VIRUS PARTICLES IN THE THYMUS OF CONVENTIONAL AND GERM-FREE MICE

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PLATES 81 TO 86

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In 1944 McEndy *et al.* (1) demonstrated that the incidence of "spontaneous" leukemia in AKR mice was markedly decreased by thymectomy. From this observation, since confirmed and developed by many investigators (2–7), it has been concluded that the thymus plays an important role in the pathogenesis of several murine leukemias as well as in normal lymphopoiesis. Since it has been convincingly established that several mouse leukemias are virus-induced diseases (8), we decided to investigate the presence of viruses in thymic cells and thereby establish a basis for a possible correlation between these two sets of experimental data. The fine structure of the viruses associated with different types of murine leukemia is well known (9–14) and electron microscopy seemed therefore an appropriate method to investigate this problem. Laboratory mice are commonly contaminated by several known and unknown viruses (15). It was therefore decided to study the ultrastructure of both conventional and germ-free[1] mouse thymuses.

Material and Method

Conventional Mice.—The thymuses of 7 young Swiss (Taconic Farms, New York) and 24 AKR (Roscoe B. Jackson Memorial Laboratory, Bar Harbor) mice were removed under ether anesthesia and immediately sliced in a drop of cold fixative, containing 2 per cent osmium tetroxide buffered at pH 7.4 with acetate-veronal (16) and supplemented with 45 mg sucrose per ml of fixative (17). Fixation was performed in the cold for 1 or 2 hours. The samples were dehydrated in ethanol and embedded in prepolymerized butyl-methyl (8/2) methacrylate with 2 per cent luperco (2,4,-dichlorobenzoylperoxide with dibutyl phthallate) as catalyst. Polymerization was carried out overnight at 50° or 60°C or by exposure to a 15 watt ultraviolet source.

Germ-Free Mice.—To avoid any possible contamination of the germ-free mice during transportation or during their brief presence in our laboratory, the thymuses of these animals were fixed in the laboratories of Dr. M. Pollard, Lobund Laboratory, Notre Dame, Indiana.

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[1] Dr. M. Pollard, Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana, kindly made germ-free mice available to us for these observations. It is our pleasure to acknowledge his stimulating interest in our work.

Fixations were always performed within 5 minutes after the mice were taken out of their sterile incubator. At autopsy all the germ-free animals showed the enlarged and dark cecum characteristic of the germ-free rodents.

In this case specimens were fixed first in 6.25 per cent glutaraldehyde (18) in 0.1 M phosphate buffer (pH 7.6). Fixation was continued for 2 to 3 hours in the cold, and the pieces of tissue were then transferred to cold 0.1 M phosphate buffer (pH 7.6) containing 0.33 M sucrose in which they were brought to our laboratory in New York where they were postfixed in 2 per cent osmium tetroxide. After ethanol dehydration, the tissues were embedded in epon 812 with 2 per cent DMP-30 as a catalyst, and polymerized at 60°C (19). The results obtained by glutaraldehyde fixation were less satisfactory than those obtained with osmium tetroxide alone used for the thymuses of the conventional mice. The frequently poorly outlined plasma membranes and the high electron density of the cytoplasmic matrix seem attributable to glutaraldehyde fixation or to long storage in the sucrose buffer solution.

Sectioning and Microscopy.—Both methacrylate- and epon-embedded samples were cut with diamond knives and Porter-Blum microtomes. Sections were mounted on bare grids (300 mesh) (20), stained with uranyl acetate alone or followed by lead hydroxide (Karnovsky method A, reference 21) and finally coated with one (epon sections) or two (methacrylate sections) carbon films. The preparations were observed in a Siemens elmiskop I electron microscope.

RESULTS

Conventional Mice (Figs. 1 to 9).—Under the electron microscope, the structure of the thymus appears complex and different cell types have to be considered. Thymic lymphocytes and epithelial cells are the major components. Reticular and endothelial cells, macrophages, and rare extravascular granulocytes are minor components of the cell population. The distribution of these cell types varies considerably from the cortex to the medulla of the thymus. However, at the magification used in electron microscopy it is sometimes difficult to delineate sharply between these two zones. It is not the purpose of this paper to give a description of all the cell types involved in thymus architecture, since this can be found in recent papers (22, 23). The present study is limited to the thymic cells associated with virus particles. At present, this association has been observed only in thymic lymphocytes and certain epithelial cells.

Fig. 1 shows, at low magnification, a portion of the thymic cortex of a 2 month old Swiss mouse. Small thymic lymphocytes are in close contact with each other and, under the conditions of fixation used, they leave practically no open extracellular spaces. Their plasma membranes, however, never make contact structures or desmosomes and therefore these cells are probably able to migrate. They are polyhedral in shape, and their nucleocytoplasmic ratio is high. The cytoplasm contains very rare elements of the endoplasmic reticulum. The few vesicles observed belong to Golgi complexes, in the center of which a pair of centrioles is frequently seen. Some elongated mitochondria and dispersed free ribosomes are present. Mitochondria are sometimes grouped on one side of the cell, as they frequently are in lymphoblasts. When fixation is performed in phosphate-buffered OsO_4 solutions, large inclusions of particulate glycogen

are preserved. The aspect of the nuclei depends to a large extent upon fixation and staining techniques and will not be described here.

