ELECTRON MICROSCOPY OF PLASMA-CELL TUMORS OF THE MOUSE

I. MPC-1 and X5563 Tumors

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ABSTRACT

An electron microscope study was made of a series of transplanted MPC-1 plasma-cell tumors carried by BALB/c mice. Large numbers of particles similar in morphology to virus particles were present inside the endoplasmic reticulum of tumor plasma cells. Very few particles were seen outside the cells or in ultracentrifuged preparations of the plasma or ascites fluid. In very early tumors particles were occasionally seen free in the cytoplasm adjacent to finely granular material. In general, the distribution of these particles inside endoplasmic reticulum is similar in early and late tumors. A few transplanted X5563 tumors of C3H mice were also examined. Large numbers of particles were found in the region of the Golgi apparatus in late X5663 tumors. A newly described cytoplasmic structure of plasma cells, here called a "granular body," appears to be associated with the formation of the particles. Particles present in MPC-1 tumors are exclusively of a doughnut form, whereas some of those in the inclusions of the late X5563 tumors show a dense center. Normal plasma cells, produced by inoculation of a modified Freund adjuvant into BALB/c mice. have been compared morphologically with tumor plasma cells of both tumor lines.

INTRODUCTION

Plasma cell tumors occur spontaneously, but rarely, in C3H (1), AK (2), BALB/c (1, 3), as well as in several other strains of mice (4). The first evidence that a virus might be associated with the X5563 C3H tumor was given by Howatson and McCulloch (5). These authors demonstrated large osmiophilic inclusions of small particles in the region of the Golgi apparatus. A more extensive electron microscope study was carried out by Dalton and Potter (6) on several lines of tumors in C3H and BALB/c mice. Virus-like particles were reported to be occasionally present in the plasma cells. To date, the evidence of the presence of virus is confined to electron microscopy only, and no reports of successful cell-free transfers have been published.

In the present report, electron microscope evidence is presented of the presence of an abundance of particles in a line of plasma-cell tumors in BALB/c mice as well as a more limited study of similar particles in the X5563 tumor in C3H mice. The morphology and distribution of the particles were studied in tumors at various stages of development. The morphology of the tumor plasma cells of both lines was compared with that of normal plasma cells produced by inoculation of modified Freund's adjuvant into BALB/c mice. No virus-like particles were seen in these normal cells. A series of acellular transmission experiments is described as well as attempts to produce purified preparations of the particles. The particles proved to possess many of the morphologic appearances of tumor viruses, and especially seemed to resemble particles found in mouse mammary tumors. In Part II of this report, evidence is presented for the successful growth of plasma cells and apparent multiplication of virus in tissue cultures of X5563 tumor.

MATERIALS AND METHODS

Concentration of Normal Plasma Cells in Adjuvant-stimulated BALB/c Mice: Normal plasma cells were obtained from a series of 12 week old BALB/c mice injected intraperitoneally with incomplete Freund's adjuvant (7). *Mycobacterium* was omitted, and the antigen used was *Salmonella choleraesuis*, phase 1, obtained from an overnight culture in a mixture of equal parts trypticase-soy broth (BBL) and tryptose broth (Difco). The bacteria were killed in 1 per cent formalin overnight, spun down, and resuspended in a small volume of saline containing 0.6 per cent formalin. The suspension was supplied to us by the Communicable Disease Center, U. S. Public Health Service, Chamblee, Georgia. The mice were given 2 injections of 0.3 ml. of the adjuvant mixture at 5 day intervals. Material for electron microscope examination was taken 12 days after the first injection.

Source of Tumors: Two tumor lines were studied: the X5563 tumor carried in C3H mice, and the MPC-1 tumor in BALB/c mice. The X5563 tumor was obtained from Dr. Potter (1). The original tumor arose spontaneously in a 22½ month old female C3H/He that had been gonadectomized at 2 months of age. The development of the tumor in the ileocecal region, and its histology, have been fully described by Dunn (8). The plasma-cell tumor MPC-1 arose in a BALB/c mouse in Dr. Merwin's laboratory (3). Seven plasma-cell tumors developed in 46 mice carrying diffusion chambers containing C3H mammary tumor tissue. In later experiments (unpublished), one plasma-cell tumor was found in a BALB/c mouse carrying an empty diffusion chamber. Merwin and Algire were successful in transplanting the MPC-1 tumor. This line (derived from experiments in which C3H mammary tumor tissue was used) has been further studied by Potter and Fahey (1). The MPC-1 tumor was generously donated to us by Dr. Potter when in its 14th transplant generation.

Mice Used as Hosts for Tumors: The BALB/c mice and C3H mice used as hosts in these experiments were not free of the mammary tumor agent. At the age during which inoculated tumors developed in C3H mice (3–4 months), the incidence of mammary tumors in control C3H mice was low, although by approximately 9 months of age 35 per cent of the mice had developed mammary tumors. In cell-free transfer experiments described in Part II of this publication, cesarean-delivered, foster-nursed C3H mothers were used to produce newborn mice free of the milk agent. The majority of the mice used were obtained from Cumberland View Farms, Clinton, Tennessee, and others from Jackson Memorial Laboratories.

Technique of Tumor Passage: The X5563 tumor was maintained by subcutaneous injection of 0.2 to 0.4 gm of minced tumor tissue, suspended in balanced salt solutions, into the axillary region of 8 to 12 week old female C3H mice. Material for the study presented here was taken from the 10th and 11th transplant generations. The MPC-1 tumor was conveniently transferred by means of the pronounced ascites that develops in inoculated BALB/c mice. Ascites fluid (0.1–0.2 ml.) (often containing a considerable amount of blood) was inoculated into the peritoneal cavity of 8 to 12 week old male and female BALB/c mice. The MPC-1 material used in this study was taken from the 23rd to 26th generations. Nearly 100 per cent takes were obtained with both tumor lines by these transfer techniques.

Tumors and Other Tissues Examined: The MPC-1 tumors, and in some cases other tissues, of a total of 13 BALB/c mice were examined by electron microscopy of thin sections. The other tissues included were liver, spleen, kidney, lung, thymus, thyroid, pancreas, salivary glands, bone marrow, buffy coat, and ascites cells, as indicated in Table 1. The X5563 tumors of 5 mice were also examined. The tissue culture results are reported in Part II.

Preparation of Tumor Extracts: Extracts of plasma-cell tumors were made in an attempt to demonstrate the presence of a possible tumor virus. A purified

TABLE I

Summary of Tissues Examined by Electron Microscopy

Days after inoculation	No. of mice	Tissues examined
MPC-1 tumor in BALB/c mice		
8	2	Very small abdominal tumor
8	2	Larger abdominal tumor; inoculation site tumor, liver, spleen
10	2	Abdominal tumor, ascites cells
11	2	Abdominal tumor, buffy coat
17	2	Abdominal tumor, liver, spleen, kidney, lung, buffy coat
18	2	Abdominal tumor, liver, spleen, kidney, thymus, thyroid, lung, pancreas, salivary glands, bone marrow (mice in terminal condition)
20	1	Abdominal tumor, ascites cells
10	6	Ultracentrifuged pellets of ascites fluid
17	6	Ultracentrifuged pellets of plasma
X5563 tumor in C3H mice		
12	2	Inoculation site tumors, tissue-cultured
20	2	Inoculation site tumors
61	1	Inoculation site tumor

ultracentrifuged preparation of particles was also required for future quantitative transmission experiments, as well as for morphologic studies by electron microscopy. In the transmission experiments with X5563 tumor extracts, filtration was not used. Instead, ultracentrifuged fractions of the homogenized tumor were inoculated into (C3H × 101)F_1 and (101 × C3H)F_1 hybrids at 8 to 12 weeks of age. Tumors induced by virus, nucleic acid, or other small-sized agent could then be distinguished from those arising from contaminant whole cells by the failure of induced tumors to transplant in the parent strains.

