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The Formation of Asbestos Bodies by Mouse Peritoneal Macrophages

An In Vitro *Study*

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For studies on the mechanism of asbestos body formation, Union Internationale Contre Cancer (UICC) crocidolite asbestos fibers were added to a culture of mouse peritoneal macrophages. Small asbestos fibers were totally ingested by the macrophages, but fibers too long to be taken up completely remained as a consequence extracellular. These long asbestos fibers became the basis for asbestos body formation. The basic mechanism underlying asbestos body formation was found to be the exocytotic activity of macrophages. The number of iron-rich inclusion bodies was dependent on the availability of iron in the culture media, and the same holds for the amount of iron in the asbestos body coat. This means that asbestos body formation is a phenomenon that occurs accidentally when macrophages come into contact with long fibers in an iron-rich environment. A time-dependent increase in the number, average size, and rate of segmentation of the asbestos bodies was observed. The present report is the first to describe asbestos body formation in vitro. ***(Am J Pathol 1990, 137:121–134)***

The presence of asbestos bodies or ferruginous bodies has been frequently reported by authors who used material obtained from patients or laboratory animals exposed to asbestos fibers or some other fibrous material with biologically inert characteristics.[1–7] In addition, the mechanism of asbestos body formation has been the subject of a number of investigations.[8–10] In an earlier study, we showed that the process of asbestos body formation was not restricted to the pleural cavity and the lungs, but could also take place in foreign body granulomas in the mouse peritoneal cavity.[7] It was concluded from that study that asbestos body formation is a phenomenon that occurs when macrophages and/or multinucleated giant cells meet indigestible fibers too large to be totally ingested by a single macrophage or giant cell. The same study provided indications that asbestos body formation is the result of exocytotic activity of the macrophages. The complex structure of the granulomas makes it difficult to determine not only the exact mechanism underlying the deposition of material around an asbestos fiber, but also whether asbestos body formation is carried out by macrophages.

To obtain more information about the process of asbestos body formation, we performed an *in vitro* study in which mouse peritoneal macrophages were cultured in the presence of Union Internationale Contre Cancer (UICC) crocidolite asbestos fibers.

Materials and Methods

Animals

The specific pathogen-free–bred Swiss male mice obtained from HARLAN CPB (Zeist, The Netherlands) were 6 weeks old and weighed 20 g at the start of the experiments. All mice were allowed 3 days to adapt before a peritoneal cell suspension was collected.

Cell Collection

The animals were decapitated, 2 ml Hank's balanced salt solution (HBSS) was injected intraperitoneally, and the abdomen was carefully kneaded for approximately 30 seconds, after which the peritoneal cell suspension was collected under sterile conditions. The cells were counted in a Coulter counter (Coulter Electronics Model DN, Mijdrecht, The Netherlands) and pelleted in a cryocentrifuge for 5 minutes at 90*g* and 4°C.

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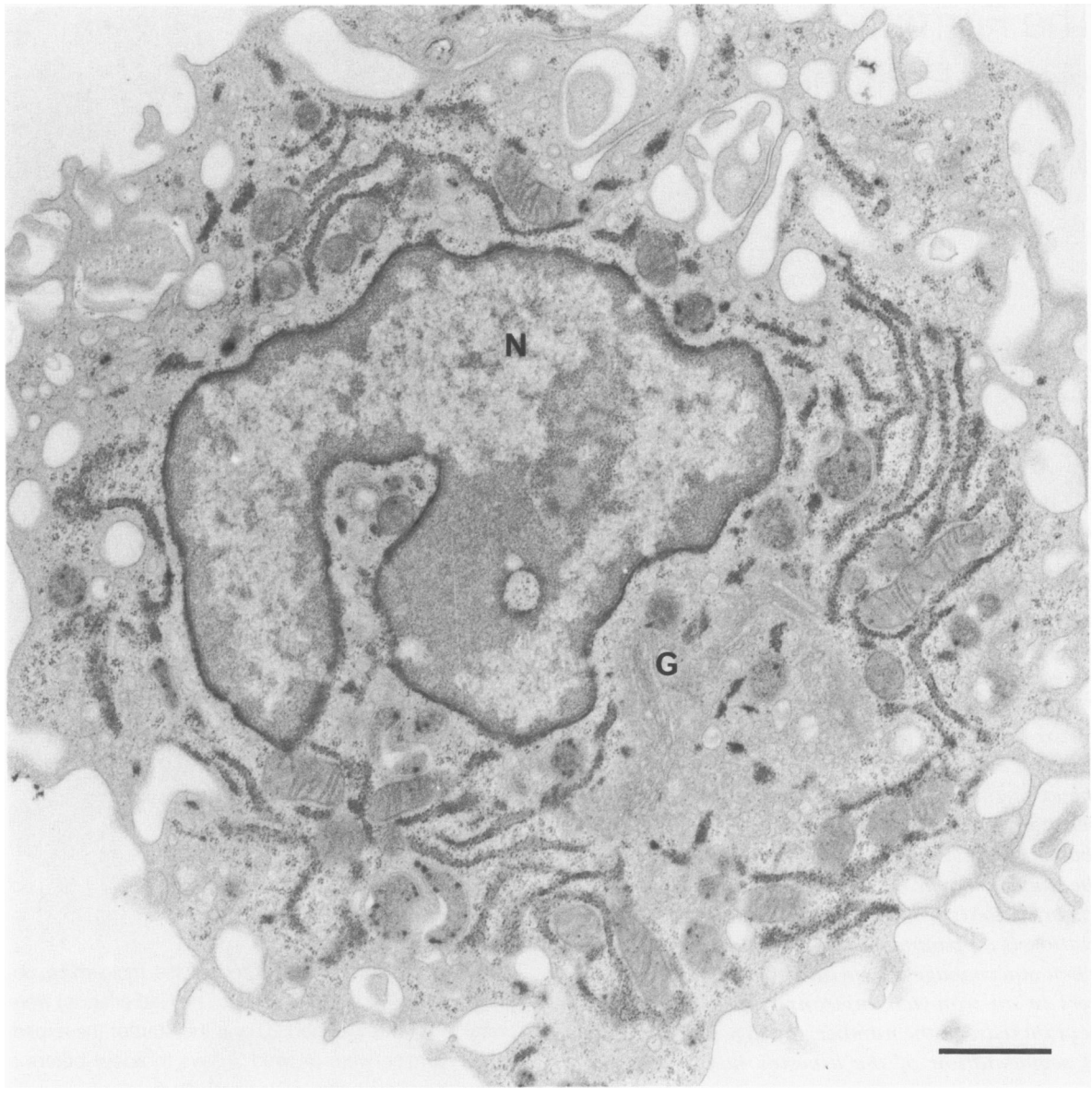

Figure 1. *Transmission electron micrograph showing a peritoneal resident macrophage from an unstimulated mouse peritoneal cavity. The preparation was processed for the demonstration of peroxidase activity. Note peroxidase reaction product in the rough endoplasmic reticulum and the nuclear envelope. Fixation: glutaraldehyde, OsO_4. N: nucleus, G: Golgi area. Bar: 1 μ.*

Cell Culture

The culture medium was HEPES-buffered Dulbecco's minimum essential medium (DMEM) containing 4.5 g/liter glucose (Gibco, Grand Island, NY), supplemented with 1% nonessential amino acids (Gibco), 2% penicillin/streptomycin, Flow Laboratories (Irvine, Scotland) (5000 IU/ml and 5000 μg/ml, respectively), and 20% heat-inactivated fetal calf serum (FCS) (Gibco). The cells were resuspended in this medium to a concentration of 0.5×10^6 cells/ml, and 2-ml samples were brought into Greiner petri dishes (Qualität FC, 35/10) containing sterile cover glasses. After incubation for 2 hours at 37°C in an atmosphere composed of 95% air and 5% CO_2, the petri dishes were washed with the culture medium to remove the nonadherent cells, and the adherent population was cultured for 16 hours in the same medium containing 5 μg/ml UICC crocidolite asbestos fibers. The petri dishes then were washed again to remove excessive asbestos and culture in the above-described medium was resumed. At 48-hour intervals, the culture medium was replaced with fresh medium. Control cultures were treated in the same way but without the addition of asbestos. The following culture media were used:

