# Immunoelectronmicroscopic Analysis of Surface Antigens Common to *Mycobacterium bovis* (BCG) and Tumor Cells [1,2]

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**SUMMARY—The presence of cell-surface antigens on line-10 hepatocarcinoma cells was demonstrated by immunoelectronmicroscopy (IEM) with the use of xenogeneic and syngeneic tumor-specific antibody and syngeneic BCG-induced antibody. This confirmed that in addition to tumor-specific antigen(s) there are common antigens between line-10 hepatocarcinoma cells and BCG organisms. The specificity of this cross-reactivity was further supported by blocking experiments and absorption of the specific antibody to BCG organisms, with subsequent IEM of the BCG and loss of reactivity to tumor cells. Complement-dependent serum cytotoxicity was correlated with the IEM assay. Cross-reactivity of the BCG-specific antibody with the surface of cells of a human melanoma culture grown from an excised subcutaneous melanoma was also demonstrated by IEM.—J Natl Cancer Inst 53: 1313–1323, 1974.**

TUMOR-SPECIFIC CELL SURFACE ANTIGENS have been demonstrated by various direct and indirect techniques. Immunoelectronmicroscopy (IEM), one of the most sensitive direct assays, has shown that tumor-specific antigenic sites of various sizes occur in discrete areas of the cell surface (*1*, *2*). This technique provides a valuable means of determining specificity of reactivities and the topographic distribution of antigens on the cell surface.

A guinea pig immunotherapy model was developed with transplantable syngeneic hepatocarcinoma cell lines (lines 1 and 10) and *Mycobacterium bovis* (BCG) (*3*, *4*). In this model, the natural or BCG-mediated development of transplantation immunity against line 1 or 10 tumor-specific antigen was detected by delayed-hypersensitivity reaction and transplantation rejection (*5–7*). Formation of xenogeneic antibodies against tumor-specific antigens of both line-1 and line-10 cells was demonstrated by complement fixation, transfer test, and immunofluorescence assays (*8*); also, antibody development to these 2 hepatoma cell lines was detectable in syngeneic hosts (*9*).

The observation by Borsos and Rapp (*10*) of an antigenic relationship between BCG organisms and line-10 cells is significant with regard to immunotherapy. We have attempted to confirm and expand this finding by IEM. The present report describes a qualitative IEM assay to determine the specificity of various antisera against 3 types of tumor cells (i.e., line-1 and line-10 hepatocarcinoma cells of the strain-2 guinea pig, and an early-passage human melanoma cell culture), and it demonstrates cross-reactivity of the BCG-specific antibody with cell-surface antigens of the line-10 hepatocarcinoma and the melanoma cells.

## MATERIALS AND METHODS

*Animals.*—Male (C57BL/6 ♀ × C3H/Anf♂)$F_1$ ($B6C3F_1$) mice 8 weeks old were housed 10 per cage. They were immunized with line-10 cells.

Inbred male guinea pigs (Sewall Wright strain 2), obtained from the Laboratory Aids Branch, Division of Research Services, National Institutes of Health (Bethesda, Md.), were syngeneic as shown by skin grafting. They were housed 5 per cage and fed Wayne guinea pig chow and kale or cabbage. Retired male breeders were immunized with BCG.

*Tumor and normal control cell lines.*—Line-1 and line-10 hepatocarcinomas were originally induced in strain-2 male guinea pigs by diethylnitrosamine in the drinking water (*4*). The ascites form of these tumor lines was used for continuous passage. Tumor cells were harvested from the peritoneal cavity of prospective donors, washed 3 times with cold Hanks' balanced salt solution (HBSS), and kept in an ice bath until used. The cells in suspension have a 90–95% viability by trypan-blue exclusion.

Human melanoma cells were grown from an excised subcutaneous melanoma and passaged 5 times in tissue culture in our laboratory. For the present studies, cells from passages 1–3 were used.

Human fibroblast WI-38 (#75, American Type Culture Collection, Rockville, Md.), a human diploid cell line derived from normal embryonic lung tissue, and a human foreskin diploid fibroblast cell culture obtained from the laboratory of Dr. James Regan (Oak Ridge National Laboratory) were used as control cells.

*BCG.*—Fresh frozen *Mycobacterium bovis* strain BCG (Phipps strain TMC1029) was obtained from Trudeau Institute, Saranac Lake, New York. The preparations (containing $1 \times 10^8$ to $2 \times 10^8$ live BCG/ml) were kept frozen at −70° C until just before use, at which time they were rapidly thawed in a water bath. This preparation was used for immunization and treatment of guinea pigs. Lyophilized BCG, Glaxo strain, obtained from Eli Lilly Pharmaceutical Co., Indianapolis, Indiana, was used for the absorption of the anti-BCG serum and in the subsequent IEM assay.

*Sera.*—Anti-BCG serum was obtained by immunization of retired male breeders (Sewall Wright

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strain 2). Guinea pigs were inoculated intravenously (iv) weekly with an increasing dose of BCG. The first dose ($1\times10^7$) was taken from a freshly thawed vial, and the rest of the suspension was kept in the cold room until its use in subsequent iv injections ($2\times10^7$ and $4\times10^7$ BCG). The animals were bled 10 days after the last injection. The serum was decomplemented at 56° C for 30 minutes and stored at −70° C.

For absorption of BCG reactivity, 0.5 ml test serum was added to 1 vial of lyophilized BCG, Glaxo strain, containing $3\times10^8$ BCG and incubated in a 37° C water bath for 1 hour with intermittent shaking. The suspension was centrifuged at 3000 rpm for 10 minutes, and the supernatant serum was saved for further assay. The pellet was rinsed twice with phosphate-buffered saline (PBS) and then incubated with ferritin-conjugated anti-guinea pig serum for IEM assay. This test was used to indicate serum reactivity to BCG membrane. Where the anti-BCG activity was low, no precipitate was observed and the suspension was passed through a 0.45-$\mu$ pore Millipore filter. The filter was processed for IEM. No titrations were done on these sera.

Anti-line-10 serum was obtained by immunization of 8-week-old B6C3F$_1$ mice with 1 ml of a 1% line-10 suspension injected intraperitoneally. The mice were bled by cardiac puncture 10 days after immunization, and the pooled serum was absorbed for 2 hours in vivo in 3-day-old strain-2 guinea pigs. The amount of mouse serum injected per guinea pig varied as a function of the body weight of the recipient but was adjusted to achieve an approximate 1:4 dilution of mouse serum. The absorbed test serum was heat inactivated at 56° C and stored at −70° C until used. Mouse anti-line-10 serum was also absorbed 1:1 on fresh guinea pig (strain 2) erythrocytes. Three absorptions, 1 hour each, were done—2 at 37° C and 1 at 4° C.

Guinea pig anti-line-10 serum was immune serum obtained from animals that had undergone a BCG-mediated regression of an established intradermally transplanted. line-10 tumor. This mode of therapy and the time at which line-10-specific antibody can be detected by immunofluorescence, complement fixation, and transfer test were previously described (*9*). Control guinea pig serum was obtained from normal strain-2 animals weighing 400–500 g.

