made out in the protoplasm. In teased preparation, the cells became cloudy, and cleared when acetic acid was added.
Langhans thought that the giant cells originated from the surrounding cells and that the spindle cells played the principal part. He advanced several arguments for his view: The nuclei were similar to those of the surrounding cells and were situated, as a rule, in the periphery of the cell. Sometimes the surrounding spindle cells were flattened out like plates on the sides of the giant cells and sometimes they extended outward from the giant cells to join with the neighboring cell groups; this observation suggested that the cells were either fusing into a giant cell or being cast off from it. Finally, he thought the included fibrillar network resembled the fibrillar arrangements of the surrounding tissues.

Ziegler (1875) implanted small glass plates cemented together in pairs in animal tissues. At first he placed the plates in the abdominal cavities of guinea pigs, later in the pleural and pericardial cavities and finally in the intermuscular and subcutaneous tissues. In the latter locations, he obtained satisfactory preparations and conducted seventy-odd experiments. He removed the plates from day to day, and was able to follow the changes in the cells between them. He did not actually produce tubercles in this way, but did obtain all the cells ordinarily encountered in miliary tubercles, including typical giant cells. He concluded that tubercles could be formed from exudative cells alone, and that giant cells were formed especially from mononuclear cells, probably from both lymphocytes and large mononuclears.
Marchand in 1888 made extensive experimental studies of foreign body giant cells by placing pieces of sponge, hardened tissues of the lung, liver, blotting paper and other substances in the peritoneal cavities of guinea pigs. He found the spaces of the sponges and the air spaces of the hardened tissue of the lung filled with exudative cells and granulations. Many of the preparations contained large foreign body giant cells. Marchand concluded that there were different kinds of giant cells, and that they were probably not all formed in the same way. He thought the large multinucleated cells were formed by fusion. He found many mitoses in the granulating areas adjoining, but none in the giant cells themselves.

Metschinkoff (1892) and later Adami demonstrated that when inert foreign particles were introduced into body cavities of Astropecten and other lower forms, multinucleated plasmodia were found about them.

Forbes (1908) in his experiments seeking the origin and development of foreign body giant cells, used injections of agar agar subcutaneously in rabbits. In order to differentiate more clearly the phagocytic cells, lamplack was added to the agar agar before injections.

In studying histologic sections Forbes found "coincident with and following an exudation of polynuclear leucocytes and serum, that there is a migration of endothelial cells from the blood stream through the vessel walls in the neighborhood of the agar." He noted that there was an extensive proliferation and hypertrophy of all endothelial cells fixed or free in the neighborhood of the injection.
He believed the free endothelial cells, and possibly some of those lining the blood vessels became free, and that these endothelial cells then migrated to the surface of the agar and surrounded small masses, coalescing to form giant cells. He further observed that proliferation and coalescence actively continued for at least twenty days, with the result that the injected agar became surrounded with a dense wall of endothelial and giant cells, and outside of them there was formed a capsule of young connective tissue cells.

Haythorn (1912) used india ink and finally ground lampblack, which was blown into the animals larger air passages. He followed tubercle formation and giant cell development from wandering phagocytic cells, which were identified by their having taken up both granules of pigment and tubercle bacilli.

Mallory (1914) described the foreign body giant cell as follows:

"When an endothelial leukocyte finds difficulty in dissolving a substance, as for instance, lime or certain fat products, it frequently fuses with other endothelial leukocytes to form a multinucleated mass of cytoplasm, commonly termed a foreign body giant cell. If the foreign body is too large for one leukocyte to incorporate (cholesterin crystals, hair, etc.) one or more giant cells are formed which surround it or cluster themselves upon its surface."

Foot (1920) demonstrated the role of endothelium in experimental tuberculosis. He used two pigments lithium-carmine and Higgin's waterproof ink, which is a colloidal suspension of carbon in water, with
an emulsifying agent and a little camphor. The former was solely used for intratracheal injections, the latter for intravenous; Foot summarized:

1. The injection of a colloidal suspension of carbon into veins of a living animal furnishes an apparently reliable means of tracing the so-called epithelioid cell of the pulmonary tubercle from its origin in the vascular endothelium to the lesion.