Fig. 2 illustrates the type of intracytoplasmic particles occasionally observed within thymic lymphocytes, in this case in a young Swiss mouse. These spherical particles consist essentially of two concentric membranes or shells, the diameters of which average respectively 70 and 52 mμ. The center of these particles has a density similar or slightly higher than that of the surrounding cytoplasmic matrix. No "nucleoid" is observed, nor any cylindrical forms. These particles have a tendency to cluster. They are free in the cytoplasmic matrix and show no attachment to any cytoplasmic organelle. They have never been seen within the nuclei or outside the cells.

Fig. 3 shows, for comparative purposes, identical particles in a transplanted lymphoma of Swiss mouse, in this case a thymic lymphoma appeared in a male DBA/2 mouse 6 months after intraperitoneal lymphoma cell transplantation. The presence of these particles in Swiss mouse lymphoma cells and the transplantation of this material in Swiss and DBA/2 mice have been the subject of previous reports (11, 24, 25). In these tumors, the particles occasionally make contact with the plasma membrane as a preliminary step in a budding out process. Since they have been seen frequently in all stages of mitotic activity of the lymphoma cells (11), they at least do not inhibit mitosis and can hardly correspond to some degenerative process. These bodies have been frequently referred to as "lymphoma particles" (25), but it should be stressed that identical particles are almost invariably present in murine mammary carcinomas (10, 13), and are frequently associated with plasma cell tumors (26) and mast cell tumors (27).

As mentioned earlier, certain thymic *epithelial* cells are also associated with virus particles as demonstrated by Figs. 4 to 7. In this case, however, the particles are localized within intracytoplasmic vacuoles, and their structure is more complex, due to the presence of one additional outer membrane or envelope.

Figs. 4 and 5 illustrate two epithelial cells of a normal Swiss mouse thymus. These cells are much larger than the lymphocytic elements, and they are frequently localized in the medulla, near the boundary between the cortex and medulla or in the pericapillary areas. These localizations do not appear clearly under the electron microscope because of excessive magnification. Semithin sections stained with alkaline methylene blue help greatly in establishing the topographical relationships of these cells which can easily be recognized under the light microscope by their vacuolated cytoplasm. Their possible relationship with the PAS-positive cells described by Metcalf (28) remains to be elucidated. Thymic epithelial cells were described recently by Clark (22). The cytoplasm of some of them shows a diversity of small and large vacuoles, dense bodies of possible lysosome nature, mitochondria, and bundles of microfibrils connected

with typical desmosomes. The large vacuoles are frequently concentrated in one area of the cytoplasm. They are limited by a typical single membrane provided with short microvilli which protrude toward the lumen. The lumen of these large vacuoles is partially filled by a finely fibrillar material which has the density and the texture of basement membranes. These vacuoles also contain a few dense spherical bodies of approximately 100 mμ in diameter (arrows). Some of these bodies are intimately attached to the limiting membrane of the vacuole (Fig. 5). Identification of these dense particles can hardly be achieved at low magnification. It seems clear, however, that they cannot be confused with the cross-sections of the short microvilli pointing in the lumen of the vacuoles.

Fig. 6 is a higher magnification of the cell seen in Fig. 4 (arrow). The dense particle is covered by a bulge of the membrane and shows a crescent of high electron density (arrow). This aspect is identical with the budding process described earlier as a maturation step of leukemia-associated viruses (9).

Fig. 7 is a higher magnification of part of the section seen in Fig. 5. The vacuole contains an apparently free particle of high electron density. The envelope of this particle has the structure of the membrane on which it probably differentiated. Unit membranes with a clear interspace of approximately 25 to 30 A can be seen. Within this envelope is a particle which in its dimension and density is identical with the lymphoma particles seen in Figs. 2 and 3.

Strikingly similar observations have been made on the virus particles associated with several murine neoplasia. Figs. 8 and 9 illustrate observations made respectively in a Swiss mouse inoculated with lymphoma cells filtrate and in a spontaneous mammary carcinoma of a C3H/HeJ mouse.

In Fig. 8, the budding process is slightly more advanced than in Fig. 6 since a pedicle is forming. In this case the plasma membrane of the infected cell clearly forms the envelope of the future mature virion. It is also apparent that the inner component of this particle is morphologically identical with a lymphoma particle as seen in Figs. 2 and 3. However, in the cytoplasm of these leukemia virus–infected cells, free lymphoma particles are never observed. We have to conclude therefore that these particles are synthesized in immediate association with the plasma membrane of the infected cells and do not accumulate in the cytoplasm to form the inclusion bodies seen in thymic lymphocytes (Fig. 2) or in lymphoma cells (Fig. 3). This immediate budding out process characterizes also the thymic epithelial cells in the cytoplasm of which free lymphoma particles have not been observed.