*IEM*.—An indirect immunoferritin labeling technique was used. Approximately $3\times10^6$ line-10 cells were incubated with 0.2 ml serum for 30 minutes in an ice bath. The cells were washed twice with ice-cold HBSS and incubated with 0.2 ml of either ferritin-conjugated rabbit anti-guinea-pig $\gamma$-globulin or ferritin-conjugated antimouse $\gamma$-globulin (Cappel Laboratories, Downington, Pa.). The mixture was incubated in an ice bath for 30 minutes. The cells were then washed twice with PBS (pH 7.4) and fixed for electron microscopy.

The cells were fixed, in an ice bath, in 2.3% *sym*-collidine-buffered glutaraldehyde (pH 7.3) for 1 hour followed by 1% *sym*-collidine-buffered osmium tetroxide (pH 7.3) and 1% aqueous uranyl acetate for 1 hour each. The cells were dehydrated in a graded series of ethanol and embedded in Spurr's low-viscosity medium (*11*). Thin sections were cut with a diamond knife on an LKB Ultratome III and mounted on naked 300-mesh copper grids. When counts of ferritin-labeled cells were made, the sections were mounted on copper grids with single 1×2-mm holes and were supported by carbon-coated Formvar (0.6%) membranes. The sections were stained with uranyl acetate followed by lead citrate and examined in a Hitachi HU-11B electron microscope operating at 50 kV.

In experiments in which the percent of ferritin-labeled cells was estimated, ≈50 cells were counted from each thin section. Thin sections were cut from 4 different regions of the block, and the values reported here represent the mean count. Only those cells with markedly distinct ferritin labels were scored as positive, regardless of the number of sites observed.

*Serum cytotoxicity test*.—Line-10 cells were labeled with $^{51}Cr$ by the addition of 100 $\mu$Ci $Na_2{}^{51}CrO_4$ to 1 ml cell suspension containing $3\times10^7$ cells, in a 50-ml conical centrifuge tube. The cells were kept for 45 minutes at 37° C in a $CO_2$ incubator with occasional shaking. The cells were washed twice with cold Eagle's basal medium and Earle's salts (EBME; Schwarz Bioresearch, Mt. Vernon, N.Y.) to remove unicorporated $^{51}Cr$. The cell pellet was resuspended in EBME containing 10% fetal calf serum (Microbiological Associates, Bethesda, Md.). Cell viability was determined by trypan blue exclusion, and the suspension was diluted to a final concentration of $1\times10^6$ live cells/ml; 0.1 ml of the suspension was incubated with 0.1 ml test serum and incubated for 30 minutes at 30° C. At the end of the incubation period, 1 ml Veronal-buffered saline with gelatin, $Ca^{++}$, and $Mg^{++}$ (VBS-G-$M^{++}$) was added to the mixture and centrifuged at 1500 rpm for 7 minutes (*12*). The supernatant was removed, and 0.1 ml of a 1:8 dilution of human serum as a source of complement was added to the pellet. The cells were resuspended and incubated for 60 minutes at 30° C. At the end of incubation, 0.7 ml cold PBS was added to each tube and centrifuged at 1500 rpm for 10 minutes. The supernatant was removed, transferred to a counting tube, and counted in a Packard Model 3002/Model 574 AutoGamma Spectrometer. Maximum $^{51}Cr$ release was determined as follows: 1 ml VBS-G-$M^{++}$ was added to 0.1 ml cell supension and centrifuged. The supernatant was removed, and 0.8 ml distilled water was added to the pellet. The mixture was frozen at −70° C, thawed in hot water 4 times, and then centrifuged at 1500 rpm. Duplicate tubes were made for each sample. Maximum $^{51}Cr$ release was measured in a fraction of the supernatant.

*Trypan blue exclusion test for cytotoxicity*.—The protocol for this assay was similar to that of the $^{51}Cr$ assay except that unlabeled cells were used. At the end of the incubation with human complement, the suspension was diluted by half with trypan blue; cells excluding the dye were scored as viable.

## RESULTS

IEM detected antibody both in serum from line-10 immune guinea pigs and in the guinea pig-absorbed mouse anti-line-10 serum that specifically reacted with line-10 cell-surface antigens. Moreover, guinea pig anti-BCG serum also reacted strongly with line-10 cell-surface sites. The detectable antigenic sites on the surface of line-10 cells incubated with all of the above-mentioned sera occurred in discrete areas and varied in size, depending on the reactivity of the test sera used (figs. 1–4).

Figures 3 and 4 are electron micrographs of line-10 cells incubated with anti-BCG serum. Although the actual size of the antigenic sites were not determined, discrete antigenic sites was closely adjacent to each other (fig. 4). No significant labeling (3 or more distinct antigenic sites) of line-10 cell-surface antigens was observed when normal mouse or normal guinea-pig serum was used (fig. 5).

We next tested these various sera against the line-1 hepatocarcinomas. The line-10 immune guinea pig serum, as well as the guinea pig-absorbed mouse anti-line-10 serum, did not react significantly with line-1 hepatocarcinoma cells. Although tests with the guinea pig anti-BCG serum demonstrated some minor labeling of line-1 cells, the pattern was not characteristic of that detected with line-10 cells. Quantitatively, the labeling of line-1 cells was not considered positive (fig. 6).

The cytotoxicity of the various sera was determined by a complement-dependent $^{51}$Cr-release assay (table 1). Mouse anti-line-10 serum thrice absorbed with guinea pig red blood cells (RBC) was included as a positive control. Line-10 cells incubated with RBC-absorbed mouse anti-line-10 serum showed a fairly uniform labeling of the cell surface, and this serum also gave the highest cytotoxicity value. In vivo absorption (in newborn guinea pigs) of mouse anti-line-10 serum removed the antibodies responsible for its cytotoxic activity (table 1). However, IEM revealed that 60% of the cells were still labeled with ferritin, which indicates that IEM is a more diagnostic assay for the tumor-specific antigen than the $^{51}$Cr cytotoxicity test. Because of the small sample size in these experiments, the data are not amenable to a statistical analysis; however, the values obtained with guinea pig anti-BCG serum and guinea pig line-10 immune serum were markedly higher than those obtained with either the normal guinea-pig serum or the normal mouse serum (table 1). Results with trypan blue exclusion test to evaluate cytotoxicity corresponded well with the $^{51}$Cr-release data (table 1).

Since the anti-BCG serum was not titered, we determined the presence of specific anti-BCG antibodies by incubating the serum with lyophilized BCG organisms (Glaxo strain) followed by ferritin-conjugated anti-guinea-pig $\gamma$-globulin. A strong ferritin-conjugated antibody reactivity with the BCG organisms was observed after this treatment (fig. 7). The serum obtained after BCG absorption was incubated with line-10 cells for IEM, and a reduction in reactivity (table 2) indicated that the anti-BCG antibodies were effectively removed by the BCG organisms.