2. Experimental tubercles are formed in the lung as in the liver, primarily by cells originating in the capillary endothelium.

3. The epithelial cell takes no active part in the process; its proliferation tends to repair denuded surfaces and is regenerative rather than combative or phagocytic in nature. This cell is free from carbon and stains only diffusely with carmine, in contradistinction to the endothelial cell which readily takes up both pigments in granular form.

4. The cells of endothelial origin not only phagocytose tubercle bacilli, but carry them into the tissues for example into lymph nodes, by way of the lymphatics, or into other lung lobules by way of the air passages, in which they are readily demonstrable.

In another study, the role of the endothelial cell in experimental tuberculosis, Foot concluded that the epithelioid cell is definitely of endothelial origin. And found that there is little evidence that the local tissue elements take an active part in the process of
tubercle formation, until after the lesion is formed; the reaction is, in a sense, exudative, since the lesion is produced from cells which migrate to the site of inflammation. The lymphocyte appears late and is not to be considered as a potential epithelioid cell, its presence in the tubercle is as yet unexplained.

Haythorn (1922) injected stained agar agar mixed with lycopodium spores, stained with gentian violet, before injection. He injected india ink to mark the cells, and it seemed to him that giant cells in these foci were formed by the fusion of mononuclear phagocytes. Later he produced lesions in similar ways, and followed them by inducing localized edemas or serous exudates about them. He separated the tubercles and particularly the giant cells, into their constituent elements. Some giant cells broke up into a central caseous mass with a margin of phagocytes and other cells, but the majority of the giant cells separated out as definite cellular entities.

Maximow and Bloom (1925) published the results of studies of tissue cultures; they concluded that lymphocytes change into large mononuclear wandering cells, which Maximow called "polyblast." In his work on the development of tubercles in vitro, he reported that "polyblasts" arise partly from local "resting wandering cells" of the tissues (such as clasmocyte and histiocyte) through rounding off and mobilization; and partly from lymphocytes, nongranulated white blood corpuscles and monocytes which may migrate from the vessels or which
may have been previously present in the tissues. The polyblasts hypertrophy join the local histiocytes, become ameboid phagocytic cells and change into epithelioid cells which may later fuse to form giant cells. He concluded that lymphocytes are slower but, nevertheless, become transformed into epithelioid cells. Mallory holds that the large mononuclear cells which become epithelioid cells come from endothelium, and that lymphocytes never become phagocytes.

Cohen (1926) observed the formation of giant cells in turtle blood culture. Using the simple hanging drop of blood method. He removed blood from the heart of a turtle by means of a syringe and a fair sized drop was placed in the center of a clean cover glass. The cover was inverted and sealed over a hollow ground slide with a vaseline-paraffin mixture. A similar preparation was made after the turtle had been injected with india ink.

Cohen observed fusion at the height of activity which occurred most commonly on the fourth day, although in some preparations, he noted that activity persisted until the eight or ninth day, and in one culture until the twenty-fifth day. From his observations Cohen concluded that:

1. Only large mononuclear wandering cells were seen to enter into the formation of giant cells.

2. Multinucleated giant cells were observed to form by fusion in hanging drop cultures of turtle blood.
3. Quiescent non-granular mononuclear type cells were observed to take an ameboid activity, to show mitochondrial granules and to participate in fusion phenomena.

4. The multinucleated giant cells behaved like true cells, showed no partitions and were merely coherent masses of single cells.