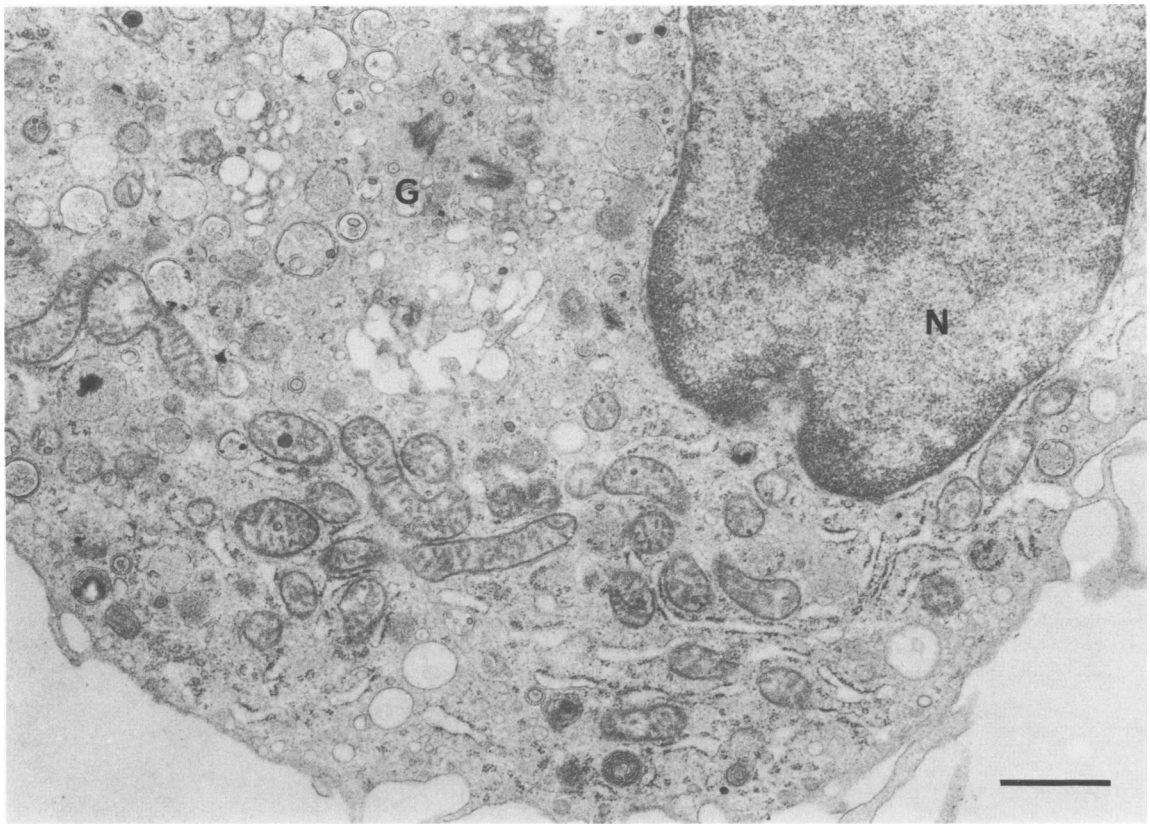

Figure 2. *Transmission electron micrograph of a mouse peritoneal resident macrophage after 48 hours of culture. The plasma membrane has become distinctly smoother. PO-positive reaction product was no longer present in cells of this type. Fixation: glutaraldehyde, osmium(VI)-iron(II) complex. N: nucleus, G: Golgi area. Bar: 1 μ.*

- Control medium: DMEM as described above with 20% FCS
- Iron-rich medium: DMEM as described above with 20% FCS and 1.8 mmol/l (millimolar) iron, in the form of iron dextran
- Manganese-rich medium: DMEM as described above with 20% FCS and 1.8 mmol/l manganese in the form of $MnCl_2$

Colloidal Gold

At the interval of 1 week, some of the cultures were refreshed with a solution containing 1% bovine serum albumin conjugated to 20 nm colloidal gold (BSA-gold). These cultures were used to study the deposition of particles into the space containing the asbestos bodies and to establish the pathway along which these particles are transported to these loci.

Fixation

For both light microscopy (LM) and transmission electron microscopy (TEM), specimens were fixed at 4, 6, 8, 10, and 12 weeks after the start of culture in 1.5% glutaraldehyde in 0.1 mol/l (molar) cacodylate buffer (pH 7.4, 330 mOsm/l) for 10 minutes at room temperature and then washed in a Ringer's solution. For TEM, the cells were postfixed in 1% OsO_4 in 0.1 mol/l cacodylate buffer. Some of the specimens were postfixed with the modified OsO_4 fixative according to de Bruijn[11] for 24 hours at 4°C. Another part was fixed with glutaraldehyde only to allow undisturbed X-ray microanalysis.

Enzyme Cytochemistry

Peroxidase

To determine the initial composition of the peritoneal cell population, we performed cytochemical staining to detect peroxidatic activity in the various cell types. For this purpose, the cells were fixed in glutaraldehyde as described above, washed at room temperature for 5 minutes in 0.1 mol/l cacodylate buffer (pH 6.9), and preincubated for 30 minutes at room temperature in a medium containing 1 mg diaminobenzidine-4HCl per milliliter cacodylate buffer. Incubation was performed in the same me-