Having demonstrated cross-reactivity of the anti-BCG serum with line-10 cells, we were interested to know if the mouse anti-line-10 serum would cross-react with BCG organisms. Incubation of BCG organisms with mouse anti-line-10 serum showed significant labeling of the BCG organisms (fig. 8). When BCG organisms were incubated with serum from a normal 3-day-old guinea pig, no labeling was observed, and only a trace labeling was seen with serum from a normal 8-week-old mouse (fig. 9). These data provide further evidence for the presence of common antigens between line-10 hepatocarcinoma cells and BCG organisms. After incubation of BCG organisms with anti-BCG serum, a visible precipitate was obtained after centrifugation of the mixture at 3000 rpm, whereas no precipitate was observed with the other sera.

From these results, an important question is whether there is a line-10 tumor-specific antigen (TSA) unique from the shared BCG antigenic site of the tumor cell. Although we do not have definitive evidence, we have performed a preliminary experiment suggesting that these are distinct antigens. Ferritin-labeled cells were scored at the ultrastructural level in a protocol attempting to block specific reactivities. When line-10 cells were incubated sequentially with guinea pig anti-BCG serum and (in vivo) absorbed mouse anti-

TABLE 1.—*Serum cytotoxicity of various sera against line-10 hepatocarcinoma cells*

| Test serum | Serum dilution | $^{51}$Cr release (% cytotoxicity) [a] | Dye exclusion (% cytotoxicity) |
|---|---|---|---|
| Mouse anti-line-10 (guinea pig RBC-absorbed) | 1:2 | 64.40±7.75 (5) | 66 |
| | 1:4 | 59.80±9.66 (7) | |
| Mouse anti-line-10 (guinea pig-absorbed in vivo) | 1:4 | 0–2.9 (2) | 7 |
| Guinea pig line-10 immune serum | Undiluted | 19.10±4.07 (4) | — |
| Guinea pig anti-BCG serum | Undiluted | 28.60±5.31 (3) | 27 |
| | 1:2 | 15.4–19.8 (2) | — |
| Normal guinea-pig serum | Undiluted | 0–2.3 (2) | — |
| | 1:2 | 1.42±1.20(4) | 11 |
| Normal mouse serum | 1:2 | 8.93±2.39(3) | — |
| | 1:4 | 7.36±1.95(3) | 9 |

[a] Values corrected for cytotoxicity due to human complement alone, which was 4.56±2.68%, and expressed as the mean ±1 SD except where only 2 samples were run and only the range is given. Number of samples run in the test is given in *parentheses*.

TABLE 2.—*Cross-reactivity of mouse anti-line-10 serum absorbed in vivo and guinea pig anti-BCG serum*

| Test sera | Percent of ferritin-labeled line-10 cells [a] |
|---|---|
| Guinea pig anti-BCG serum + mouse anti-line-10 serum absorbed in vivo + ferritin-conjugated rabbit antimouse γ-globulin | 33 |
| Mouse anti-line-10 serum absorbed in vivo + guinea pig anti-BCG serum + ferritin-conjugated rabbit anti-guinea pig γ-globulin | 16 |
| BCG-absorbed guinea pig anti-BCG serum | 16 |
| BCG-absorbed mouse anti-line-10 absorbed in vivo | 40 |

[a] *See* "Materials and Methods" for details of sequential serum incubation and BCG absorption.

line-10 serum (containing antibody specific for both line-10 TSA and common BCG antigenic sites), followed by ferritin-conjugated rabbit antimouse γ-globulin, 33% of the cells had more than 4 ferritin conjugate-reactive sites per thin section of the cell surface. In contrast, sequential incubation of line-10 cells with mouse anti-line-10 serum followed by guinea pig anti-BCG serum and ferritin-conjugated anti-guinea pig γ-globulin showed only 16% of the cell cross sections labeled. Also, in this group only 1 or 2 reactive sites were detected per cell section.

The 2 control groups in this experiment included Glaxo BCG-absorbed guinea pig anti-BCG serum reacted with ferritin-conjugated rabbit anti-guinea pig γ-globulin and Glaxo BCG-absorbed mouse anti-line-10 serum. The former treatment group resulted in 16% of the sections being labeled with 1 or 2 positive sites. This is identical with the results obtained when we assumed blocking had occurred, which indicates that 16% is a background level. The BCG-absorbed mouse anti-line-10 reaction caused 40% labeled cells with several reactive sites per cross section; we consider this to be the level of resolution for line-10 TSA sites alone. The twofold reduction in ferritin labeling in the blocking experiment, though not a definitive result, suggests that mouse anti-line-10 serum masked the BCG-reactive sites on the tumor cell surface. Thus, from the reciprocal test, it could be assumed that the line-10 TSA is distinct from the common BCG antigenic sites of line-10 cells.

Because BCG is being used clinically in immunotherapy of human melanoma (*13*), we investigated the possibility of a cross-reactivity of the anti-BCG serum with a single in vitro-passaged human melanoma culture. Reactivity of the guinea pig anti-BCG serum was strong with melanoma cells scraped from the tissue culture plates of melanoma passages 1–3 (fig. 10), as well as in situ with cells grown on coverslips or Millipore filters (fig. 11). Incubation of melanoma cells with normal guinea pig or normal mouse serum showed no reactivity. Incubation of melanoma cells with BCG-absorbed anti-BCG serum also showed no significant labeling (fig. 12). Melanoma cells were also incubated with mouse anti-line-10 serum, and this showed significant labeling (fig. 13); however, the reactivity was less than that detected with guinea pig anti-BCG serum. Control normal human fibroblasts (fig. 14) and foreskin cells (fig. 15) grown under identical tissue culture conditions showed no labeling when tested with guinea pig anti-BCG serum. Quantitation of the degree of labeling was not attempted in this study because of the difficulty in dispersing the cells without trypsinization. In an attempt to absorb the anti-BCG serum with human melanoma cells, we observed that, even after the third absorption of serum on overgrown plates, the serum still had considerable activity as shown by immunoferritin assay.

## DISCUSSION

Utilizing IEM, we demonstrated specific cell-surface antigens on guinea pig line-10 hepatocarcinoma cells. Furthermore, we confirmed that these cells also possess a cell-surface antigen common to BCG cell walls. Despite the limitations of quantitating labeled cells at the ultrastructural level, this assay was used to determine the immunologic cross-reactivity of line-10 or BCG-immune sera with respect to both anti-BCG and anti-line-10 reactivity. Cross-reactivity was also supported by complement-dependent serum cytotoxicity tests. The specificity of BCG-immune sera for common antigenic sites on line-10 cells was further verified by absorption of specific antibody to BCG organisms, with subsequent positive IEM reactivity of the absorbed sera with BCG organisms and negative IEM reactivity with line-10 cells.

Results from sequential incubation of line-10 cells with guinea pig anti-BCG serum, which should have a single specificity on line-10 cells, and mouse anti-line-10 serum absorbed in vivo, which should have multiple reactivities including the anti-BCG reactive sites, indicate that the line-10 TSA sites may be distinct from those shared with BCG. This confirms and supplements a previous report that there are common antigens between the line-10 tumor cell and BCG (*10*). The lack of anti-BCG reactivity with the highly antigenic line-1 cells is of interest, since line-1 tumor, although syngeneic, is rejected when transplanted to strain-2 guinea pigs (*6*). Borsos and Rapp (*10*) also showed by complement fixation and transfer test that guinea pigs immunized to BCG alone developed antibodies to the transplantable guinea pig line-10 hepatocarcinoma cells but not to line-1 cells.