Aronson and Elberg (1962) found fusion of peritoneal histiocytes were collected from the rabbits and maintained on cover slips in Leighton tubes, in a medium consisting of forty percent normal rabbit serum in Tyrode solution. Smears examined by autoradiography showed frequent instances of histiocytes containing two or more nuclei. It became apparent that at least some of these binucleate and poly-nucleate cells did not arise from dividing cells which failed to separate because in many cases only one of the nuclei was labeled. From their experiments, they showed that the percentage of multinucleated cells increased in time. They stated, "It is interesting to note that the oil vacuoles merged in the "giant cells" as if the cells were re-organizing their structure. One may perhaps consider this as analagous to the in vivo formation of "giant cells" the task of which is to isolate indigestible foreign material."

Silverman and Shorter (1963) investigated the mechanism of giant cell formation in rats, by the combined use of colchicine and autoradiography. Lycopodium powder was used as the foreign body material; it was introduced into subcutaneous spaces through incisions in both lower quadrants of the abdomen.
Their experimental results in the rat suggest that the multinucleated foreign body giant cell that forms in the presence of lycopodium spores arises, at least initially, by fusion of histiocytes.

Burstone (1958) demonstrated that acid phosphatase is localized in macrophages and foreign body type giant cells.
PART III
MATERIALS AND METHODS

White C57 mice twenty-five to forty grams in weight were used in all the experiments and were housed under standard conditions at a temperature of 72°F, on a diet of Wayne's Laboratory Block Food and water ad libitum.

Under ethyl ether anesthesia incisions were made in both lower abdominal quadrants of the animals with a No. 15 B. P. scalpel. Approximately one gram of lycopodium powder U.S.P. (foreign body material) was introduced into the subcutaneous areolar spaces of the left incisional wound.

Twenty mice were divided into four groups; A, B, C, D. Each mouse was weighed and marked for identification. (Refer to appendix for additional information.)

Group A, eight in number were given intraperitoneal injection of tritiated thymidine (H³T) with an activity of 1.9 c/m mole (Schwarz Biological Research Inc., Mountain View Avenue, Orangeburg, New York.) at a dose rate of 0.5 c per gram of animal weight on the first day. These animals were sacrificed four hours, one, two, three, seven, nine, twelve, and fifteen days after injection of H³T.

Group B consisting of four animals, were injected on the second post operative day with H³T as group A, and were sacrificed four hours, one, three and five days after injection of H³T.
Group C comprised of six mice, were injected on the seventh post operative day with $^3$H as the above A, and B groups, and were sacrificed four hours, one, three, five, nine and fifteen days after injection of $^3$H.

The animals were killed by an overdose of ethyl ether; the abdominal wall was excised including the wound area and surrounding tissues, immediately after removal the tissues were placed into ten percent neutral buffered formalin solution having a pH of 6.9.

After fixation in cold neutral formalin the specimens were processed in the usual manner and embedded in paraffin and sectioned six micra.

Autoradiographs were prepared using NTB 3 Kodak liquid emulsion exposed fourteen to twenty-one days at $4^\circ$C. After development the sections were fixed, washed and stained with hematoxylin and eosin.

Cell counts were made at the site of lycopodium deposition. While labeled inflammatory cells were present, only labeled histiocytes were included in the labeling indices. A ratio of labeled and unlabeled cells was determined for each group.

Group D consisting of two mice were sacrificed on the third and eight post operative day. The abdominal wall was excised including the wound area and surrounding tissues, immediately upon removal the tissues were placed into ice cold acetone. (For acetone fixation procedure see appendix.)
After fixation the specimens were processed in the usual manner and embedded in paraffin, sectioned at six microns, washed and stained for acid phosphatase.

PROTOCOL OF MICE TREATMENT

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Mice</th>
<th>PODI-H&lt;sup&gt;3&lt;/sup&gt;T</th>
<th>Mice Killed</th>
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<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>1d</td>
<td>4hr  1d-2d</td>
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<td>3d - 7d-9d</td>
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<td>12d - 15d</td>
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<td>B</td>
<td>4</td>
<td>2d</td>
<td>4hr - 1d</td>
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<td>3d - 5d</td>
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<tr>
<td>C</td>
<td>6</td>
<td>7d</td>
<td>4hr - 1d-3d</td>
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<td></td>
<td></td>
<td></td>
<td>5d - 9d-15d</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>NONE</td>
<td>3d - 8d</td>
</tr>
</tbody>
</table>

Legend:

hr = hour

= day

PODI = Post operative day of injection.