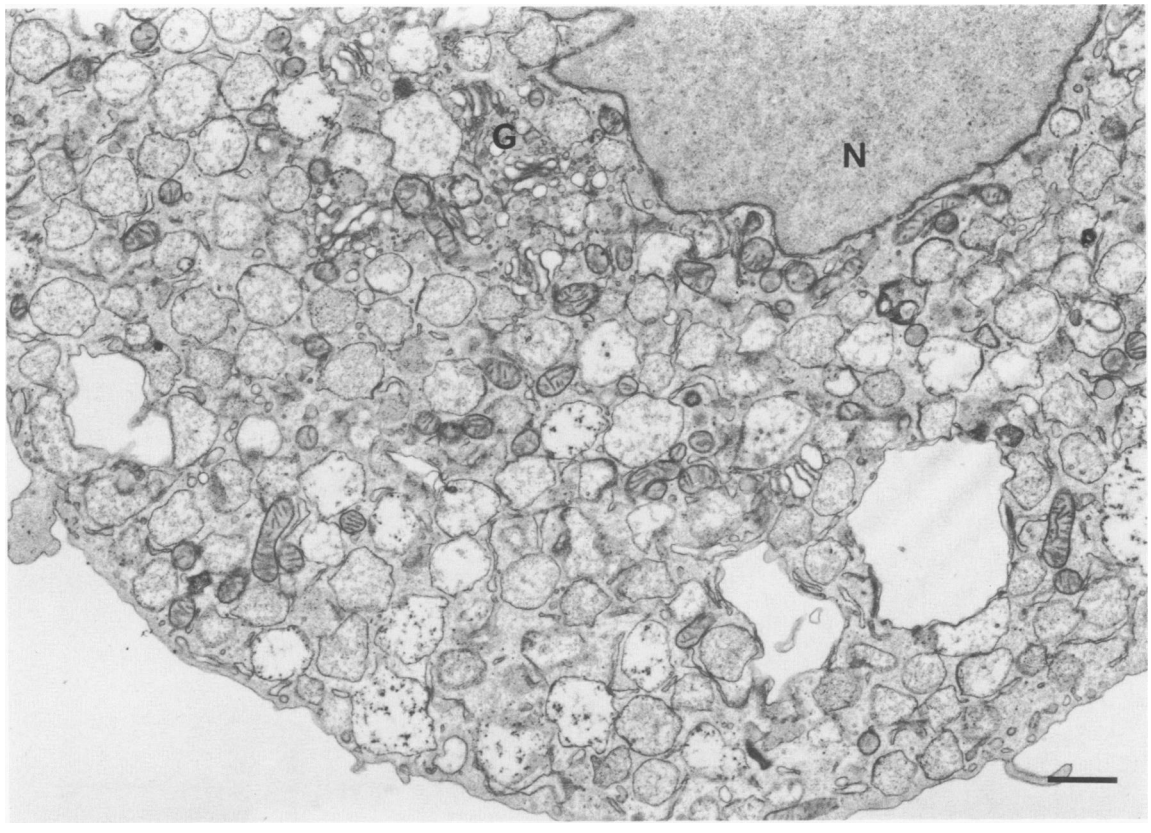

Figure 3. *Transmission electron micrograph of a mouse peritoneal resident macrophage after 4 weeks of culture. Blebs were sometimes observed on the plasma membrane of such cells. Many cytoplasmic vacuoles are visible. Fixation: glutaraldehyde, osmium(VI)-iron(II) complex. N: nucleus, G: Golgi area. Bar: 1 μ.*

dium supplemented with H_2O_2 to a final concentration of 0.01%.

Acid Phosphatase

The method described by Hulstaert et al[12] was used to detect acid phosphatase activity in the macrophages. The cells were prefixed in glutaraldehyde, washed for 5 minutes in 0.1 mol/l cacodylate buffer (pH 7.4), followed by 5 minutes in 0.08 mol/l TRIS-maleate buffer (TMB) (pH 5.0), frozen at −24°C in 0.08 mol/l TMB, and preincubated for 30 minutes at 37°C in 0.08 mol/l TMB containing 1 mmol/l $CeCl_3$. Incubation was performed in the same solution to which 1.5 mg/ml β-glycerophosphate had been added. The specimens then were washed in a Ringer's solution before being further processed for TEM. $CeCl_3$ was added to the incubation solution as a tracer to demonstrate the localization of the β-glycerophosphate.

For the controls, the procedure was the same but β-glycerophosphate was omitted.

Light Microscopy

After fixation with 1.5% glutaraldehyde, the specimens were rinsed with a Ringer's solution and stained with Prussian blue according to Bunting[13] for the demonstration of iron. The preparations were stained with Kernechtrot according to Domagk[14] to visualize the nuclei. The stained specimens were dehydrated in a graded alcohol series up to alcohol 100% and embedded in Entellan embedding medium (Merck, Amsterdam, The Netherlands).

The preparations were examined by conventional brightfield light microscopy as well as with a Bio-Rad Lasersharp MRC-500 laser scanning microscope.

Transmission Electron Microscopy

The preparations were dehydrated in a graded alcohol series up to 100% and embedded in epoxy resin (Epon, Ladd Research Industry, Burlington, VT); ultrathin sections were cut on an LKB microtome (Bromma, Sweden), stained with lead hydroxide, and examined in a Philips EM 410 transmission electron microscope (Eindhoven, The Netherlands).

X-ray Microanalysis

X-ray microanalysis was performed with a Tracor (TN) 2000 X-ray microanalyser attached to a Philips EM 400

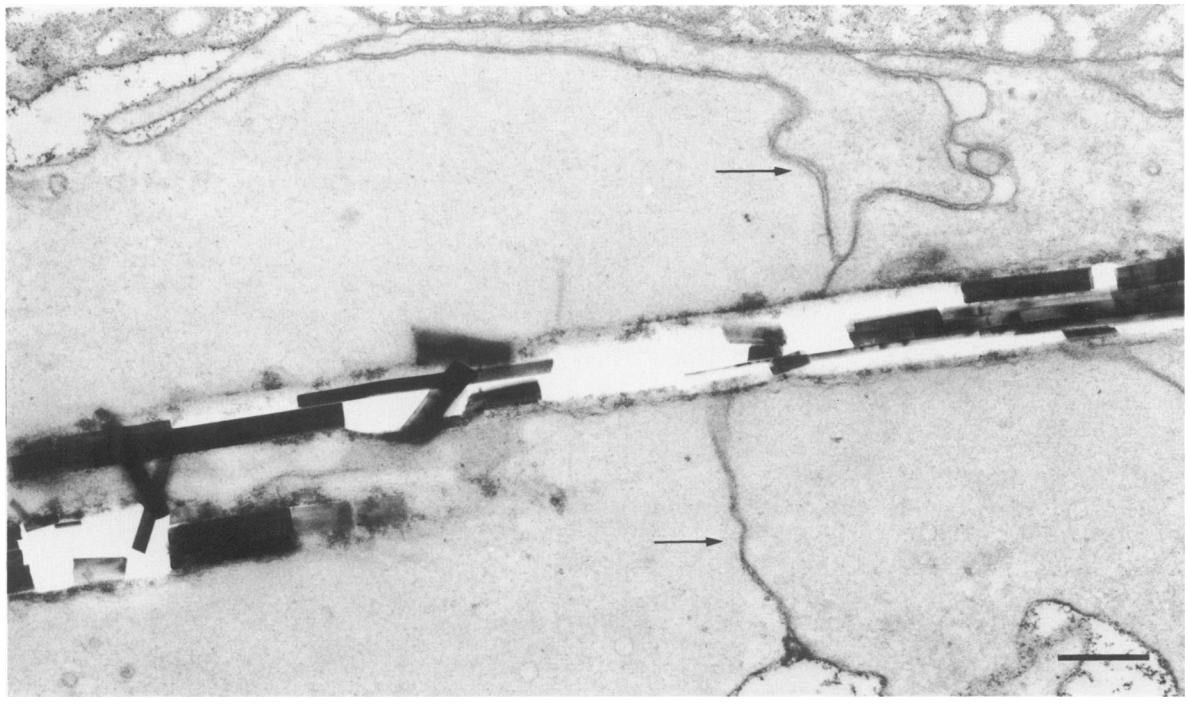

Figure 4. *Transmission electron micrograph showing part of an asbestos fiber surrounded by a number of cells. The place where two macrophages make contact can be seen. Communication between the space containing the asbestos fiber and the extracellular space can also be seen (arrows). Note the organelle-free cytoplasm surrounding the asbestos fiber. Fixation: glutaraldehyde, osmium(VI)-iron(II) complex. Bar: 0.25 μ.*

scanning transmission electron microscope. The system geometry of this instrument has been described elsewhere.[15] The sections were collected on copper grids that were placed in a low-background holder. The holder was tilted at an angle of 108 degrees relative to the electron beam. Measurements were performed for 100 s lifetime; the spot diameter was 100 nm and the accelerating voltage 80 kV.

Results

Cell Suspensions

In earlier works we have investigated the composition of the cell population of unstimulated mouse peritoneal cavities.[16,17] These studies showed that about 60% of the cells in the peritoneal cell suspensions were resident macrophages and about 25% were lymphocytes. Also, in the present investigation, TEM showed that according to the localization of reaction product after incubation for peroxidatic activity, these numbers of peritoneal resident macrophages (Figure 1) and lymphocytes were present in the isolated cell suspensions.