The demonstration of common antigens between the weakly antigenic, transplantable line-10 hepatocarcinoma and BCG organisms raises some interesting points regarding the biologic aspects of the BCG-mediated regression of these established tumors and metastases in the strain-2 guinea pig immunotherapy model. Results of studies of line-10 tumor regression after intralesional injection of BCG have showed that the early development of specific bacterial immunity is required in this model (*9*, *14*). Whereas this might have been considered one of the nonspecific features of the model, it must now be considered a positive and immunologically specific aspect of the reaction at the transplantation site. We have also demonstrated that a BCG-mediated granulomatous reaction contributes to the complete elimination of established line-10 tumors after the intralesional injection of BCG (*15*, *16*). Thus in a tubercular lesion produced within an established tumor, the cell changes attributed to the ability of "activated" cells of the macrophage-histiocyte compartment to destroy the organisms can also be associated with their ability to destroy tumor cells. Ultrastructural evidence indicates sizable areas of apparent fusion of the cell membranes of activated histiocytes and line-10 cells, which supports the idea that the cytotoxic effect of the histiocyte includes cell-surface contact, possibly microphagocytosis, of cell-surface antigens (*17*). From the observation of common antigens between line-10 cells and BCG, it is conceivable that microphagocytosis of these tumor cells by activated histiocytes might occur, predominately at the BCG-reactive sites of the tumor cell surface.

The cross-reactivity of anti-BCG serum with human melanoma cells is of special interest, since BCG scarification or intralesional injection is a mode of immunotherapy of melanoma. We believe we have described an immunologically specific reaction and not an artifact of the cell culture conditions, because under identical experimental conditions 2 control human cell lines had negative reactivity with BCG-specific antibody. No generalizations can be made from our data regarding the spectrum of common antigens of microorganisms and tumor cells, since we have only tested the serum against one type of melanoma; however, screening of other types of human tumors is now in progress.

Meyer et al. (*18*) and Azuma et al. (*19*) recently isolated and characterized a BCG cell-wall skeleton fraction (CWS-I) as effective as the original cell wall in the suppression of tumor growth in strain-2 guinea pigs. The chemical characterization of the active fraction indicated the presence of neutral sugars, arabinose and galactose, as well as glucosamine. Complex oligosaccharides are important constituents of cell-surface antigens, and specific cell-surface saccharide residues have now been identified and localized by use of ferritin-conjugated plant agglutinins. The most widely used agglutinins are concanavalin A and *Ricinus communis* which are specific for the $\alpha$-D-mannopyranosyl and $\beta$-D-galactopyranosyl configurations, respectively (*20*). Burger and Goldberg (*21*) reported that *N*-acetylglucosamine is an important part of the tumor cell-surface receptor interacting with wheat germ agglutinin. Thus it would be important now to further determine the correlation between the cross-reactivity and the chemical nature of the antigenic sites of the tumor cells and BCG or other types of organisms.

Although the present study has verified the presence of common antigens between BCG organisms, line-10 hepatocarcinoma cells of strain-2 guinea pigs, and an early human-melanoma cell culture, several important questions are now raised with regard to BCG immunotherapy. The most important question is whether the humoral immune reactivity to the common antigens might, under certain conditions, antagonize development of cell-mediated immunity in a BCG-immunotherapy model. An early and pronounced humoral immune response to BCG may block cell-mediated immunity either on the sensitized lymphocyte or on the tumor cell level. This could depend on the nature of the BCG used for therapy and the quantitative level of common antigenic sites on the tumor cell. Another question is whether the BCG-specific antibody can be cytotoxic to tumor cells in vivo. From the present results as well as the studies of Borsos and Rapp (*10*), the general nature of immunologic cross-reactivity between tumors of different histologic types with BCG or other microorganisms needs to be investigated.

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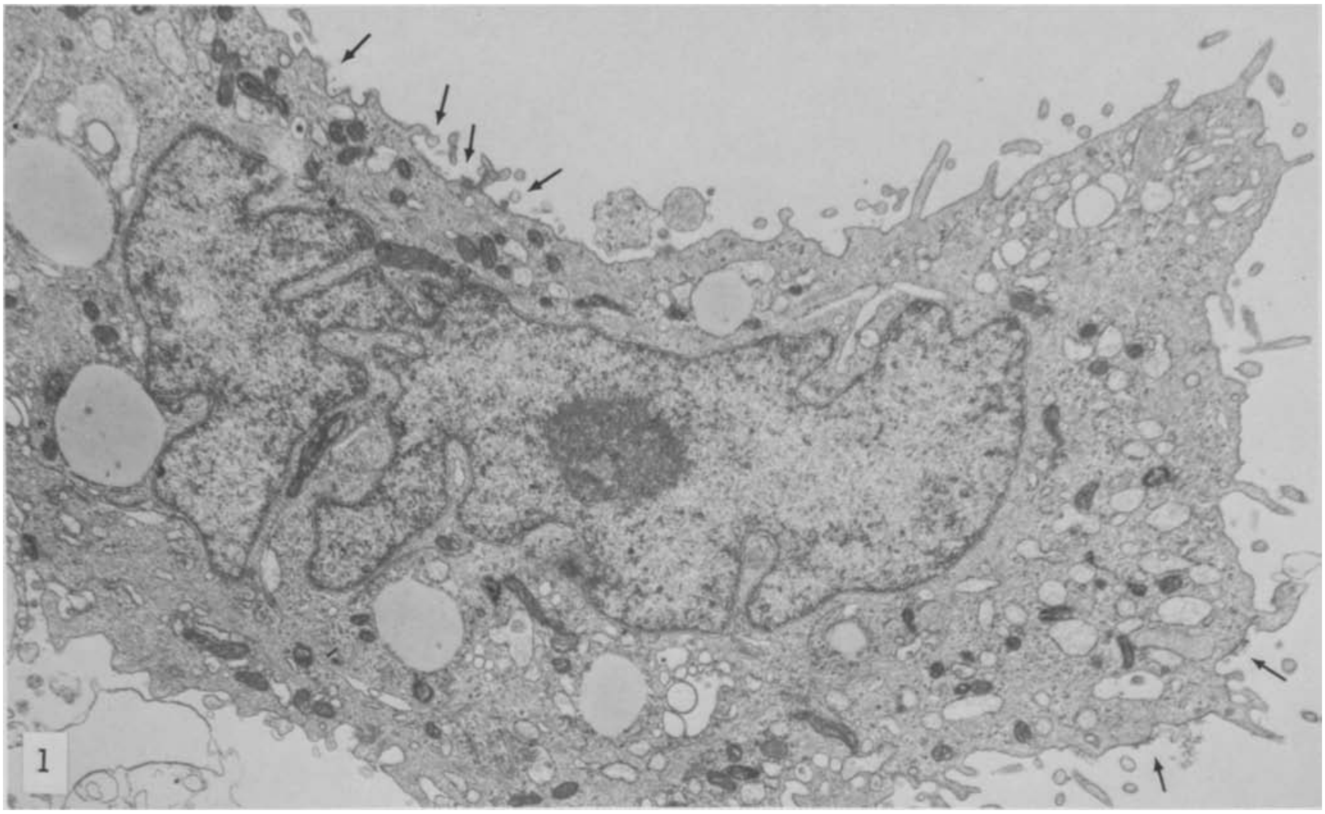
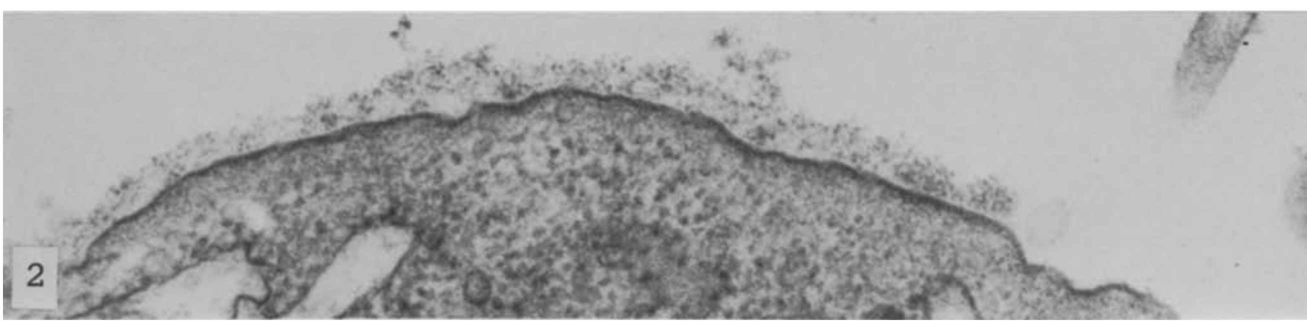