In general, the tumor tissue from 8 to 10 C3H donors was weighed and made up to a 20 per cent suspension in balanced salt solution. The suspension was homogenized in a Waring blendor, or a teflon tissue homogenizer, or a mortar and pestle. The first spinning was at low speeds to remove pieces of tissue, whole cells, and large cell fragments. This pellet was discarded in some experiments and in others was injected to test for complete homogenization. Part of the supernatant of the first spinning was injected into a few mice to test for the presence of whole cells. The remainder of the supernatant was then spun at about 7000*g* for 10 to 30 minutes; the pellet was resuspended in small volume of saline and injected into about 10 mice. The supernatant was then spun at 40,000 to 105,000*g* for ½ to 3 hours; the pellet obtained from this spinning was also injected into 10 mice. The final supernatant from this spinning was also injected into 10 to 25 mice. All fractions were injected intraperitoneally or subcutaneously or by both routes. Some variations of this basic experiment were as follows: (*a*) Sodium lauryl sulfate, an anionic detergent, was in the homogenizing medium to aid in rupture of the cells and release of virus (9). (*b*) Tween 80, a nonionic detergent, was used in a similar way.

Twenty-five mice also received intraperitoneal implants of X5563 tumor tissue in plastic capsules covered with millipore membranes (0.2–0.5 μ pore size) (10).

Ultracentrifuged preparations from X5563 and MPC-1 tumors were also examined with the electron microscope. In one experiment, a Waring blendor homogenate was used; in two others, the tumor was ground gently in a mortar and pestle. A 15 per cent suspension was prepared in balanced salt solution. All manipulations were carried out in the cold room. After a preliminary low-speed centrifugation at 3000*g*, the supernatant was spun successively at 10,000*g* for 20 minutes, 60,000*g* for 45 minutes, 105,000*g* for 60 minutes. The pellets were fixed for 90 minutes in osmic acid and then dehydrated and embedded as described in a following section. The weight of tumor tissue used (1–2 gm.) was adjusted to give small pellets (3–5 mm. diameter) on ultracentrifugation. The pellet material could then be divided among only a few blocks, and the embedded material could be sectioned almost serially to provide a fairly quantitative picture of the amount of virus-like particles in a pellet. The particle content of the combined ascites fluid from 6 BALB/c mice, 10 days after inoculation, was examined in this way, centrifuging at the speeds listed. In a similar manner, the combined heparinized plasmas of 6 BALB/c mice, taken 17 days after the tumor transfer, were ultracentrifuged and examined with the electron microscope.

Light Microscopy: BALB/c tumors were examined by light microscopy at 11, 17, and 18 days. Other tissues (liver, spleen, kidney, lung, salivary glands, thymus, thyroid, bone marrow) were examined for metastases and other pathology. Sections were fixed in Zenker-formol and stained with hematoxylin and eosin.

Electron Microscopy Technique: Tumors at various stages of development were removed from the mice, as listed in Table 1, and cut into ~1 mm. cubes while immersed in ice-cold Palade's osmic acid (pH 7.2–7.4). Fixation was carried out in the cold for 90 minutes. The tissue was washed briefly in Tyrode's solution and then dehydrated in 30, 50, 70, 90 per cent alcohols for 10 minute periods. Two 10 minute washes in absolute alcohol were given, and the tissue then soaked for 15 minutes in a 1:1 monomer-alcohol mixture. The monomer mixture consisted of 85 per cent butyl methacrylate, 15 per cent methyl methacrylate, and 1.5 per cent Luperco CDB catalyst. Butyl and methyl metacrylates were dried over Linde Type 4A molecular sieve and then vacuum distilled separately at 50 to 70 mm. pressure through a glass bead-packed, 28 cm. fractionating column. After the monomer-alcohol treatment, the tissue was soaked for 3 periods of 15 minutes in undiluted monomer-catalyst mixture. Polymerization in gelatin capsules was carried out by heating at 65°C. overnight. Gray (40–60 mμ) sections were cut on a Porter-Blum microtome with a 1.8 mm. 50° angle diamond knife (Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela). The majority of the sections used in this study were stained with semisaturated lead subacetate solution for 7 minutes in an atmosphere of nitrogen (11). Carbon support films were prepared by evaporation onto grids covered with formvar film, and the latter was dissolved off with ethylene dichloride.

An RCA EMU-3E electron microscope was used, operating at a voltage of 50 kv. The condenser aperture was 250μ in diameter and the objective aperture 25 μ. Low magnification pictures of whole cells were taken at 3000 to 4000 times. The routine magnification for examination of cell structure and virus-like particles was 8000 times. Areas of particular interest were also photographed at 18,600 to 35,600

times direct magnification. Eastman Kodak Contrast anti-abrasion lantern slide plates were used, and prints made on Kodabromide F5 paper. In most cases, 4 blocks of any particular tissue were selected by scanning from a total of 8 to 10 embedded, in order to find areas of tumor that were not necrotic and that were well fixed. Suitable blocks were sectioned at 3 or 4 levels. Other tissues, especially ultracentrifuged pellets, were scanned from a larger number of blocks.

RESULTS

I. Development of Plasma-Cell Neoplasms in BALB/c and C3H Mice After Transfer of the Tumor

MPC-1 Tumor in BALB/c Mice: Visible tumors first developed in the abdomen, after intraperitoneal inoculation of ascites fluid and cells, at about 8 days. Tumors consistently arose in the area bounded by the spleen, pancreas, and posterior wall of the stomach. The smallest tumor detected, at 8 days, extended in a thin line of pink tissue along the splenogastric ligament and posterior wall of the stomach. Large tumors formed a roughly spherical mass adjacent to the hilum of the spleen. Somewhat later, a tumor nearly always developed at the site of inoculation (through the upper groin into the abdomen). Frequently this tumor arose between the skin and the peritoneum, presumably owing to leakage of fluid from the hypodermic needle. Less often it was attached to the peritoneum. The tumors increased rapidly in size from 8 to 21 days after inoculation, when most mice succumbed. Together with the growth of the tumor, a pronounced ascites developed, which contained tumor cells and a variable number of red cells. In the terminal stages of the disease, the tumor spread widely through the abdominal cavity and, to a lesser extent, to the thoracic cavity. The main tumor masses, 17 days after inoculation, showed extensive areas of necrotic cells, often with pyknotic nuclei. As early as 11 days after inoculation, many tumor cells were seen in the spleen, and small groups of cells were scattered in the liver, lung, kidney, and thymus. Few tumor cells were present in the buffy coat. Only adjacent lymph nodes were involved.

X5563 Tumor in C3H Mice: The main tumor developed in the subcutaneous tissues at the site of inoculation. The earliest tumors were palpable and visible at 6 days, and increased to as large as 4 cm. in diameter at 60 days after inoculation. The tumors in the later stages also showed extensive areas of necrosis.

II. Electron Microscopy of Normal (Stimulated) Plasma Cells

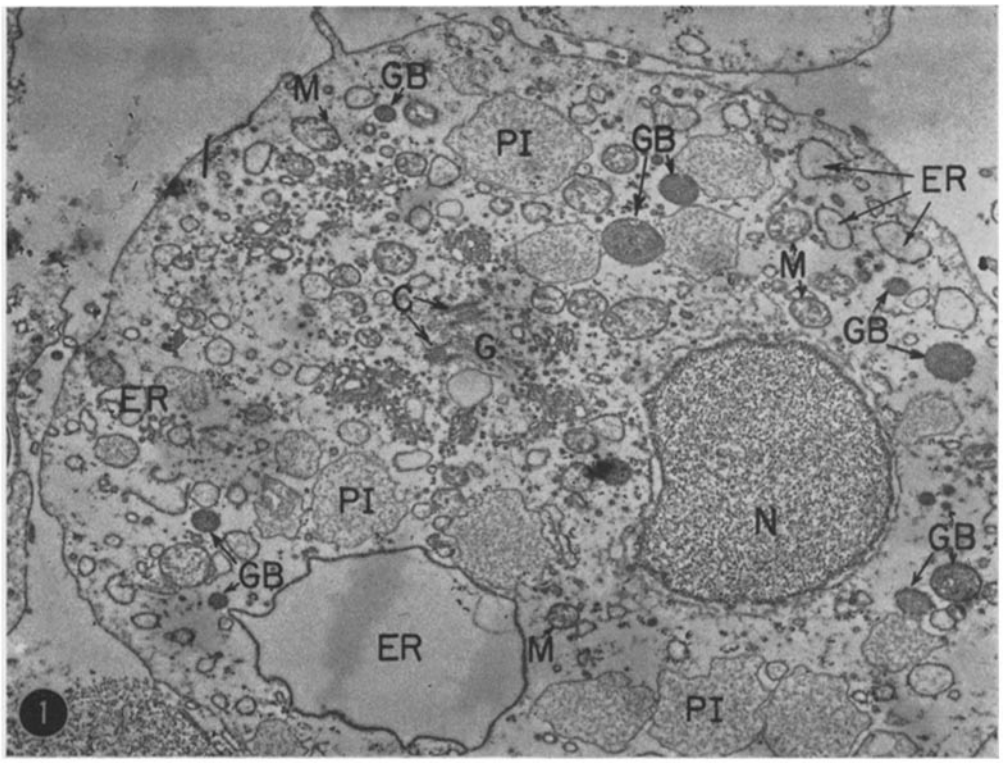
Normal plasma cells were examined for virus-like particles and also to allow a cytological comparison between the normal and the neoplastic cell Mice inoculated with the adjuvant were followed by preparing Wright's stained smears of the ascites fluid. The mice were sacrificed 12 days after inoculation, when the smears showed the presence of plasma cells. At this time an extensive peritonitis was present with adhesions between all abdominal organs. Specimens for the electron microscope were obtained from the adhesions and lymphoid tissue. Numerous plasma cells were found in the adhesions and somewhat fewer in the lymphoid tissue. One of these plasma cells is illustrated in Fig. 1. The eccentric nucleus (*N*) is cut to one side, and the section passes centrally through the large Golgi apparatus (*G*) The chromatin of the nucleus is fine, an ap-