Control Cultures

The macrophages were still vital at the end of the longest interval studied (12 weeks), and the total number of macrophages determined at various intervals after the start of the experiment did not alter significantly. Some of the culture dishes contained very flat cells with a large oval to round nucleus. On basis of their morphology, these cells were identified as fibroblasts. The fibroblasts gradually increased in number during the course of the experiments, but their presence or absence had no noticeable effect on the vitality of the macrophages, as indicated by their structural appearance, or on the investigated phenomena, ie, the number of asbestos bodies formed.

On the eighth day of culture, a number of multinucleated giant cells were seen. Initially, there were only two to three nuclei in the cytoplasm of these giant cells. During the further course of the experiment, the number of multinucleated cells increased slowly to 13.4 ± 0.9% at the longest interval (12 weeks), but the total number of nuclei per giant cell remained relatively low. Giant cells containing more than five nuclei were not seen.

Transmission electron microscopy showed that the morphology of the macrophages underwent characteristic alterations during culture. At 24 and 48 hours, the nuclei became circular and the plasma membrane had a

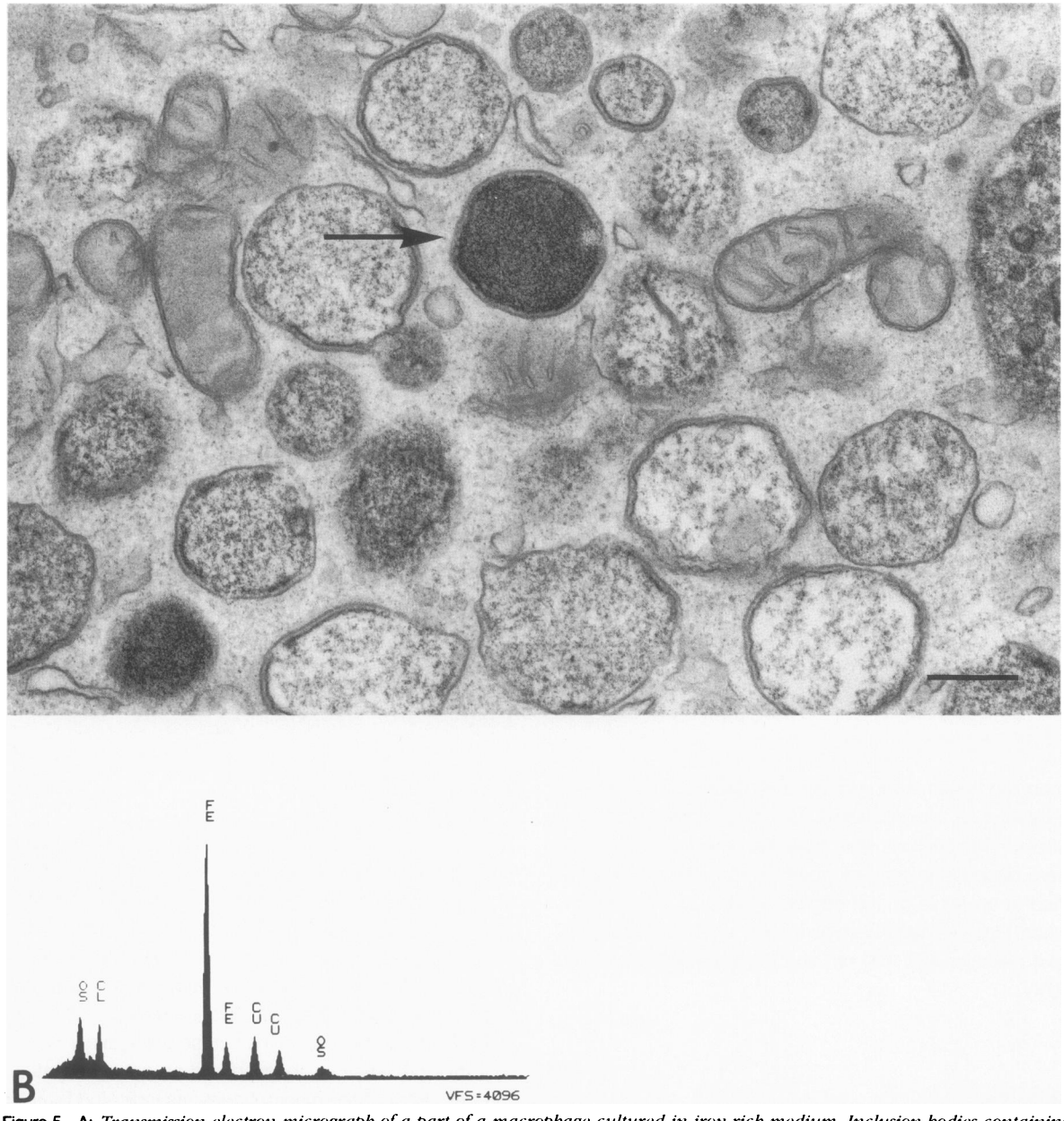

Figure 5. **A:** *Transmission electron micrograph of a part of a macrophage cultured in iron-rich medium. Inclusion bodies containing variably dense material are visible. Fixation: glutaraldehyde, osmium(VI)-iron(II) complex. Bar: 0.5 μ.* **B:** *x-ray microanalysis spectrum of the dark inclusion body in* **A** *(arrow), showing the presence of iron. (Vertical full scale: 4,096 counts).*

rather smooth contour. An increasing number of cytoplasmic vacuoles were present (Figure 2). After the longer culture periods, there were many of those (Figure 3) and the formation of blebs (cytoplasmic bulges) was sometimes observed. Occasionally, lipid droplets were seen. The cells in cultures incubated to demonstrate peroxidatic activity were peroxidase-negative at 24 hours or longer after the start of the culture.

Endocytosis of Asbestos Fibers

At 16 hours, the macrophages had endocytosed the majority of the asbestos fibers. A number of the asbestos fibers were too long to be completely ingested by one macrophage or multinucleated cell. In general, a number of macrophages were attached to these long fibers at the same time. Total engulfment of a relatively small fiber (about 10 μ long) by one macrophage or multinucleated cell was sometimes impossible because two macrophages had started to endocytose it simultaneously.

A small proportion of the asbestos fibers were endocytosed by fibroblasts if such cells were present in the culture dishes. However, these fibers were always small enough to be completely ingested by a single cell.