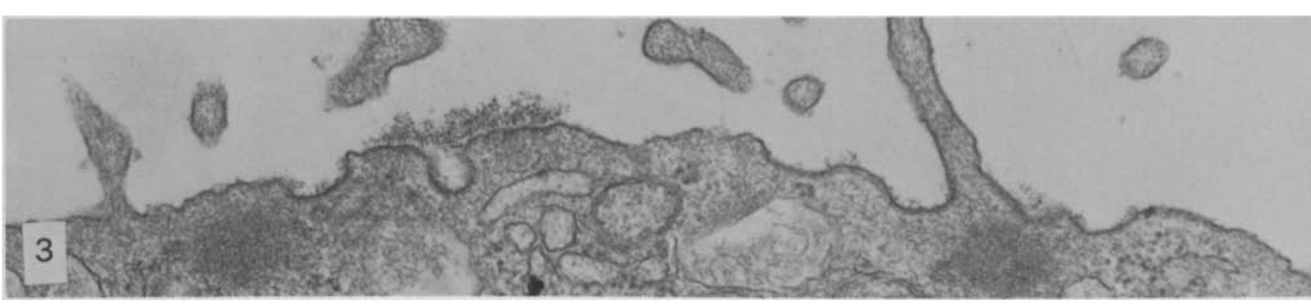
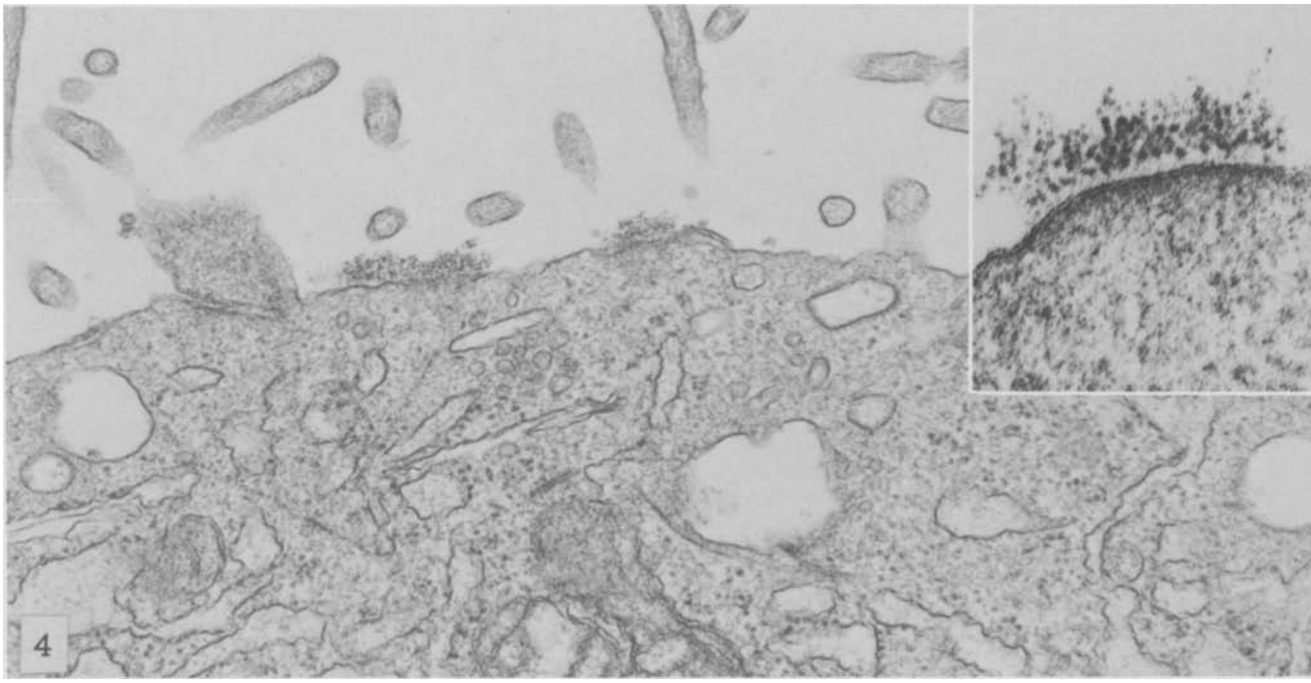
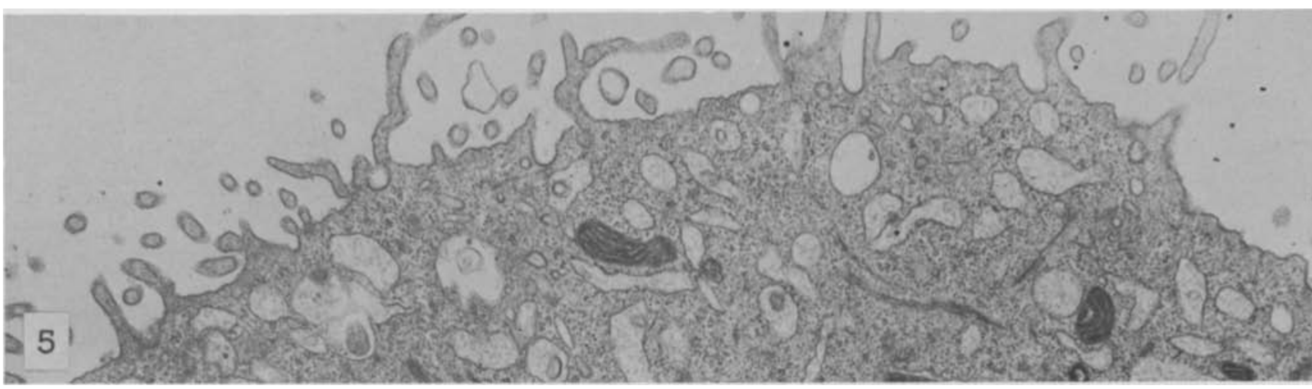