Time for mice killed given is after injection of tritiated thymidine.
PART IV

RESULTS

Group A.

1. Four hours after injection of $H^3T$. This section shows the surgically induced wound. The cut epithelial surface is widely separated, the cells at the cut surfaces have hyperchromatic nuclei, reduced cell size which are characteristic of cell degeneration. In the surface of the area between the cut edges of the epithelium is a coagulum comprised of necrotic inflammatory cells.

The injured corium is edematous infiltrated by many polymorphonuclear leucocytes and filled by a fibrin clot. This edema extends into the adjacent superficial muscle layers. The inflammatory exudate completely infiltrates the dermis, resulting in a loss and unraveling of the collagen fibers. Many dilated capillaries and hemorrhage is evident in the corium and muscle layer. Margination of leucocytes in dilated capillaries was noted in the above layers.

The lycopodium spores located in the corium, are almost completely surrounded by dead or dying inflammatory cells. The overwhelming majority of the acute inflammatory cells are polymorphonuclear leucocytes.
The cells of the basal layer of the epithelium and cells of the hair follicles are labeled with \( \text{H}^3 \text{T} \), some undifferentiated connective tissue cells situated perivascularly and a few polymorphonuclear leucocytes found in the dermis and muscularis are also so labeled.

2. One day after injection this section receives essentially the same description as in section 1, with slightly less edema. In addition to the pmns, lymphocytes were evident. It was noticed that there was a proliferation of reserve connective tissue cells as few mitotic figures were seen in these cells situated around dilated capillaries in the dermis, in the loose connective tissue and between the muscle fibers. Many histiocytes were labeled, as were some fibroblasts and pmns. Labeled endothelial cells at the lumen of some capillaries (bulging in) were seen. Some labeled basal cells have migrated to prickle cell layer, some basal cells were still labeled.

3. On the second day the epithelium was proliferating over the coagulum. Dilated blood vessels, heavy fibroblastic activity, collagenous fibers, clot organization and capillary proliferation was most obvious in this section. Increase in the lymphocytic and histiocytic population was observed. The histiocytes adjoining the capillaries were found to be oval or fusiform in shape with a prominent cell membrane, having a round or oval nucleus,
fine chromatin network, big nucleoli, and definite nuclear membrane. There was almost a two fold increase in the labeled histiocytes present, as compared to earlier sections. Some labeled pmns, fibroblasts and endothelial cells were still seen. Labeled epithelial cells were now found in the granular layer.

Lycopodium spores were now surrounded by degenerating and necrotic inflammatory cells. Labeled histiocytes appeared to be migrating towards and arranging themselves around the lycopodium spores.

4. Three days post-operatively epithelium continuing proliferation over coagulum and inflammatory cells. Markedly less edema. Tremendous vascularization. Histiocytes continue to increase in number. Lymphocytes and some plasma cells were noted. Labeled histiocytes in contact and surrounding individual lycopodium spores and groups of lycopodium spores. Labeling now consisting of fine grains, as seen in autoradiograms. Almost entire high power field filled with labeled cells, most appear to be histiocytes. Continued collagenous fiber formation, labeled fibroblasts and endothelial cells. Labeled viable cells now occupy the spaces between individual spores, spaces that had been previously filled with degenerating inflammatory cells. The labeled histiocytes are in such close proximity one to each other that they can give the impression of forming giant cells.
5. On the seventh day there was a decrease in edema and it was noted that there was diminishing inflammatory reaction, few pmns present. Multinucleated giant cells with some labeled nuclei. From two to fifteen nuclei per giant cell. The nuclei have the same shape and size, are oval or round, and active chromatin. The giant cells are in contact or immediate vicinity of lycopodium spores. There is a marked reduction of labeled histiocytes. It appears that there was no increase in the number of histiocytes. Small capillaries with some lightly labeled nuclei and continued apposition of collagenous fibers was noted.