FIGURE 1

Normal plasma cell from a 12 week old BALB/c mouse after intraperitoneal inoculation of incomplete Freund's adjuvant 12 days previously. The section passes through the Golgi zone (*G*) and the centrioles (*C*). The nucleus (*N*) is cut to one side. Granular bodies (*GB*) are fairly numerous. *PI* may represent inclusions of protein. Endoplasmic reticulum (*ER*) and mitochondria (*M*) and mitochondria (*M*) are visible × 9000.

FIGURE 2

Typical plasma-cell from an abdominal plasma-cell tumor 8 days after inoculation of ascites cells. Particles (*VP*) are abundant inside the endoplasmic reticulum (*ER*). The mitochondria (*M*) are swollen and sometimes lacking in structure on one side. The particles are not present in the central part of the Golgi apparatus (*G*). The nucleus (*N*) is indented. × 9000.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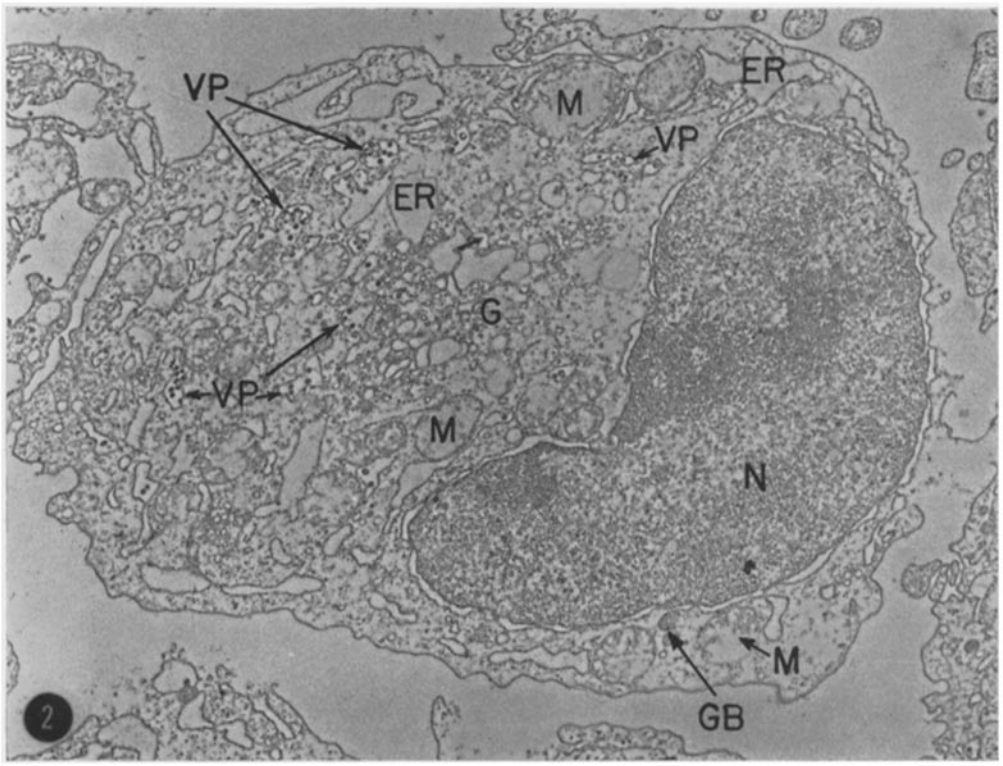
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pearance not easily correlated with the clumped "cartwheel" nucleus usually observed in human plasma cells as seen by light microscopy, or in electron micrographs of human plasma cells (12). A nucleolus is usually present, but is not included in this section. Attention is specially directed to the small dense structures (*GB*), which will be called "granular bodies." They are frequently seen in sections of normal and neoplastic plasma cells. The irregular vacuoles marked *Pl* are assumed to be inclusions of protein in endoplasmic reticulum sacs. Only a few Palade granules are seen attached to the walls of these sacs. In sections through the Golgi region, as in Fig. 1, the endoplasmic reticulum (*ER*) does not appear as abundant as in sections at other levels. Small clusters of free Palade granules are distributed in the cytoplasm. The mitochondria (*M*) are of a small spherical type and rarely show many well-defined cristae. The inner density of the mitochondria is variable, and may, infrequently, be quite high. The Golgi apparatus is large. The centrioles (*C*) are surrounded by an almost structureless region and then a periphery of smooth elongated saccules and small vesicles. The Golgi apparatus usually flattens the adjacent side of the eccentric nucleus. This appearance of a clear area (archoplasm) adjacent to a flattened nucleus is quite distinctive for mouse plasma cells in the light microscope. In Figs. 3 and 4 some examples of granular bodies are shown at higher magnification. Fig. 3 is an enlargement of portion of Fig. 1. One granular body shows curved structures (*CR*) in the dense inner material. Fig. 4 illustrates two large granular bodies. One shows a double membrane (*DM*). In these examples the inner dense material is fine in structure. In others, especially those showing evidence of inner membraneous structures, the dense material is more granular.

III. Electron Microscopy of MPC-1 Tumors at Various Stages

A. Morphology of Tumor Plasma Cells: The normal plasma cell described in Section II is 7 to 16 μ in diameter. Fig. 2 illustrates a typical plasma cell from an abdominal tumor 8 days after inoculation of ascites fluid. This cell is about 14 μ in diameter, but cells of nearly twice this size were frequently encountered. In Fig. 2 the endoplasmic reticulum is abundant, as in normal plasma cells. Dense particles (*VP*) are widely distributed in the endoplasmic reticulum (these particles are discussed in detail in Section III B). The section passes through the voluminous Golgi zone. The mitochondria were distributed in a manner similar to that of the normal plasma cell and were frequently surrounded by lamellae of ergastoplasm. As will be seen in subsequent pictures at higher magnification, the mitochondria are often swollen, lacking in cristae, and possess a broken outer wall. Structures identical to the granular bodies described in normal plasma cells are present in tumor cells. Their number is variable but they may be fairly numerous, especially at the periphery of the cell. The nuclei frequently assume bizarre shapes, with convolutions of the nuclear membrane and deep notches. Often they contain large dense areas (Fig. 2) or even larger masses stretching from one side of the nucleus to the other. These areas are more prominent in the tissue-cultured tumor cells. Cell borders of the tumor plasma cells are often ill defined, giving an impression of large syncytial groups. The tumor contains a minimum of collagen and other supportive tissue. Blood sinuses are frequently lined with tumor cells only.

In the MPC-1 tumors studied, no aggregates of particles in the region of the Golgi apparatus were encountered. This is in striking contrast to the X5563 tumor in which large numbers were frequent in the Golgi region in the larger neoplasms. In MPC-1 tumors, in areas free of necrosis, the distribution of particles in the endoplasmic reticulum was surprisingly uniform. Nearly all sections showed at least a few particles. The loss of structure of mitochondria appeared to be sufficiently marked to be classified as a possible cytopathogenic effect, although many of the mitochondria examined in normal cells did not seem to have very distinct cristae. The general features distinguishing the neoplastic plasma cell from the normal were the increased size, bizarre-shaped nucleus, the presence of dense masses in the nucleus, and a large number of particles in the endoplasmic reticulum. A rather surprising feature was the rarity of presumed protein inclusions, as described for the normal plasma cells (Fig. 1). However, the tumor-bearing mice show characteristic, abnormal globulin in the plasma (1).