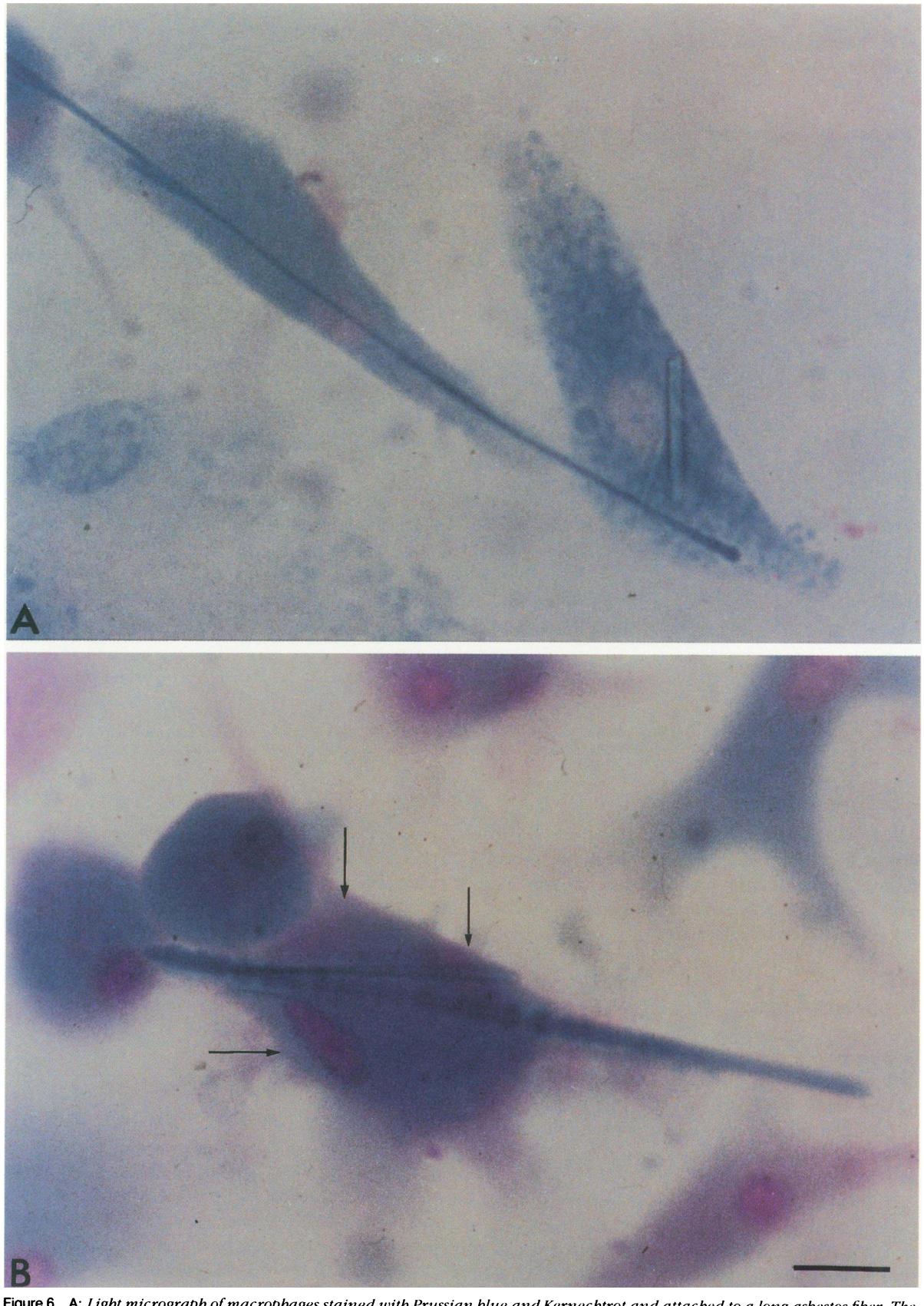

Figure 6. A: *Light micrograph of macrophages stained with Prussian blue and Kernechtrot and attached to a long asbestos fiber. The cells were fixed after 4 weeks of culture. A blue stain indicating iron deposition is visible along the asbestos fiber.* b: *Light micrograph showing a segmented asbestos body fixed after 8 weeks of culture. The preparation was stained with Prussian blue and Kernechtrot. Note the presence of at least three nuclei in the cytoplasm of the large cell containing the greater part of the asbestos body (arrows), and also the intense staining of the asbestos body coat, pointing to a relatively high iron concentration. Bar: 5 μ.*

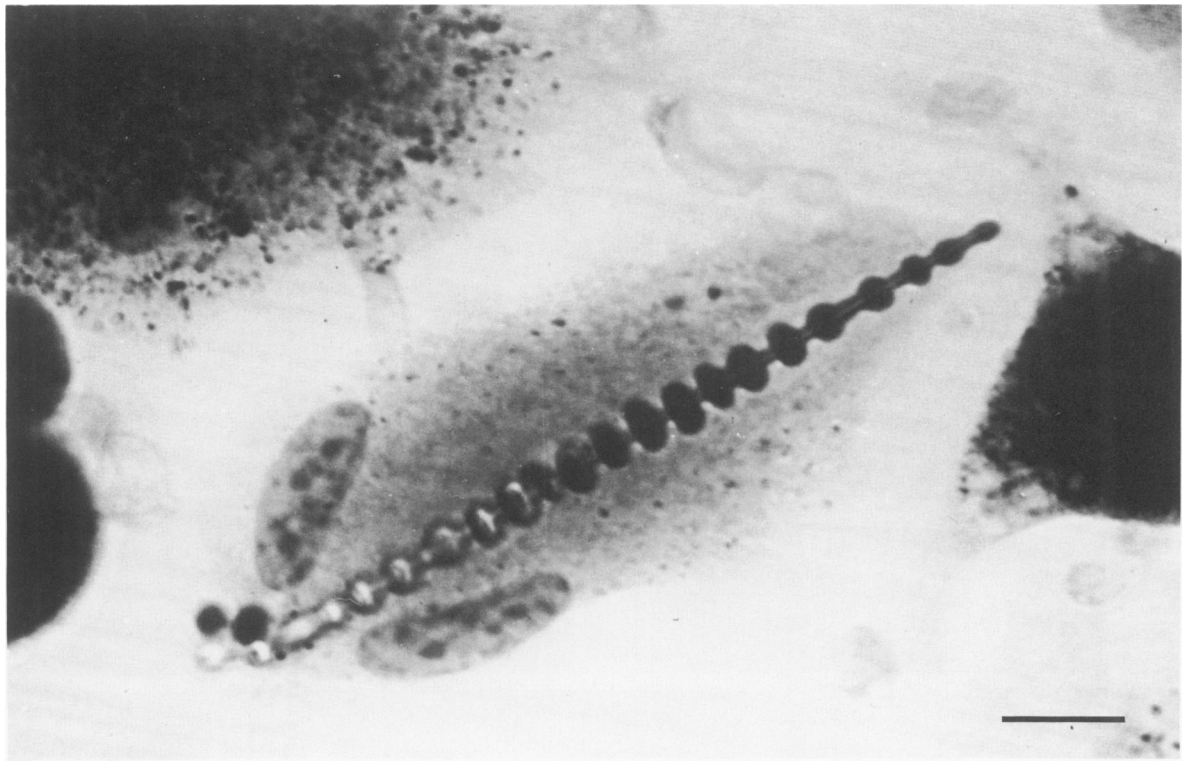

Figure 7. *Light micrograph showing a segmented asbestos body fixed after 12 weeks of culture. The micrograph was made with a Bio-Rad Lasersharp MCR-500 laser scanning microscope, which revealed the beaded appearance of the asbestos body clearly. Bar: 5 μ.*

Transmission electron microscopy of the macrophages attached to asbestos fibers showed the typical organelle-free zones seen in the *in vivo* experiments[7] (Figure 4). At places where two macrophages met, the contact between them was intimate.

Deposition of Iron

Despite the low concentration of iron in the standard DMEM culture medium, light microscopy performed on material fixed 6 weeks or longer after the addition of asbestos fibers occasionally showed extremely thin layers of Prussian blue stained material along asbestos fibers (not shown).

The addition of iron dextran to the culture media led to the formation of numerous electron-dense inclusion bodies in the macrophages. Transmission electron microscopy showed that the number of inclusion bodies per macrophage was variable (110.5 ± 56.1 per cell section) and that within one cell-type the electron density of the inclusion bodies was variable as well (Figure 5A). These electron-dense bodies contained iron, as was demonstrated by XRMA (Figure 5B). The values obtained for the net count rate with XRMA showed that variation in electron density referred to variations in the iron concentration. Although additional iron was present in the culture media throughout the total culture period, a persisting increase in the number of inclusion bodies per macrophage was not seen.

Like the cells in the control cultures, the cells incubated in the media containing asbestos and higher concentrations of iron were still vital at the end of the longest culture period.