6. On the ninth day there was a continued increase in the number of giant cell formation, increase in the number of nuclei in these cells. Collagenous fiber formation. Labeled fibroblasts, histiocytes and endothelial cells were noted.

7. Twelve day post-operatively the giant cells are better formed, there is an increase in the amount of cytoplasm and the border of the cell is better outlined, there is an increase in the number of nuclei, some are labeled lightly. Continuation of collagenous fiber formation. Labeled fibroblasts, lipid filled histiocytes, foam cells and endothelial cells were present.

8. On the fifteenth day the lesion has more compact connective tissue, with a decrease in the number of monocellular elements. Better giant cell differentiation, with an increase in
the number of nuclei. Labeling of some of the nuclei consisting only of a few fine grains.

GROUP B.

At **four hours** an acute inflammatory reaction was noted. The nuclei of the basal cells of the epithelium cells of the hair follicle, pmns, and histiocytes were heavily labeled.

At **one day** continued inflammatory reaction. Dense accumulation of pmns in and about the lesion. Labeling seen in the nuclei of some pmns, endothelial cells, basal cells of the epithelium, fibroblasts and histiocytes.

At **three days** epithelium proliferating over coagulum and inflammatory cells. Dense accumulations of pmns around the lycopodium spores. The spores of lycopodium vary in diameter, small discrete clumps separated by fibrin and many mononuclear cells. Capillary formation was noted. Epithelial cells, pmns, endothelial cells, fibroblasts and histiocytes were labeled. These cells did not show as heavy a labeling as was seen in the twenty-four hour specimen.

At **five days** giant cell formation was noted. Organization of the connective tissue. Some of the nuclei of the giant cells, fibroblasts, endothelial cells and histiocytes were labeled. The labeling consisted of only fine grains as seen in the autoradiograms.
GROUP C.

At four hours aside from the basal cells of the epithelium and the cells of the hair follicle only about ten to twenty percent of the cell population was labeled. Designating the fact that the cells were differentiating, to continue to perform their respective duty. The labeled nuclei were fibroblasts, histiocytes, endothelial cells, and of a few giant cells.

At three days increased number of giant cells with increased number of nuclei. Continued connective tissue formation. A few endothelial cells and a few lipid filled histiocytes were labeled.

At five days and nine days continued fibrin formation diminution of lycopodium spores. Increased number of giant cells, decreased number of individual cells. No recognizable labeling.

At fifteen days more differentiated giant cells, with increased number of nuclei within giant cell, and increased amount of cytoplasm. Plagues of giant cells, the nuclei of giant cells appear hyperchromatic. Only a rare histiocyte with labeled nucleus was seen.

GROUP D.

This section shows that the histiocytes and the giant cells contain acid phosphatase and no evidence of alkaline phosphatase.
PART V
DISCUSSION

Lycopodium powder proved to be a good material to bring about a foreign body reaction in the skin of the mouse.

The immediate reaction was an inflammatory one. The fibrin clot and the polymorphonuclear leucocyte infiltrate served as a barrier between the lycopodium and subcutaneous tissue. The nuclei of some histiocytes were labeled, indicating DNA synthesis before cell division and proliferation of these cells.

The polymorphonuclear leucocytes immediately around the lycopodium did not contain radioactive nuclei, as they had been in the peripheral circulation prior to margination and emigration from the capillaries. The labeled polymorphonuclear leucocytes seen at twenty-eight hours and later represent those cells arising from the bone marrow which were in DNA synthesis when tritiated thymidine was injected.