B. Localization and Morphology of Particles at Different Stages of MPC-1 Tumor Development: The particles were studied in a series of MPC-1 tumors ranging from a few millimeters in diameter (8 days after

inoculation) to approximately 1 cm. (18–21 days after inoculation). Localization of the particles to the endoplasmic reticulum is very striking. Equally remarkable is the rarity of particles outside the cells in the intercellular spaces or in the blood sinuses of the tumor. A typical field showing part of the cytoplasm of a plasma cell of an early abdominal tumor (8 days after inoculation) is shown in Fig. 11. Particles of 72 to 80 mμ diameter are seen free inside and attached to the wall of the endoplasmic reticulum sacs. At VP_1, 3 particles are attached in close proximity. At VP_2, a particle lies inside the perinuclear space and is attached to the outer of the 2 nuclear membranes. Particles are also found attached to the inner of the 2 nuclear membranes. The mitochondria show extensive degenerative changes. At M_1, the wall on one side is lacking in contrast, and the cristae are missing. Few cristae are present in M_2, M_3, and M_4. A few small granular bodies are present. The smooth, single-walled vesicles (G) probably represent part of the Golgi apparatus. The endoplasmic reticulum is densely lined with Palade granules, and small clusters of particles are also distributed between cytoplasmic structures. The distribution of particles in the Golgi region of a plasma cell of an abdominal tumor, 8 days after inoculation, is shown in Fig. 14. A few particles are usually found scattered around the periphery of the Golgi apparatus inside endoplasmic reticulum sacs. Occasionally, particles are seen in association with smooth-walled sacs, but no large inclusions of particles are to be seen in the Golgi area, as is the case with late X5563 tumors. Fig. 14 shows particles (VP_1, VP_2) attached to smooth portions of the wall of endoplasmic reticulum and adjacent to collections of smooth vesicles (V), assumed to represent part of the Golgi apparatus. Free Palade granules are often seen inside endoplasmic reticulum which may show breaks in the wall of the sac.

Occasionally, large groups of particles were encountered. These appeared to almost cover the wall of a portion of endoplasmic reticulum. Such an area is illustrated at low magnification in Fig. 12. Nearly 100 particles (VP) are present in this area of endoplasmic reticulum. The mitochondria (M_1 and M_2) show considerable loss of structure on one side. These particles are shown at higher magnification in Fig. 13. Inside the endoplasmic reticulum they are circular in cross section. Those attached to the wall of the saccule appear to be in various stages of formation. Some, as at VP_2, appeared as thickened, double-walled convexities at the boundary of the saccule. Others, as at VP_3, appear to have an almost completely spherical inner membrane, but the outer one is continuous with the wall of the endoplasmic reticulum as a narrow neck. The particles are particularly concentrated on indentations of the wall into the sac, as at S_1 and S_2. In these areas, there are fewer Palade granules than in the surrounding cytoplasmic matrix. The inner membrane of the virus-like particles is well defined and dense. It occasionally shows evidence of being double in nature. The density of this membrane is especially enhanced by lead subacetate staining. The outer membrane appears to be made up of clusters of small dense particles.

The morphology of the particle remained the same from the smallest tumor, 8 days after inoculation, to the largest terminal tumors at 20 days after inoculation. Particular attention was paid to a possible variation in the number of virus-like particles per section in non-necrotic areas at different stages of development. However, early and late tumors showed little variation in this respect. A tumor of 5 to 7 mm. diameter, 8 days after inoculation, showed 443 particles per 44 electron microscope fields (magnification 8000); a large 20 day abdominal tumor showed 412 particles per 44 fields. Even with a very early abdominal tumor (8 days after inoculation) of only a few cubic millimeters volume, quite large groups of particles were seen in endoplasmic reticulum sacs, as shown in Fig. 5. However, a suggestion of a different mode of particle formation was seen at this stage. The cytoplasm sometimes showed areas of fine, slightly dense material where no Palade granules were present. This occurred most frequently in the Golgi zone. Particles were present in vacuoles at the borders of such areas (Fig. 6). In these areas, in the very early tumors, distinct particles were seen occasionally between vacuolar structures.

The distribution of tumor cells and particles was studied in other tissues in some of the BALB/c mice with larger tumors. The special association of the particles with the endoplasmic reticulum of plasma cells made an examination of other cells with abundant ergastoplasm of particular interest. The pancreas, thyroid, liver, and salivary gland were examined (Table I). No particles

were seen in these tissues except inside metastatic plasma cells. Particles of similar appearance were seen in the cytoplasm of a spleen cell (possibly a reticular cell) of a mouse 8 days after inoculation (Fig. 7). Rarely, similar particles were seen in kidney cells.

C. Morphology of Other Cytoplasmic and Nuclear Structures: GRANULAR BODIES. Structures identical in appearance to the granular bodies described in normal mouse plasma cells were seen in greater number in the tumor cells. These structures range in diameter from 80 mμ to 1 μ.

FIGURE 3

An enlargement of Fig. 1 to show the granular bodies (*GB*) of a normal plasma cell. One granular body contains doubled curved elements (*CR*). Mitochondria (*M*) are shown and *PI* probably represent inclusions of protein. × 16,000.

FIGURE 4

Two granular bodies (*GB*) from the same series of adjuvant inoculated normal mice as the example of Fig. 1. One granular body shows a double membrane (*DM*) and one end of the structure appears to be broken up. Endoplasmic reticulum (*ER*) is also shown. × 50,800.

FIGURE 5

A large group of particles (*VP*) are shown in a saccule of endoplasmic reticulum (*ER*) of an MPC-1 tumor. This tumor was the earliest found having a volume of only a few cubic millimeters (8 days after inoculation with ascites cells). The particles appear to be budding from the wall of the saccule. × 35,000.

FIGURE 6

The same early MPC-1 tumor as illustrated in Fig. 5. The fine granular material (*GM*) was abundant in the Golgi zone, and distinct particles are present at the junction of this area with a vacuole of the Golgi apparatus (*GV*). × 38,100.

FIGURE 7

Cell from the spleen (not a plasma cell) of a mouse carrying the MPC-1 tumor (8 days after inoculation). 66 to 80 mμ particles are present in the cytoplasm. Some show a double outer membrane similar to the particles of Fig. 5. × 34,700.