Formation of Asbestos Bodies

As already mentioned, macrophages cultured in the control and the iron-rich media contained small asbestos fragments, and macrophages exposed to asbestos fibers too long to be completely ingested attached themselves to such fibers. Light microscopy of material cultured with asbestos fibers for 4 weeks and longer before being studied with Prussian blue showed that asbestos body formation had occurred (Figure 6A). At 4 weeks the coats were still thin and segmentation was only seen occasionally, but the thickness of the asbestos bodies increased with increasing duration of the culture period and segmentation became more prominent (Figure 6B). Confocal laser mi-

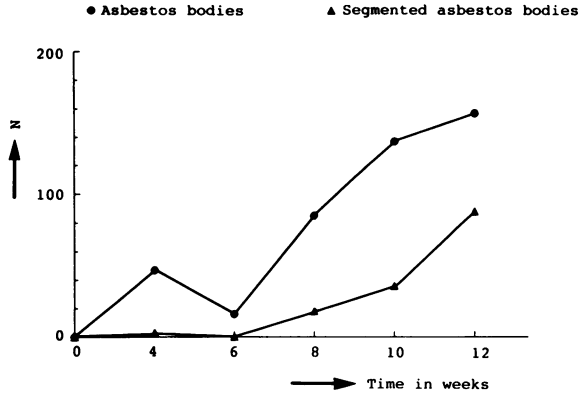

Figure 8. *Graph showing the proportion of asbestos bodies with a beaded appearance among the total number of asbestos bodies detected in a randomly chosen area measuring 10,000 × 3,000 μ.*

croscopy was found to be valuable for studies concerning the rate of segmentation, as it revealed the typical beaded appearance of asbestos bodies (Figure 7). As the graph in Figure 8 shows, the number of asbestos bodies of the beaded type increased with time. This graph is based on the data obtained from randomly chosen fields measuring 10,000 × 3,000 μ.

The relative number of coated fibers also increased with time, as shown in Table 1. The data in this table represent the findings in a randomly chosen field in which fibers and asbestos bodies were counted until a total of 10 asbestos bodies had been found. It is evident from Table 1 that the proportion of coated fibers increased with time too, and that almost all of the fibers with a length of 25 μ or more had given rise to asbestos body formation by the end of the 12th week. Coated fibers shorter than 25 μ were rarely observed.

Transmission electron microscopy showed that the coats were composed of the same characteristic electron-dense material as in the *in vivo* study[7] and invariably coincided with the appearance of type I asbestos bodies (Figure 9A, B). Asbestos bodies with the same morphology as asbestos bodies of types II, III, or IV as found in the *in vivo* study were not seen in this material. X-ray microanalysis showed the presence of iron and frequently also of chlorine in the coats of the asbestos bodies. Calcium and phosphorus were not detected.

The time-dependent increase in the thickness of the asbestos-body coats observed in the light-microscopical preparations was confirmed by the TEM observations, which also showed an increase in the number of iron micelles (compare Figure 9A with Figure 10B).

Culture in Manganese-rich Medium

Coating of the fibers in manganese-rich media remained at the level of the control cultures. Here, too, very thin Prussian blue-positive coatings were sometimes detected. Manganese was not detectable by XRMA in inclusion bodies in the macrophages or in deposits on long asbestos fibers.

Incorporation of Colloidal Gold

For investigation of the involvement of inclusion bodies in the process of asbestos body formation, a number of cultures were given a medium containing colloidal gold particles at the end of the first week of culture.

The gold particles were endocytosed by the macrophages and then enclosed in the inclusion bodies (Figure 10A). These gold particles too were incorporated into the coats of the asbestos bodies (Figure 10B).

Acid Phosphatase Activity

To determine the nature of the inclusion bodies, we processed a number of cultures for the demonstration of acid phosphatase activity. The tracer used for the localization of the β-glycerophosphate was $CeCl_3$. A cerium signal was demonstrated with XRMA in the inclusion bodies and in the coat of the asbestos bodies (Figure 10C). Point analyses performed in the nucleus and cytoplasm were negative for cerium, which was also found to be

Table 1. *Results of an Evaluation Yielding the Relative Proportion of Asbestos Fibers Giving Rise to the Formation of an Asbestos Body*

	Percentage of coated asbestos fibers				
Length in μ	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
0–25	0.0	0.0	0.4	0.0	0.0
25–50	9.0	2.0	25.7	24.5	69.9
50–75	33.8	36.4	100	100	100
>75	47.8	100	100	100	100

It is clear that this proportion increases with time. This is accompanied by a decrease in the average length of fibers that became coated. It is evident that after the longest interval studied (12 weeks) almost 100% of the fibers measuring 25 μ and longer have become asbestos bodies.

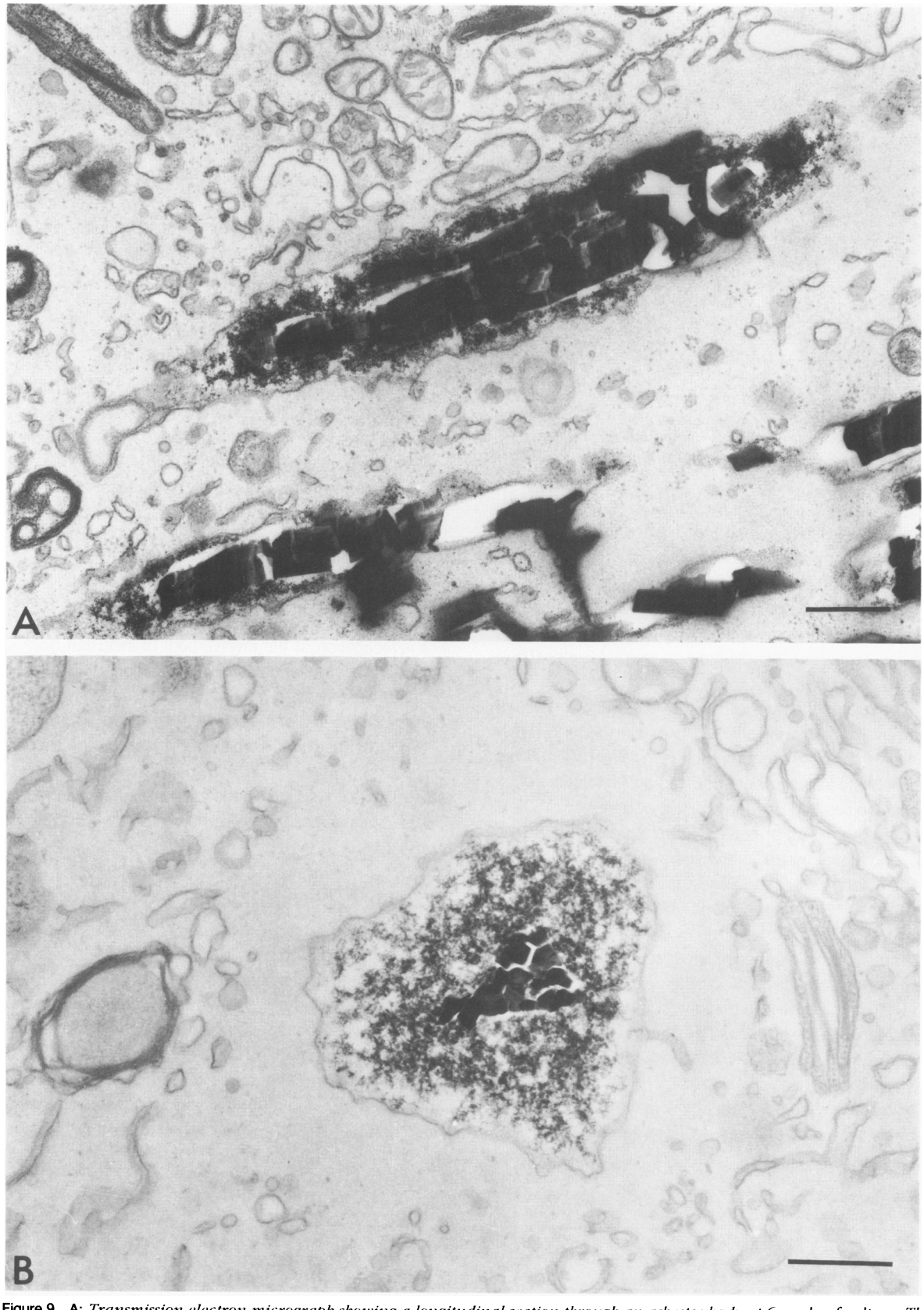

Figure 9. **A:** *Transmission electron micrograph showing a longitudinal section through an asbestos body at 6 weeks of culture. The deposits of iron along the fiber are clearly visible. Note the characteristic organelle-free zone surrounding the asbestos fibers.* **B:** *Transmission electron micrograph showing a transverse section through an asbestos body at 6 weeks of culture. Similar dark deposits and an organelle-free zone are clearly visible. Fixation: glutaraldehyde, osmium(VI)-iron(II) complex. Bar: 0.5 μ.*