There was a continued proliferation of histiocytes a few mitotic figures were noted around dilated capillaries in the dermis, in the loose connective tissue and between muscle fibers. Many histiocytes were labeled, as were some fibroblasts and endothelial cells. Collagenous fiber formation was evident. Proliferation of histiocytes was very great at four days, almost the entire high power field was filled with labeled histiocytes. The histiocyte is a precursor cell of the fibroblast, and in some cases also the endothelial cell.
As the clot was invaded by new capillaries and histiocytes the lycopodium spores were being trapped.

The labeled and unlabeled histiocytes fused in and around individual spores and clumps of spores to form multinucleated foreign body giant cells. The nuclei of both the histiocytes and giant cells have the same shape and size, oval or round and staining intensity. As some nuclei were labeled, the origin of such cells can only be from the pre-existing labeled histiocytes. Furthermore, as some nuclei were free of labeling, the giant cell could only form by the fusing of cells.

The labeling of some of nuclei of the giant cell was light as compared to the heavy labeling of the first histiocyte seen in earlier sections. This is due to the mitotic division of the labeled cells which transfer one-half of its nucleic acids to each of its daughter cells, then as each daughter cell divides it further decreases the radio-activity to one-quarter to that of the originally labeled cell. Concurring with the findings of Toto and Abati (1963).

We have found labeled histiocytes throughout the granulation tissue and labeled nuclei in the giant cell, supporting the theory that the multinucleated foreign body giant cell is formed by fusion of the histiocyte.

The presence of acid phosphatase in the histiocyte and in the giant cell is further evidence that the histiocytes fuse to form foreign body giant cells. The acid phosphatases are functional in
other tissues such as the prostate gland and kidney. However their role is not yet clear, nevertheless, they appear to be an important component of all mononuclear, and multinuclear cells showing phagocytic function. - Burstone (1958). The presence of acid phosphatase in the histiocyte before they fuse to form giant cells clearly demonstrates that phagocytic functions of the giant cell is predicated upon differentiation of the histiocytes.
PART VI
SUMMARY AND CONCLUSIONS

Twenty white C57 mice, divided into four groups (A.B.C.D.) were used in this investigation of cytogenesis of the multinucleated foreign body giant cell.

Incisions were made in the lower abdominal quadrants of the animals. Lycopodium powder was used as the foreign body agent and introduced into the incisions.

The first three groups (A.B.C.) were given intraperitoneal injections of tritiated thymidine, one, two and seven days respectively post-operatively, for the purpose of identifying cells in DNA synthesis.

After the animals were killed, the wound area including the surrounding tissue of the abdominal wall was removed and immediately placed in ten percent neutral buffered formalin solution.

Autoradiographs were prepared using NTB 3 Kodak liquid emulsion exposed fourteen to twenty-one days at 4°C. After development the sections were fixed, washed and stained with hematoxylin and eosin.

The tissue of the killed animals of the fourth group (D) were placed in ice cold acetone. After fixation the specimen were embedded in paraffin, sectioned and Burstone’s method for the demonstration of acid phosphatase was used.

In the formation of multinucleated foreign body giant cells in the skin of the mouse the only cells that synthesize DNA locally are 25.
the histiocytes. They are the only cells labeled with tritiated thymidine.

The failure of the giant cell to incorporate the nucleic acid precursor tritiated thymidine is evidence that mitotic activity does not occur in the multinucleated foreign body giant cell. This supports the findings of Silverman and Shorter (1963).

Proof of the presence of acid phosphatase in the histiocyte and then in the giant cell gives further support that giant cells form by fusion of histiocytes.
APPENDIX

Autoradiographic Preparatory Technic

The histologic sections were cut at four to six microns and mounted with albumin on slides.

Emulsion coating was done in a light proof dark room about three feet from a Wratten safe light number 2. The flask containing gelled NTB 3 was removed from the refrigerator and placed on the table, when it was at room temperature, the flask was placed into a water bath at 40° - 45°C for approximately twenty minutes to change it from a gel to liquid state. The emulsion was then poured into a coplin jar. One or two drops of a surface active agent (Tween 20) were added. The solution was stirred and allowed to stand until the emulsion was 40 - 45°C.