FIGURE 1.—Line-10 cell showing distribution of ferritin-labeled sites on the cell surface (*arrows*) after incubation with mouse anti-line-10 serum absorbed in vivo. × 13,200

FIGURE 2.—Portion of line-10 cell surface showing extensive labeling with ferritin. Cells were incubated with mouse anti-line-10 serum absorbed in vivo. × 86,800

FIGURE 3.—Line-10 cell incubated with guinea pig anti-BCG serum showing discrete antigenic site labeled with ferritin. × 43,400





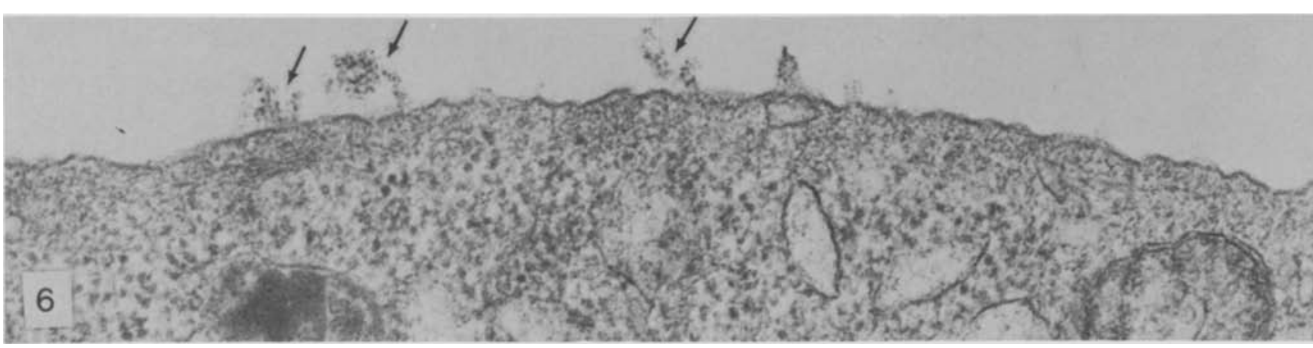


FIGURE 4.—A. Adjacent sites labeled with ferritin on line-10 cell surface after incubation with anti-BCG serum. × 46,200. B. *Inset* is high magnification of cluster of ferritin on reactive site on cell surface. × 150,000

FIGURE 5.—Portion of line-10 cell surface after incubation with normal guinea-pig serum. Labeled sites resembled those shown in figure 3. × 18,000

FIGURE 6.—Selected region of a line-1 cell surface after incubation with anti-BCG serum. Although this picture overexpresses amount of labeling detected in line-1 cells, it indicates qualitative nature of labeling. *Note* unevenness (*arrows*) of ferritin labeling as compared to labeling sites of line-10 cells shown in figure 4. × 62,700