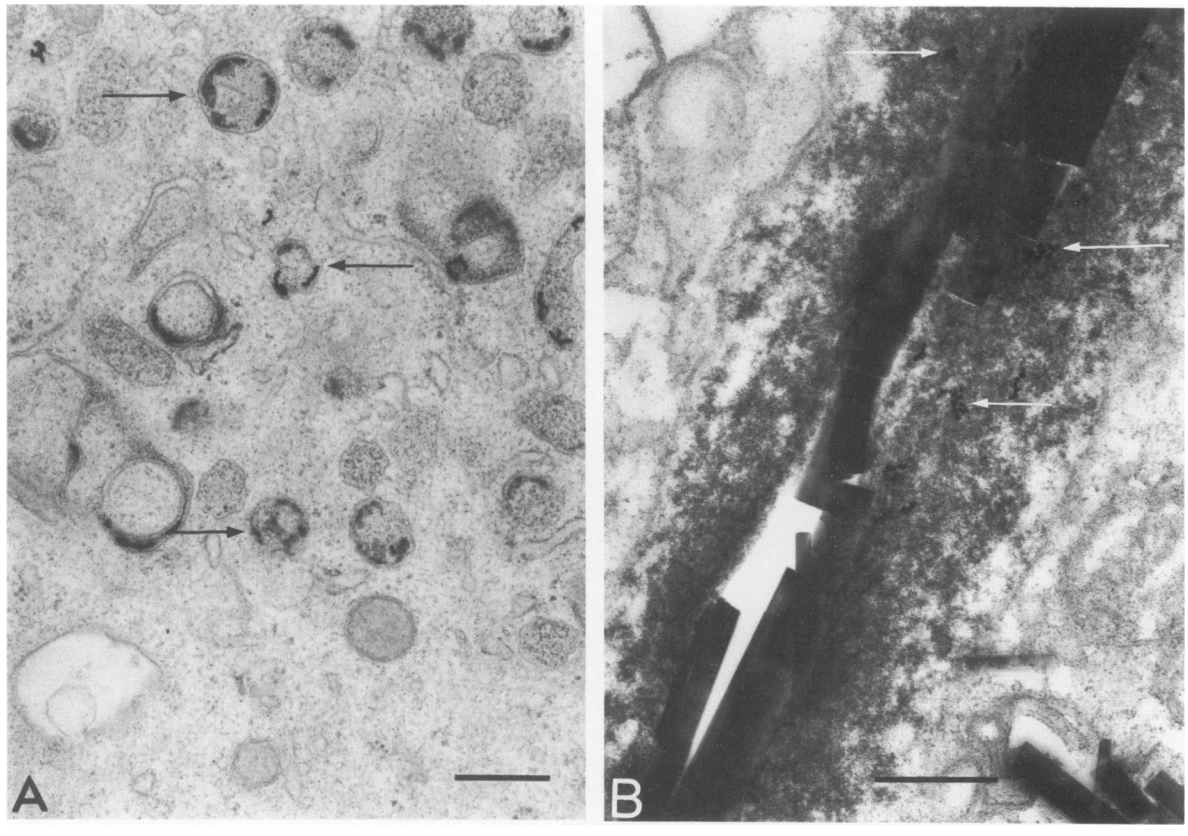

Figure 10. A: *Transmission electron micrograph showing a part of a macrophage fixed at 4 weeks of culture. The macrophages had been exposed to a culture medium containing colloidal gold particles for 24 hours. Note that the colloidal gold has been incorporated into the inclusion bodies (arrows).* B: *Transmission electron micrograph showing an asbestos body from the same culture at 12 weeks. Colloidal gold particles according to our hypothesis indicative for fusion of inclusion bodies (like those in* A*) with the space containing the asbestos body, are visible at various places in the coat of this asbestos body (arrows). Fixation: glutaraldehyde, osmium(VI)-iron(II) complex. Bar: 0.5 μ.* C: *X-ray microanalysis spectrum of the asbestos body in* B. *This technique shows not only an iron signal but also a cerium signal, indicating the presence of acid phosphatase in the asbestos body coat. (Vertical full scale 1,024 counts.)*

absent in inclusion bodies and the asbestos bodies in the control experiments where β-glycerophosphate was omitted.

Formation of Cell Aggregates

Aggregation of cells was seen in culture dishes in which both fibroblasts and macrophages were present (Figure 11). Light microscopy and especially TEM showed that these relatively large concentrations of cells contained both fibroblasts and macrophages. Numerous asbestos fibers as well as asbestos bodies were present in the aggregates. Collagen was as a rule present in the extracellular space within the aggregates.

Discussion

The effects of asbestos fibers on cells in culture have been studied.[18–22] It has been reported that crocidolite asbestos added to macrophages in culture is cytotoxic and leads to increased cell death.[23–25] These cytotoxic effects were, however, related to high concentrations of asbestos. Bey and Harington[26] reported that macrophages in culture exposed to low concentrations of crocidolite asbestos fibers remained vital for a period of 10 days. The formation of asbestos bodies in a macrophage culture assay has, to the best of our knowledge, never been reported.

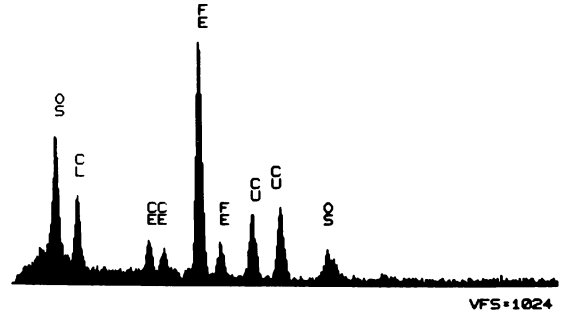

Figure 10 (*continued*)