The slides were dipped for five to ten seconds in the melted emulsion. After withdrawal of the slide from the emulsion the excess was allowed to drain into the container and the back of the slide was wiped clean with soft paper. The slide was then allowed to dry in a vertical position.

When drying was complete the slides were placed in black plastic boxes containing perforated capsules sealed with black plastic tape. They were then stored in the refrigerator (5°C) for fourteen to twenty-one days.

27.
Development of the autoradiographic emulsion on the slides was done in the dark room using a Wratten number 4 safelight at a distance of four feet. The slides were placed in a standard staining dish, containing Kodak D-19 developer for two minutes. The slides were rinsed for ten seconds and placed into Kodak fixer for two minutes. Washed for ten minutes and allowed to dry. The prepared sections were stained with hematoxilyn and eosin.

**Histochemical Method for Acid Phosphatase**

Procedure for paraffin embedding and sectioning of tissue for enzyme studies.

Immediately upon removal of tissue from the animal the tissue was placed in ice cold acetone and stored in the refrigerator. Fixed for twenty-four hours and dehydrated in three changes of acetone. Cleared in benzene for two changes, over thirty minutes each. The tissue was embedded quickly after one to one and one-half hours in each of two changes of paraffin below $55^\circ C$.

For sectioning, the water bath was heated gradually to the desired temperature in the range of $45^\circ$ to $50^\circ$C. with a thermometer in the water bath. Each time a set of sections was floated out and mounted on slides after being flattened out, the temperature of the water bath was observed on the thermometer at the beginning and at the end of the procedure.
Histochemical Technique

Approximately 5 mg. of substrates As-Bl (derived from naphtols) were dissolved in 0.5 ml. N, N-dimethylformamide (DMF) Matheson, Coleman, and Bell) followed by 25 ml. distilled water and 25 ml. of buffer (0.2 M acetate, pH 5.2-5.6) to make a total volume of 50 ml. Thirty-five mg. of diazonium salt (Red Violet LB salt diazotized 5-benzamido 4-chloro 2-toluidine) and two drops of ten percent Mn Cl₂ were added. The mixture was then shaken and filtered into a coplin jar. Incubation period was twenty hours at 37°C.
Photomicrograph one-day after lycopodium placement four hours after injection of $\text{H}_3^3\text{T}$.

Showing labeled cells of the basal layer of the epithelium and labeled histiocytes.
Photomicrograph one-day after lycopodium placement.

Showing labeled histiocytes situated perivascularly in the loose connective tissue.
Photomicrograph one-day after lycopodium placement.

Showing labeled inflammatory cells, polymorphonuclear leucocytes in the loose connective tissue.
Photomicrograph three-days after lycopodium placement.

Showing granulation tissue, labeled histiocytes, and capillary proliferation.
Photomicrograph seven-days after lycopodium placement.

Showing lycopodium spores, labeled histiocytes and giant cells.
Photomicrograph fifteen-days after lycopodium placement.

Showing fibrous capsule formation labeled giant cells.
Photomicrograph seven-days post-operatively, four hours after injection of $\text{H}^3\text{T}$.

Showing labeled histiocytes adjacent to lycopodium and giant cells, polymorphonuclear leucocytes in loose connective tissue.
Photomicrograph fifteen-days after injection of $\text{H}^3\text{T}$.

Fibrous capsule formation, giant cells about lycopodium spores.
Histochemical Demonstration of Acid Phosphatase with Naphtol AS - Phosphates

Low Power

Intense staining of histiocytes and giant cells.
Histochemical Demonstration of Acid Phosphatase with Naphtol AS - Phosphates

High Power
Intense staining of histiocytes and giant cells.


40.


The thesis submitted by Dr. Mitchell V. Kaminski has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 19, 1965

Date

Patrick J. [Signature]

Signature of Adviser