FIGURE 8

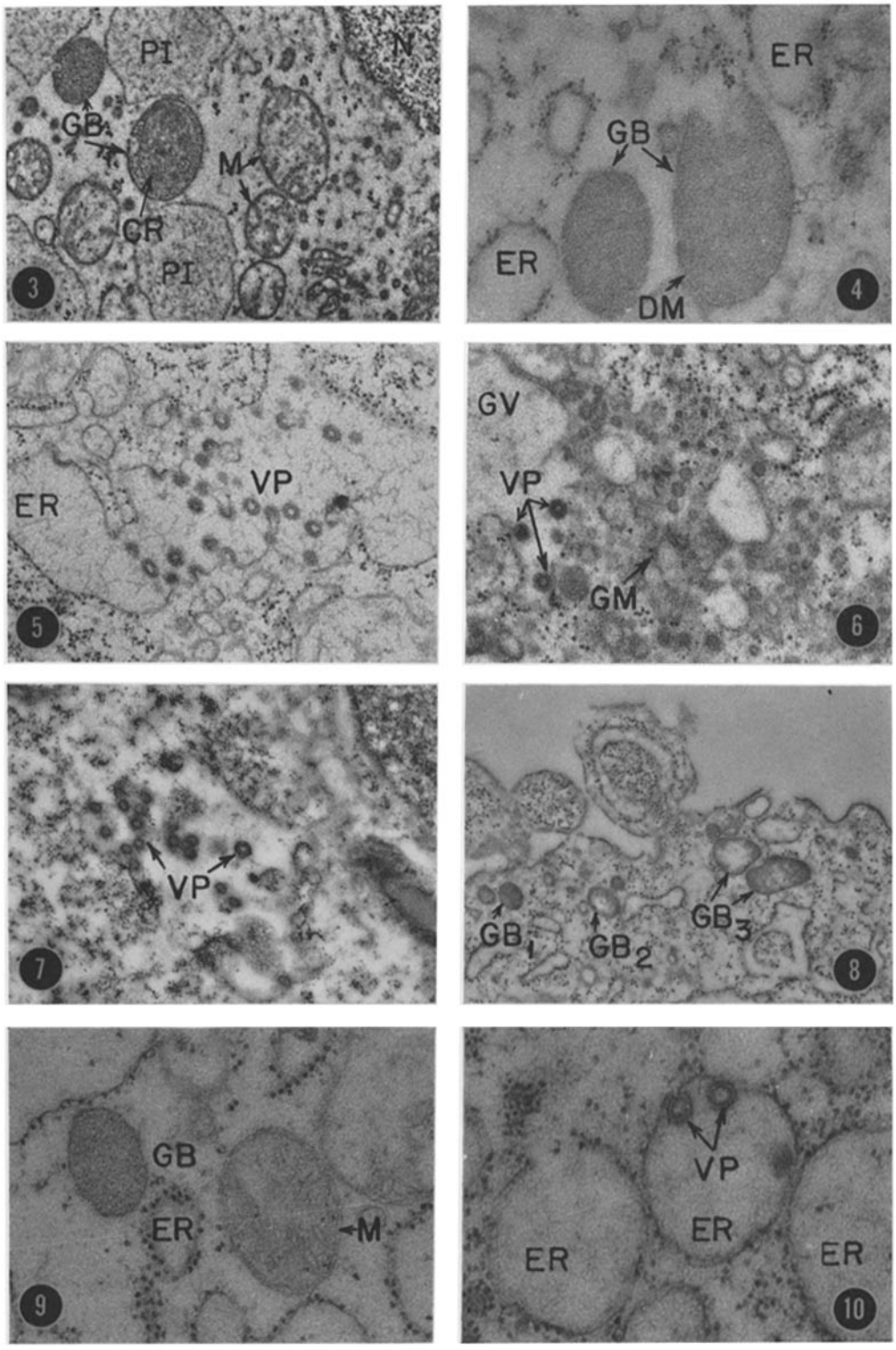
A group of granular bodies (*GB*) in an early MPC-1 abdominal tumor. The bodies are typically located at the periphery of the cell but here some have a light center (GB_2, GB_3) and others (GB_1) are dense. The granular body at GB_2 appears to have some features of a mitochondrion. × 22,800.

FIGURE 9

A portion of the cytoplasm of an MPC-1 tumor plasma cell (18 days after inoculation), showing a dense mitochondrion (*M*) with indefinite cristae and a typical granular body (*GB*). Endoplasmic reticulum (*ER*) is also shown. × 55,000.

FIGURE 10

Portion of the cytoplasm of a plasma cell from a large X5563 tumor (61 days after inoculation), showing the apparent budding of particles (*VP*) from the wall of endoplasmic reticulum (*ER*). The particles are more commonly found as large inclusions in the late stage tumors, but this appearance was common in a tumor formed 20 days after inoculation. × 63,500.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They appear to be more frequently located near the plasma membrane. Fig. 8 shows examples in a very small abdominal tumor, 8 days after inoculation. In this tumor, the granular bodies appeared to have light centers more frequently than in older and larger tumors (GB_3). GB_2 shows a double membrane and a suggestion of cristae. In Fig. 9, a dense mitochondrion is shown with a distinct double membrane and cristae. Adjacent to this is a typical granular body. The dense mitochondrion appears to be intermediate in structure between that of a mitochondrion and a granular body. In Fig. 15, a double-membraned structure (VP_1) lies inside a granular body. This structure has a denser inner membrane and is similar to the particles inside endoplasmic reticulum (VP_2). Several other examples were found in MPC-1 tumors, but this modification of granular bodies was more frequently encountered in tissue-cultured tumor plasma cells (*see* Part II).

PARTICLES IN THE NUCLEUS. Large dense masses were frequently encountered in the nucleus, but only one example was found showing virus-like particles in association with it. In most cases, the appearance was more of large nucleoli, sometimes of the reticulated type and with some finer dense material, possibly chromatin, attached to the periphery. A plasma cell from an early MPC-1 tumor showed particles of approximately 50 mμ diameter. A few particles of 60 mμ diameter with small dense centers were also present in the nucleus. These did not resemble the particles that occur in the endoplasmic reticulum.

IV. Electron Microscopic Examination of X5563 Tumors

The most detailed study of X5563 tumors was made by the method of tissue culture, but a preliminary study of mid-stage subcutaneous tumors (20–25 days after inoculation) and late-stage tumors (61 days after inoculation) is reported here. The general morphology of the tumor plasma cells and the distribution of virus-like particles in endoplasmic reticulum, in the 20 to 25 day X5563 tumors were very similar to those in the MPC-1 tumors. Bizarre-shaped nuclei containing prominent dense masses were frequent. The late-stage tumor, however, showed a striking difference in the site of occurrence of the particles. Fig. 16 illustrates at low magnification a typical plasma cell from such a tumor. A large aggregation of particles (*IB*) is shown in the region of the Golgi apparatus. This type of aggregation has been previously reported in X5563 tumors by Howatson and McCulloch (5). The mitochondria frequently showed similar degenerative changes to those described for MPC-1 tumors.

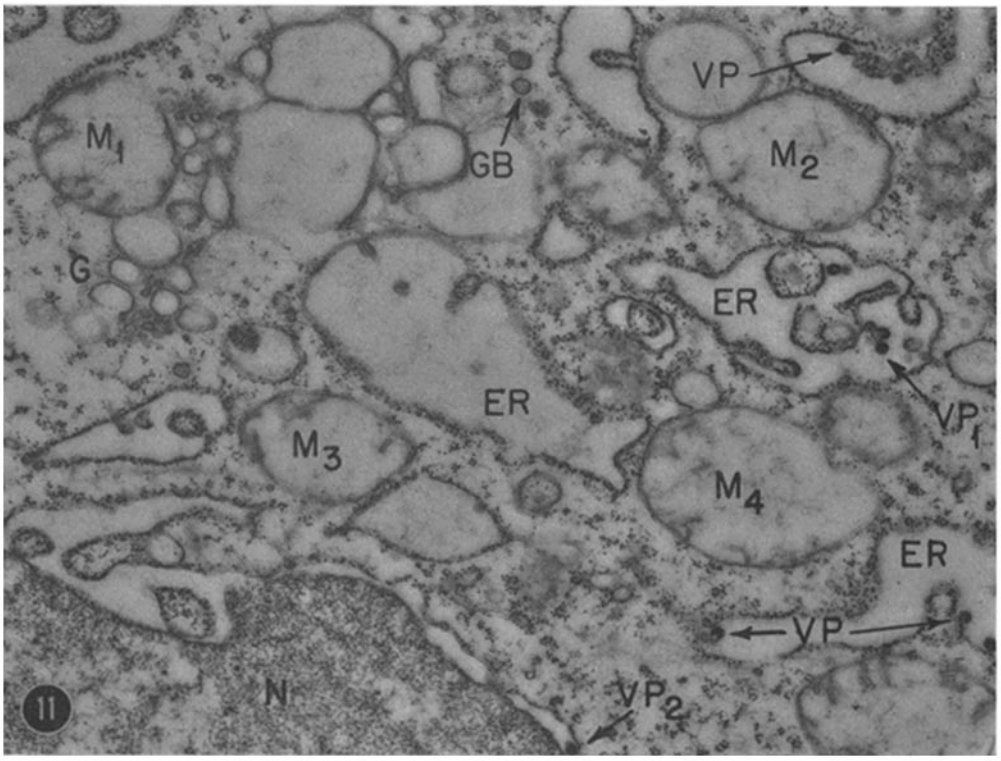
The distribution and morphology of the particles in the inclusions of the late X5563 tumor showed distinct differences from those described inside endoplasmic reticulum of the MPC-1 tumor. However, the particles still occur inside endoplasmic reticulum (Fig. 10), where they appear to form by the same budding process. A portion of an inclusion is shown at higher magnification in Fig. 17. Here the particles occur mainly in the cytoplasmic matrix between structures. Some particles (VP_3) do not have a dense center; others, as at VP_1 and VP_2, show a distinct dense nucleoid inside the double membrane. A large granular body is shown with a suggestion of cristae (*CR*). Small granular bodies are widely distributed throughout the inclusion. Fig. 18 illustrates the structure of the particles that do not show a dense nucleoid. At this magnification, the inner dense region appears to have a membranous (VP_1) or granular (VP_2) ultrastructure. The particles from these inclusions also differ con-