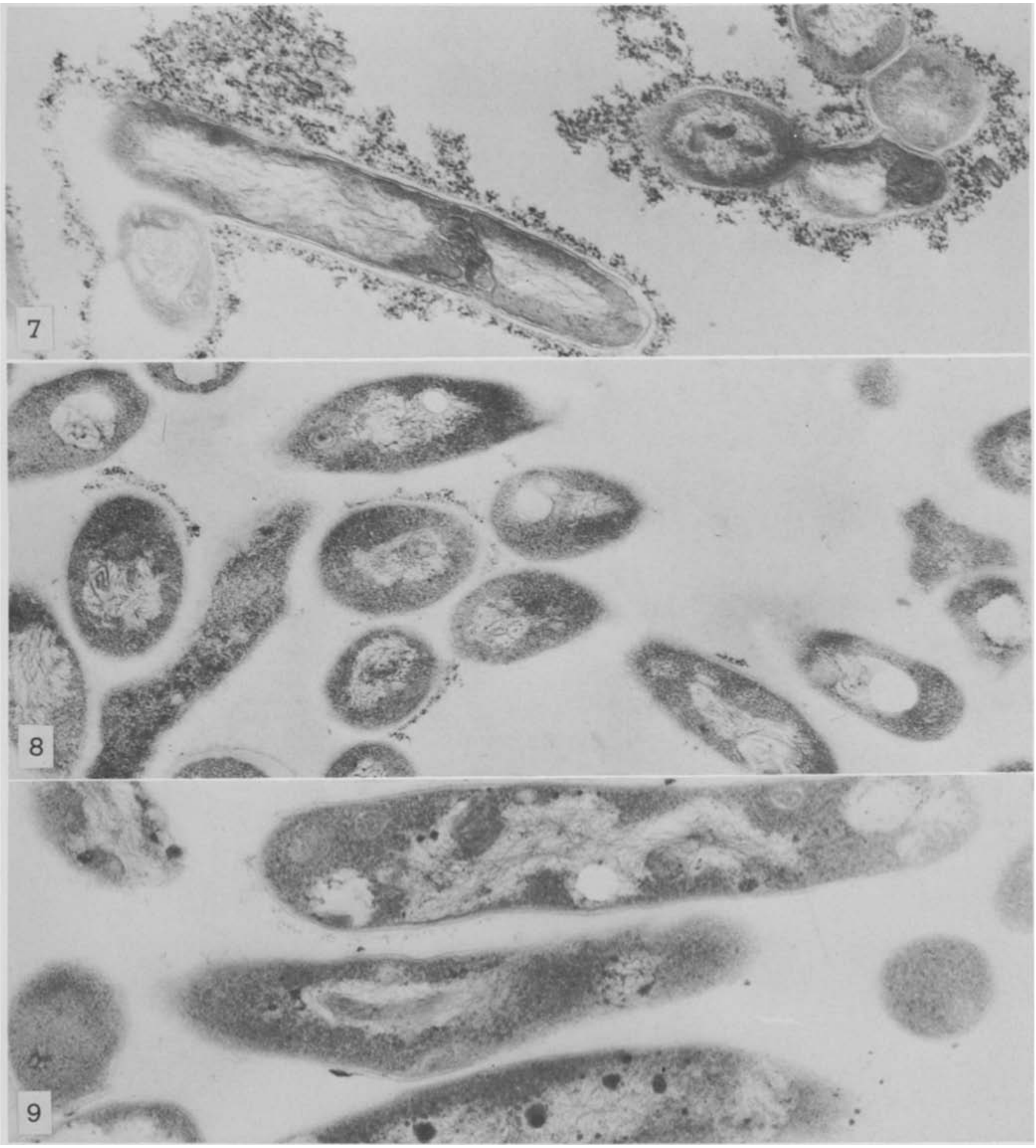
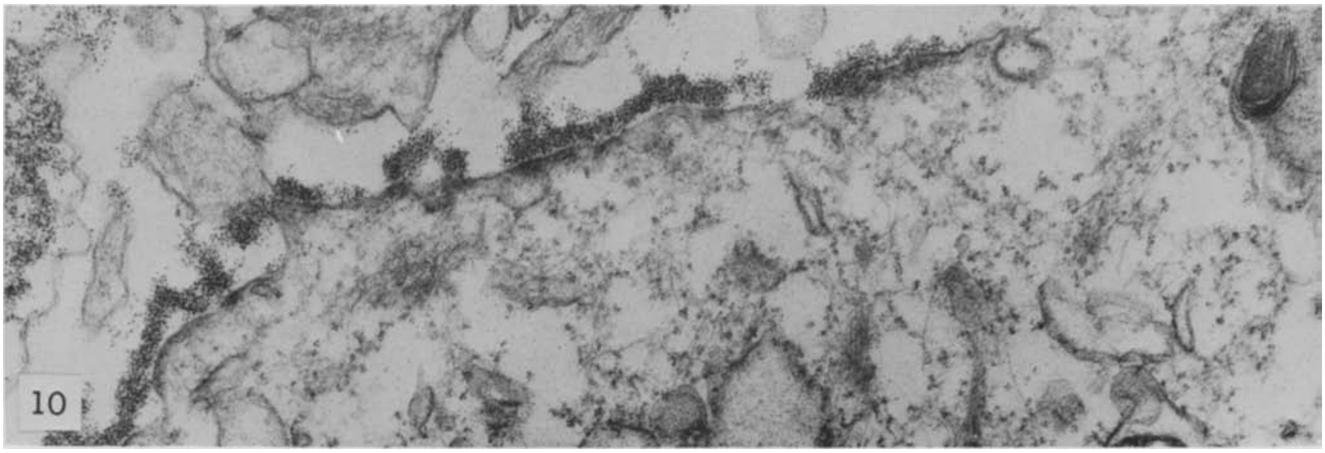
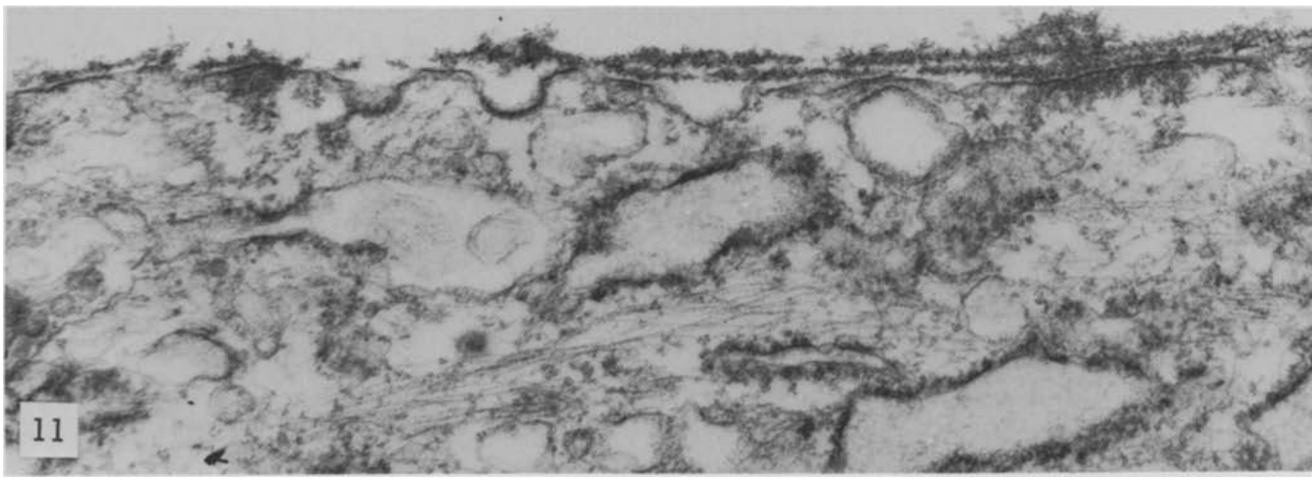



FIGURE 7.—BCG organisms after incubation with anti-BCG serum. × 84,000

FIGURE 8.—BCG organisms incubated with mouse anti-line-10 serum absorbed in vivo. × 54,000

FIGURE 9.—BCG organisms incubated with serum from normal 8-week-old mouse. × 69,600