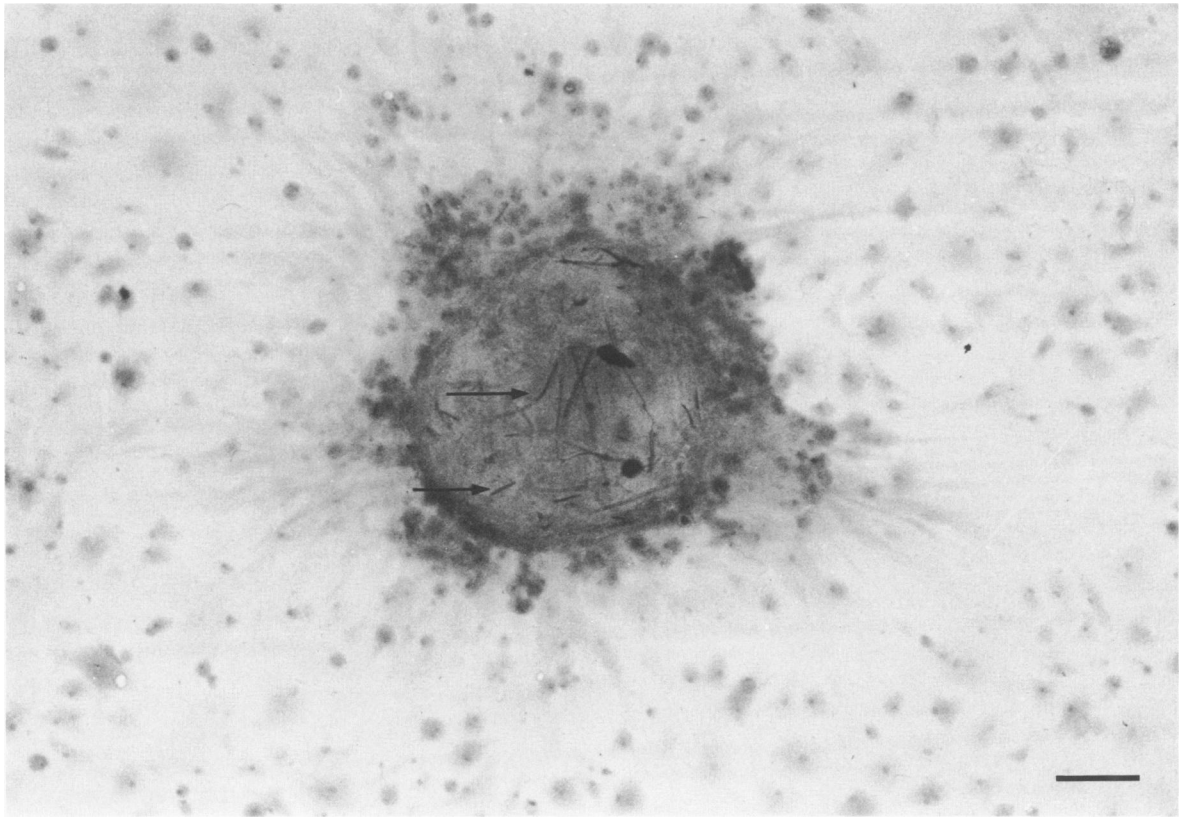

Figure 11. *Light micrograph showing a cell aggregate containing many asbestos fibers (arrows). Aggregates of this type were frequently seen in culture dishes in which not only macrophages but also fibroblasts were present. The relatively large number of asbestos fibers in such aggregates suggests an active transport of fibers to these locations. Staining: Prussian blue and Kernechtrot, Bar: 50 μ.*

In the present study, macrophages survived after exposure to relatively low concentrations of crocidolite. Even after a culture period of 84 days, the cells were still vital, as deduced from their morphology and from several features: they did not round off, they remained adherent, were still capable of endocytosing gold particles, and the process of asbestos body formation continued throughout the culture period. Further, cell numbers did not decrease markedly. The ingestion of asbestos fibers did not reduce the endocytotic capacity of the macrophages either, as shown by the uptake of gold particles 1 week after the start of the experiment.

The present report describes the formation of asbestos bodies by mouse peritoneal macrophages in culture. The sequence of the separate steps in asbestos body formation seen in this study was similar to that observed *in vivo* by Botham and Holt,[8] who distinguished a number of steps in the process of asbestos body formation around anthophyllite asbestos fibers in the lungs of guinea pigs. The next steps described by Botham and Holt, and also by us in an earlier report,[7] are successively the development of giant cells, the formation of an iron-containing coat along the fibers, the development of an irregular outline of the coat, the formation of young asbestos bodies, and finally the formation of mature asbestos bodies. These steps, and roughly also the duration of each step as observed in the present study, are very similar to those reported by Botham and Holt as well as the data obtained in our *in vivo* study. According to Botham and Holt, the source of the iron essential for the formation of the iron-rich coat is hemolysis. Although, in our opinion, the digestion of erythrocytes by macrophages was not the source for the iron,[7] the present investigation has shown that an increase of the iron concentration in the culture media is indeed related to increase in asbestos body formation. The present study showed also the occurrence of a number of phenomena:

1. Macrophages cultured in iron-rich media contain many iron-rich inclusion bodies.
2. Iron-rich inclusion bodies are lysosomal in origin, as can be demonstrated by the use of an acid phosphatase reaction.
3. Small gold particles endocytosed by the macrophages are found in the inclusion bodies and also in the asbestos bodies.

On the basis of these observations and the results of our previous work, the following mechanism of asbestos

body formation is postulated. Macrophages in the vicinity of asbestos fibers attach to these fibers in an attempt to ingest them. If, however, a fiber is too long to be completely ingested, the macrophage can only surround part of that fiber, and this gives rise to a micro-environment between the macrophage and the asbestos fiber. This type of contact between the macrophages and the long fibers leads to exocytosis of the lysosomal contents, which are deposited in the space between the fiber and the macrophage. Thus, when an iron compound is present in the lysosomes, an asbestos body comes into existence. Enhancement of the iron concentration in the culture media led to an increase in the number and average size of the asbestos bodies. This observation supports the hypothesis that asbestos body formation is dependent on the contents of the lysosomes and is therefore a coincidental phenomenon. This hypothesis is also supported by the findings in cells cultured in media containing colloidal gold particles. These particles initially present in lysosomes were later also found in the asbestos body coats. In material cultured in a medium rich in manganese, an element that does not accumulate in macrophage lysosomes, the manganese did not reappear in the inclusion bodies or the asbestos body coats. No indication pointing to increased asbestos body formation was obtained from this material.

Because coating of asbestos fibers was found only at places where contact occurred between macrophages and asbestos fibers, and because the lysosomal contents accumulated at that site, we concluded that the macrophages seal off this part of the extracellular space, thus forming a separate compartment from which the exocytosed lysosomal contents cannot escape.

The formation of such a compartment and the exocytosis of lysosomal contents into it suggests analogy with the process of bone resorption by osteoclasts, where the osteoclasts form an extracellular micro-environment into which the lysosomal enzymes are released.[27]

Asbestos bodies were sometimes formed on asbestos fibers that were actually short enough to be ingested by a single macrophage or giant cell. These were fibers that had been approached by two macrophages simultaneously, making engulfment of the fiber by only one of them impossible. This means that such fibers will not be ingested and can serve as the basis for asbestos body formation.

As Figure 8 shows, an increase in the number of asbestos bodies showing the characteristic beaded appearance was observed in the material studied in this investigation. Because the rate of segmentation increased as a function of time, we conclude that this phenomenon is related to maturation and does not depend on mechanical forces as suggested by Mace et al.[28]

Asbestos bodies with the characteristic morphology or chemical composition of types II, III, and IV[7] were not seen in this study. This means that the formation of different types of asbestos bodies is related to systemic variations that are not initiated in stable and strictly controlled macrophage cultures.

The occurrence of cell aggregates resembles the aggregation and granuloma formation found in our *in vivo* experiments.[7] A chemotactic response induced by the interaction between asbestos fibers and macrophages or fibroblasts is probably responsible for this aggregation. Such a factor is indeed known to be released in the supernatant of cultures of rat alveolar macrophages exposed to crocidolite asbestos fibers.[29] The presence of relatively large numbers of asbestos fibers in these aggregates suggests that the fibers were transported to these loci by macrophages.

In sum, the present findings show that the formation of asbestos bodies by mouse peritoneal macrophages can be initiated *in vitro.* Further, the results support our earlier assumption that asbestos body formation is at least partially the result of exocytotic activity of macrophages. Finally, this study has yielded a model making it possible to obtain more information on the mechanism of asbestos body formation.

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