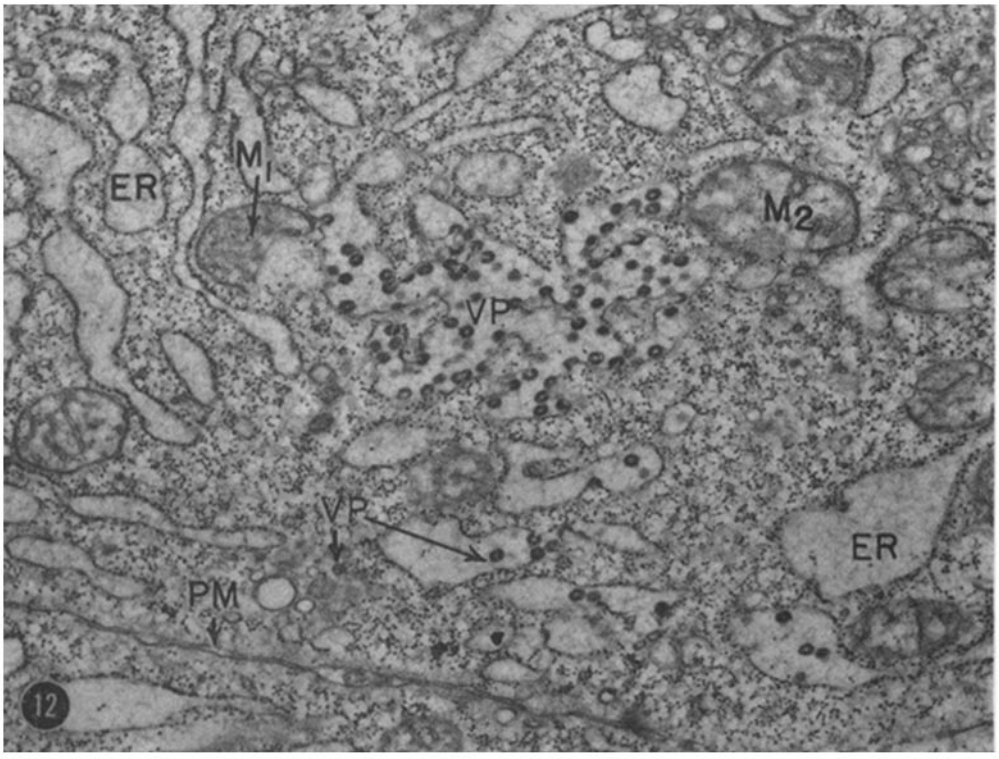
FIGURE 11

Portion of a typical plasma cell from an abdominal MPC-1 tumor (8 days after inoculation). The particles (*VP*) of size 77 mμ are apparently budding from the wall of the endoplasmic reticulum (*ER*). At VP_1, 3 particles are located in close proximity, and at VP_2 1 is located in the perinuclear space. The mitochondria (M_1–M_4) show extensive loss of structure. A few small granular bodies (*GB*) are present. × 25,600.

FIGURE 12

Large group of particles (*VP*) in abdominal MPC-1 tumor (8 days after inoculation). The mitochondria M_1 and M_2 show some loss of structure. The plasma membrane (*PM*) is lined with fine granular material. × 28,000.


VP
M1
GB
M2
G
ER
ER
VP1
M3
M4
ER
VP
N
VP2
11


ER
M1
M2
VP
VP
ER
PM
12

siderably in size from those found in the MPC-1 tumors. They are more pleomorphic and vary in size from 70 to 90 mμ. The particles found inside endoplasmic reticulum (Fig. 10), however, had the same size and appearance as those from MPC-1 tumors.

V. Particle Purification and Cell-Free Transfer Experiments

Cell-free Transfer: No positive results can be reported at present. A variety of extraction and inoculation techniques were carried out with the X5563 tumor and (C3H × 101)F_1 or (101 × C3H)F_1 mice used as recipients. Approximately 425 mice have received various ultracentrifuged fractions of homogenized tumor tissue. These experiments have been in progress for 6 to 12 months. Merwin and Algire (3), however, report finding tumors up to 17 months after the beginning of their experiments. The experiments in which filtered X5563 tissue culture supernatants were used (*see* Part II) have been under way for a shorter period.

Electron Microscopy of Ultracentrifuged Fractions of MPC-1 and X5563 Tumor Homogenates: Surprising difficulty was found in concentrating the particles from the tumors. Scattered particles were seen in 45,000 or 60,000*g* fractions of MPC-1 tumors. These seem to be diluted out by a large microsome or Palade granule fraction. In the cell fragments of the 10,000*g* fraction, many particles were still present inside endoplasmic reticulum, whether Waring Blendor homogenization or mortar and pestle grinding was used. Considerable numbers of particles were seen in the 60,000*g* fraction from a terminal (61 days after inoculation) X5563 tumor. The suspension of the tumor was prepared by grinding with a mortar and pestle. In most cases, the particles were clumped in a manner suggesting that portions of an aggregation had been sedimented. No particles were detected in ultracentrifuged fractions of the plasma of 6 mice or of the ascites fluid of 6 mice (Table I).

DISCUSSION

The electron microscopic examination of the MPC-1 tumor demonstrated that particles are abundant in the tumor plasma cells at all the stages of evelopment examined. In the absence, to date, of any positive results from cell-free, or relatively cell-free, transmission experiments, it cannot be claimed that the described particles are, in fact, viruses or the etiologic agents. The localization of the particles inside the endoplasmic reticulum in the MPC-1 or early X5563 tumors is a striking phenomenon. The possibility was

FIGURE 13

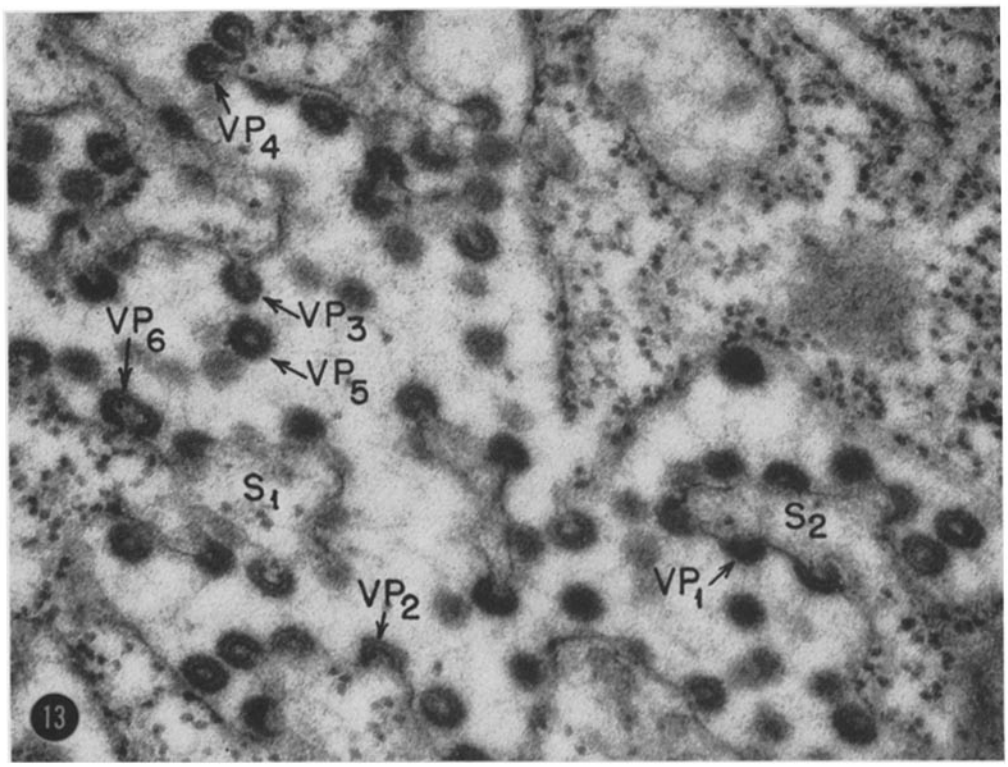
A portion of the endoplasmic reticulum of the cell shown in Fig. 12 at higher magnification. The particles (*VP*) appear to be in the process of budding from the wall of the endoplasmic reticulum. At VP_1 there is only a thickening of the wall, whereas particles VP_2–VP_4 appear to demonstrate the formation of neck attachment to the wall. At VP_6, 2 particles appear to be joined by a single neck. Particles free in the saccule (VP_5) are also shown. The indentations S_1 and S_2 appear to contain fewer Palade granules than the surrounding cytoplasm. × 85,000.