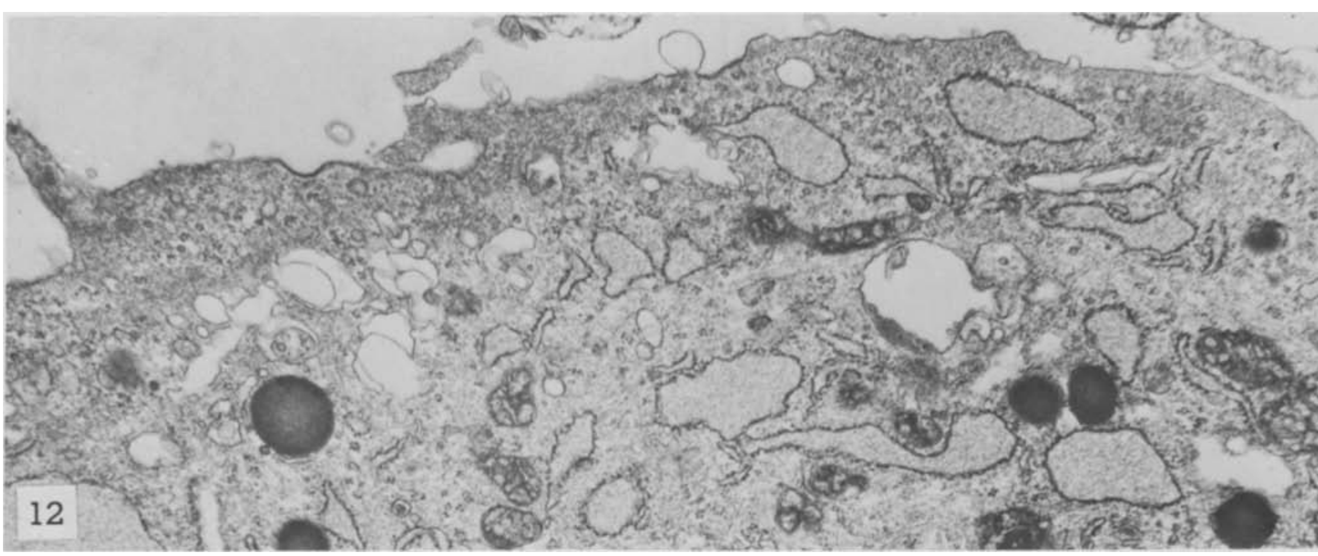
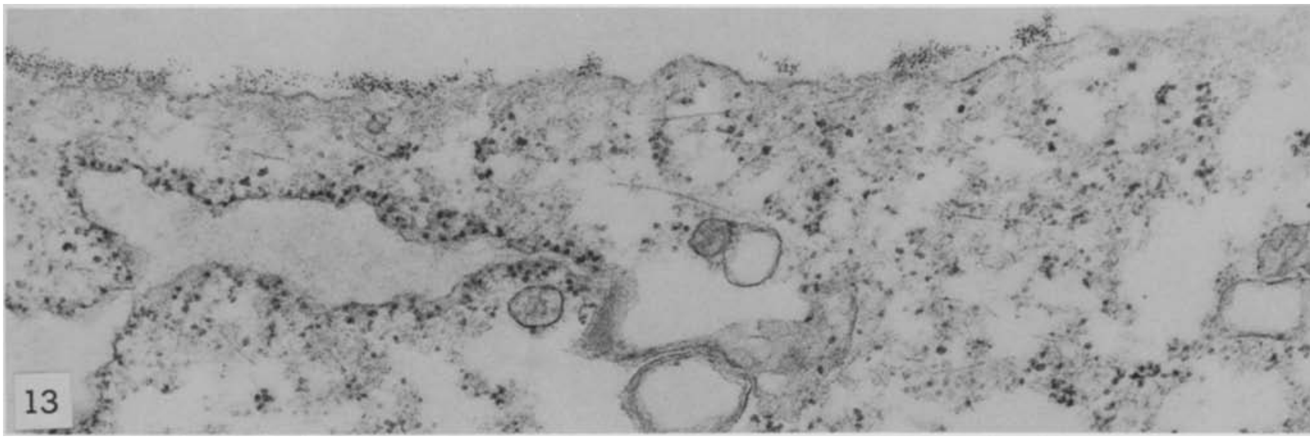
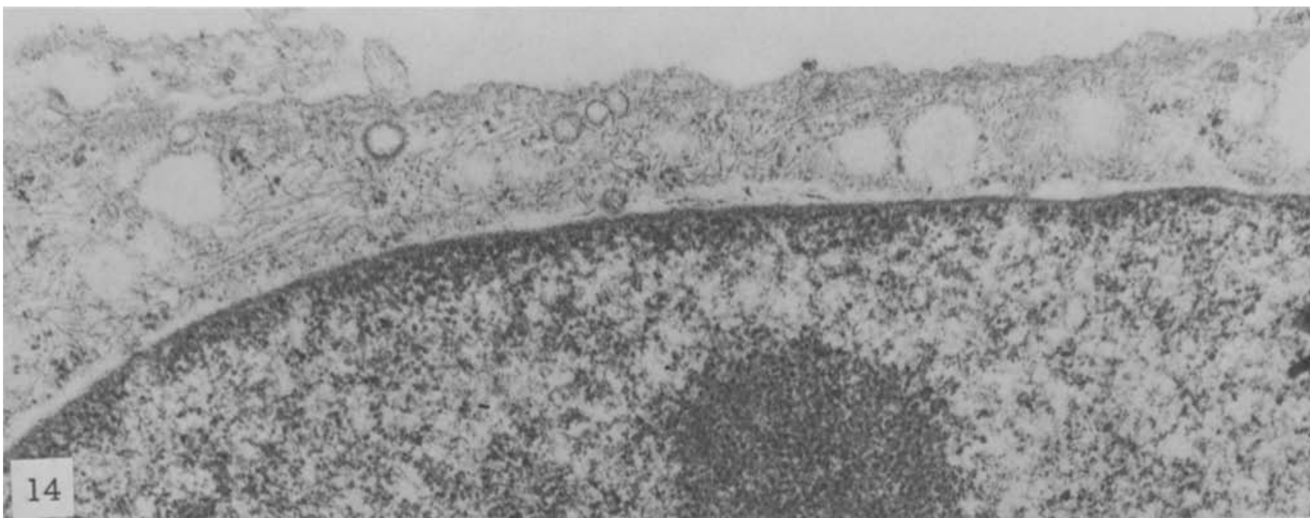


FIGURE 10.—Portion of melanoma cell surface showing extensive labeling after incubation with guinea pig anti-BCG serum. ×54,000

FIGURE 11.—Melanoma cell grown on glass coverslip and assayed for immunoferritin labeling in situ after incubation with guinea pig anti-BCG serum. *Note* extensive labeling of cell surface. × 42,000

FIGURE 12.—Melanoma cell after incubation with BCG-absorbed anti-BCG serum. × 22,800





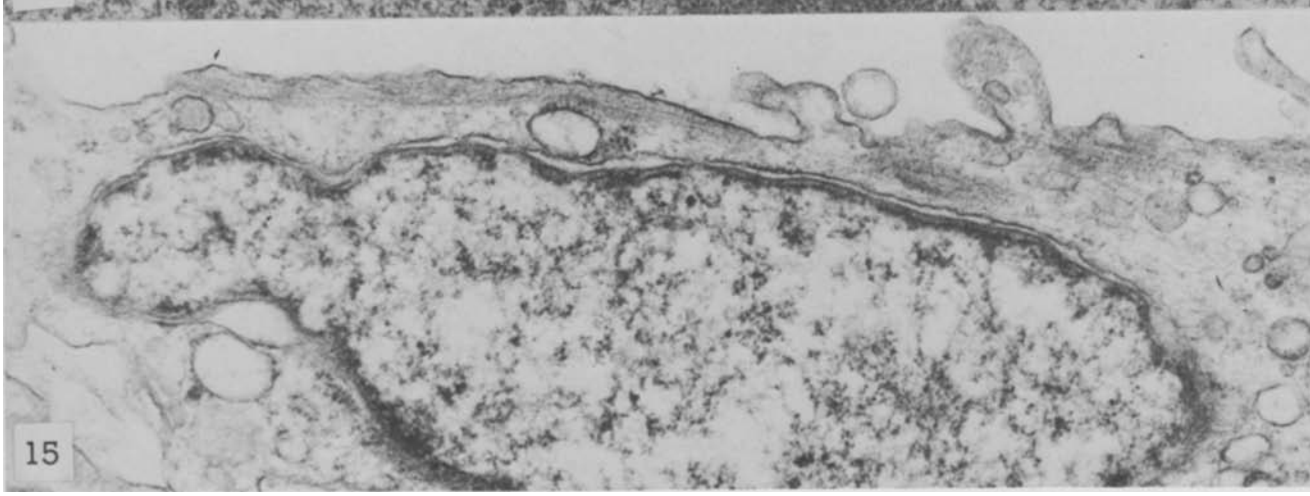


FIGURE 13.—Portion of melanoma cell showing sporadic labeling of cell surface after incubation with anti-line-10 serum. × 40,000

FIGURE 14.—Region of the human foreskin cell after incubation with guinea pig anti-BCG serum. × 40,000

FIGURE 15.—Region of the WI-38 fibroblast after incubation with anti-BCG serum. × 45,000