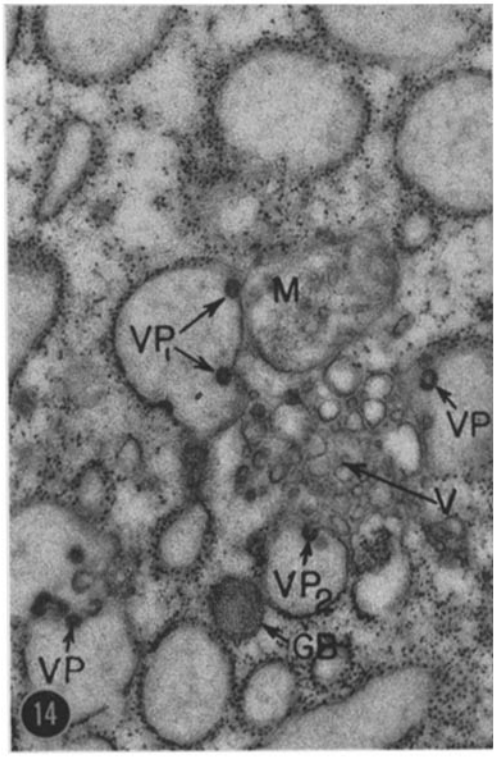
FIGURE 14

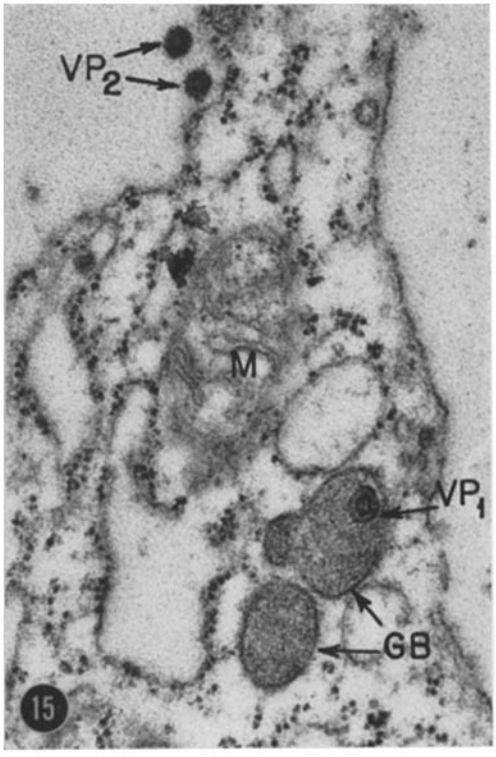
Particles (*VP*) in the Golgi region (MPC-1 tumor, 8 days after inoculation). The collection of smooth-walled vacuoles (*V*) is assumed to be part of the Golgi apparatus. The particles VP_1 and VP_2 appear to be budding from a smooth portion of the saccule. A degenerated mitochondrion (*M*) and the granular body (*GB*) are also shown. × 32,600.

FIGURE 15

A particle (VP_1) inside a granular body (*GB*). This particle may be compared with 2 particles (VP_2) lying inside the endoplasmic reticulum. (Early abdominal tumor 8 days after inoculation.) × 52,000.


VP4
VP6
VP3
VP5
S1
S2
VP1
VP2
13


M
VP1
VP
V
VP2
GB
VP
14


VP2
M
VP1
GB
15

considered that these particles represented a local proliferation of the wall of the endoplasmic reticulum. To investigate this, we examined stimulated normal plasma cells. These showed no evidence of such particles. Other cell types of the tumor-bearing mice having a large amount of endoplasmic reticulum in the cytoplasm (pancreas, thyroid, liver, and salivary glands) were also examined for a possible similar proliferation, with negative results. Close re-examination of the MPC-1 tumor, particularly at a very early stage, showed that particles do occur in the cytoplasmic matrix and often in association with a dense, finely granular material. The majority of the particles of the late X5563 tumor were present also in the cytoplasmic matrix and often so closely packed in the Golgi area as to form an inclusion body. In both tumor types, particles were sometimes seen inside the perinuclear space and were attached to either inner or outer nuclear membranes. The inner membrane is not usually considered to be part of the endoplasmic reticulum structure, but the outer membrane in this material can frequently be seen to be continuous with it. Rarely, in the MPC-1 tumor, a particle appeared to be budding from the plasma membrane.

The particles are doughnut shaped (similar to viruses of Type A in Bernhard's classification (13)) whenever they are present at a cell surface (endoplasmic reticulum, nuclear membranes, and plasma membrane). They are remarkably uniform in size (77 mμ) and do not show a dense center. When the particles are present in the cytoplasmic matrix, however, they are larger and more pleomorphic. The center is usually dense and may show the presence of a small nucleoid, or other fine structure. Some portions of the wall of the endoplasmic reticulum are thickened but other portions show two dense layers that bulge out into the saccule. The inner layer of some particles forms a complete sphere but the outer layer is constricted to a neck and is continuous with the wall of the endoplasmic reticulum.

The granular bodies described seem to have an association with the particles. The nature of the granular bodies as they occur in normal cells is not yet fully understood. Similar structures have been described in normal and neoplastic myeloblasts of the chicken (14, 15). Weissenfels (16) describes identical structures in several mouse tumor cells and believes them to be precursors of mitochondria. In the present examination of normal and tumor plasma cells, evidence of a double outer membrane and internal structures resembling cristae was frequently obtained. The conclusion that the granular bodies, at least those of smaller size, are early forms of mitochondria seems justifiable. These structures seemed of particular interest in the plasma-cell tumors studied because of the occasional finding of particles inside them (Fig. 15) and the large numbers distributed in the inclusion bodies of late X5563 tumors (Fig. 17).

FIGURE 16

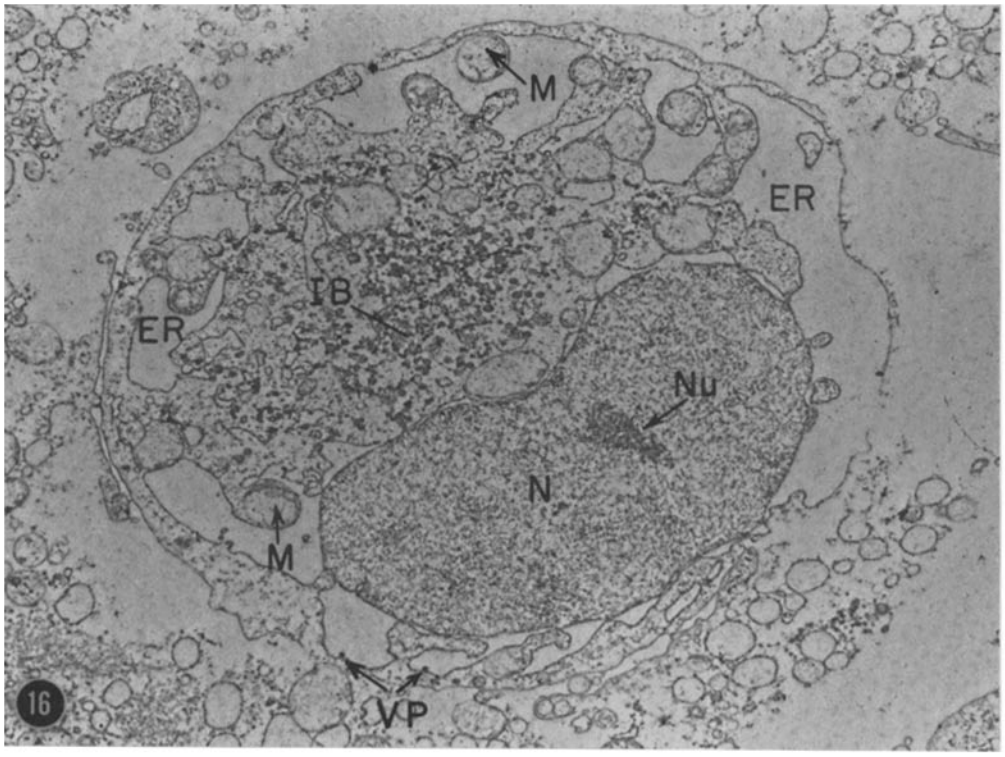
Low magnification view of typical plasma cell from a late (61 days after inoculation) X5563 tumor. A large aggregation of particle (*IB*) lies in the region of the Golgi apparatus. A few particles (*VP*) are found inside endoplasmic reticulum (*ER*). The mitochondria (*M*) show loss of structure. × 8900.

FIGURE 17

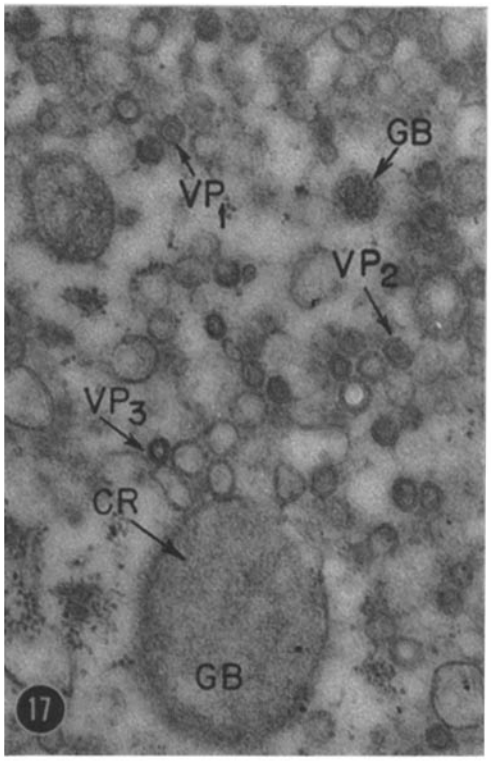
A portion of an aggregation of particles similar to that illustrated in Fig. 16 (*IB*). The particles are pleomorphic. Those at VP_1 and VP_2 have a dense nucleoid. The particle at VP_3 has a hollow center. The granular body (*GB*) shows a suggestion of cristae (*CR*). × 49,200.

FIGURE 18

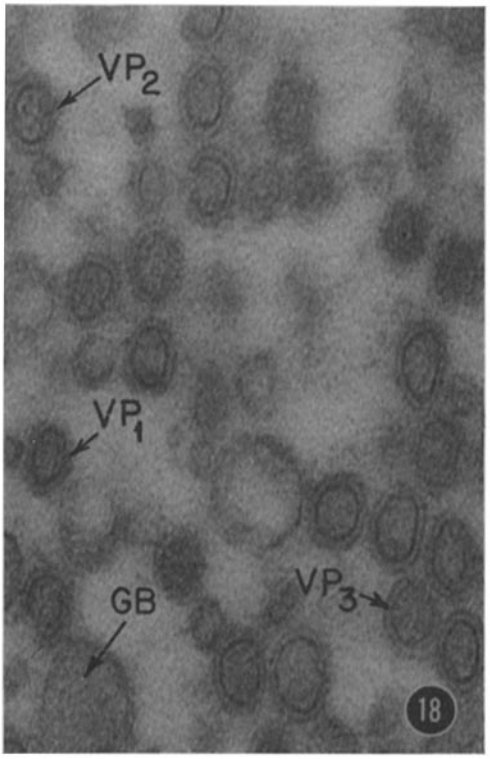
Particles (*VP*) at higher magnification. Taken from an inclusion present in a plasma cell of large X5563 tumor (61 days after inoculation). The particles VP_1 and VP_3 show additional linear structures in their center, and particle VP_2 appears to have a granular ultrastructure at its center. × 124,000.



16



17



18

Particles are found more frequently inside granular bodies in tissue cultures of X5563 tumors (*see* Part II). In avian myeloblastosis, the granular bodies of the myeloblasts *in vivo* contain very few recognizable virus particles; in a tissue culture in an enriched medium (14, 15) large numbers of mature particles seem to develop from these structures.

Peculiar difficulties seem to be connected with the isolation and purification of these particles. The MPC-1 and X5563 tumors contain large numbers of particles in the cytoplasm, but particles were very infrequent outside of the cells. An extensive search for particles in the plasma and ascites fluid of the mice bearing the MPC-1 tumor was also negative. Relatively few particles were seen free inside the endoplasmic reticulum (the majority being attached to the wall of the saccule) and, apparently, none outside the cells or in the body fluids. It does not seem surprising that only a few particles could be obtained by the usual purification procedures. The extraordinarily high yield of microsomes in these preparations tends to dilute the particles further.

As a result of this morphologic examination by the electron microscope of these two tumor lines at different stages of development, indicating that particles of a more complex type are to be found only in the late stage X5563 tumor, acellular transmission experiments using extracts of this late tumor are now being carried out.

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REFERENCES

1. POTTER, M., and FAHEY, J. L., Studies on eight transplantable plasma-cell neoplasms of mice, *J. Nat. Cancer Inst.*, 1960, **24**, 1153.
2. BICHEL, J., A transplantable plasma-cell leukemia in mice, *Acta path. et microbiol. scandinav.*, 1951, **29**, 464.
3. MERWIN, R. M., and ALGIRE, G. H., Induction of plasma-cell neoplasms and fibrosarcomas in BALB/c mice carrying diffusion chambers, *Proc. Soc. Exper. Biol. and Med.*, 1959, **101**, 437.
4. RASK-NIELSEN, R., GARMSEN, H., AND CLAUSEN, J., A transplantable plasma-cell leukemia in mice associated with the production of β-paraprotein, *J. Nat. Cancer Inst.*, 1959, **22**, 509.
5. HOWATSON, A. F., and MCCULLOCH, E. A., Virus-like bodies in a transplantable mouse plasma-cell tumor, *Nature, London*, 1958, **181**, 1213.
6. DALTON, A. J., and POTTER, M., Some of the features of the fine structure of a series of plasma-cell tumors of mice, *Proc. Am. Assoc. Cancer Res.*, 1959, **3**, 14.
7. LIEBERMAN, R., DOUGLAS, J. O. A., and MANTEL, N., Production in mice of ascitic fluid containing antibodies induced by Staphylococcus or Salmonella-adjuvant mixtures, *J. Immunol.*, 1960, **84**, 514.
8. DUNN, T. B., Plasma-cell neoplasm beginning in the ileocecal region in strain C3H mice, *J. Nat. Cancer Inst.*, 1957, **19**, 371.
9. CARTWRIGHT, S. F., and THORNE, H. V., Some applications of detergents to the study of the virus of foot-and-mouth disease, *J. Gen. Microbiol.*, 1959, **20**, 61.
10. ALGIRE, G. H., BORDEN, M. L., and EVANS, V. J., Studies of heterografts in diffusion chambers in mice, *J. Nat. Cancer Inst.*, 1958, **20**, 1187.
11. PARSONS, D. F., and DARDEN, E. B., JR., A technique for the simultaneous dirt-free lead staining of several electron microscope grids of thin sections, *J. Biophys. and Biochem. Cytol.*, In press.
12. BESSIS, M., Cytology of the blood and blood-forming organs, New York, Grune & Stratton, 1956, 538–539.
13. BERNHARD, W., Electron microscopy of tumor cells and tumor viruses. A review, *Cancer Res.*, 1958, **18**, 491.
14. PARSONS, D. F., BEAUDREAU, G. S., BONAR, R. A., and BEARD, J. W., Cell multiplication, virus liberation, and ultrastructure of avian myeloblastosis in tissue culture. *Acta Unio internat. contra cancrum*, 1959, **15**, 826.
15. HADDAD, M. N., WEINSTEIN, D., BONAR, R. A., BEAUDREAU, G. S., BECKER, C., BEARD, D. and BEARD, J. W., Virus of avian myeloblastosis. XV. Structural loci of virus synthesis and adenosinetriphosphatase activity by electron and light microscopy of myeloblasts from tissue culture, *J. Nat. Cancer Inst.*, 1960, **24**, 971.
16. WEISSENFELS, N., Über die funktionelle Entleerung, den Feinbau und die Entwicklung von Tumor-Mitochondrien, *Ztschr. Naturforsch.*, 1957, **12B**, 168.