Fig. 9 shows a group of extracellular particles in a spontaneous mammary carcinoma of a C3H/HeJ mouse. The similarity to the particle seen in Fig. 7 and the presence of inner components identical with lymphoma particles is remarkable. In mammary tumors these particles will ultimately transform into type B particles by a swelling of the envelope and by a condensation of the

inner components forming an eccentric nucleoid. Whether this last transformation is a final step in a maturation cycle or a first sign of degeneration remains to be established.

In the thymuses of AKR mice the findings have been similar to those made in Swiss mice, except for the fact that no intracytoplasmic particles were observed. All the particles seen in AKR mice thymuses were of the extracellular type. They were frequently seen budding along the plasma membranes of thymic lymphocytes or apparently free in the extracellular spaces.

Germ-Free Mice (*Figs. 10 to 14*).—Our samples of thymic tissue from germ-free mice were fixed in glutaraldehyde and embedded in epon. Obviously, preservation is different from that obtained by direct osmium tetroxide fixation for the membranes are frequently poorly outlined. This is unfortunate since the virus particles comprise a series of membranous components. Other structures like cytoplasmic microfibrils and ribosomes show up distinctly.

TABLE I

Frequency of Positive Observations

Mice		No. of thymuses in which virus particles were observed			
Type	No.	Intracytoplasmic particles only	Extracellular particles only (or budding)	Intracytoplasmic and extracellular	Total No. of thymuses
Conventional					
Swiss (young adults) ♀ and ♂	7	1	2	3	6
AKR/J	24	0	12	0	12
Germ-free					
Swiss	10	0	5	0	5
C3H	2	0	0	1	1

Fig. 10 illustrates an epithelial cell in the thymus of a germ-free C3H mouse. Despite the difference in fixation, the similarity to Figs. 4 and 5 is striking. The large vacuoles contain the same type of particle, easily distinguished from cross-sections of microvilli. The arrow points at a thickening of the limiting membrane which represents an early step in the differentiation of a particle.

Figs. 11 to 13 show different vacuoles of epithelial cells, all from germ-free mice and all containing the characteristic particles, the inner membranes of which are well preserved in Figs. 11 and 12. A particle still attached to the membrane by a narrow pedicle is seen in Fig. 11 (*P*) where an early step of budding can also be observed (arrow). In Fig. 12 unit membranes are well preserved, but this is not the case for Fig. 13 in which, however, an early step of the budding process is seen (arrow).

Fig. 14 is not an epithelial cell, but a thymic lymphocyte of a germ-free mouse which shows the characteristic particle budding to the extracellular space from the plasma membrane.

Frequency of These Observations.—The particles observed in both conventional and germ-free mice are much less numerous than those present in murine neoplasms. Long study of many sections has been necessary, which is in sharp contrast to the abundance of the particles in murine lymphoma or mammary tumors. Although thin sections represent an extremely limited sample and negative results are therefore of little significance, the incidence of positive observations is relatively high, as shown in Table I.

DISCUSSION

Three questions will be considered in the discussion of the findings reported here: 1. Are these particles viruses? 2. Are the intracytoplasmic and extracellular particles different phases in the development of the same agent? 3. If these particles are viruses, can they be identified?

1. The particles described here do not resemble any known cellular component. The pitfalls of viral identification are now well known by electron microscopists and have been recently reviewed by Haguenau and Hollmann (29). Small vesicles, nuclear pores, cross-sections of microvilli, and collagen fibers obviously bear no possible comparison with the particles now under consideration. Furthermore, these particles are identical in their dimensions, fine structure, and localizations to several viruses, and especially those associated with the murine leukemias (9–14), the physicochemical properties of which are very similar to those of well known infectious viruses.

Therefore, it seems highly probable that the particles observed in the thymus of both conventional and germ-free mice are indeed viruses. It follows that the germ-free mice subjected to our investigation were not virus-free. This conclusion had to be expected since several routes of vertical transmission of viral infection (30, 31) are not eliminated by the germ-free technique. It follows also that germ-free mice are not yet the ideally "clean" system available for experimental purposes.

2. Two types of virus particles have been observed in the thymuses of our mice: (*a*) intracytoplasmic particles, present in thymic lymphocytes, and (*b*) extracellular (or intravacuolar) ones, present occasionally at the surface of thymic lymphocytes but most frequently in some thymic epithelial cells. This association of intracytoplasmic and extracellular particles characterizes two well known murine spontaneous tumors, the mammary carcinoma (10, 13) and the lymphoma (25). In neither case has it been concluded that multiple infection is present, because all transitions between the intracytoplasmic and extracellular forms have been observed in the same infected cells. The difficulty in the case of the thymus stems from the fact that the two types are observed in

completely different cells. However, the inner components of the extracellular particles resemble very closely the intracytoplasmic particles (Figs. 2 to 9). It seems therefore that the two cell types are infected by the same, or by at least closely related agents which develop following different patterns in lymphocytic or epithelial cells. In lymphocytic elements they frequently accumulate within the cytoplasm as probably incomplete viruses and occasionally bud out of the cells. In epithelial cells they form from the beginning in contact with the plasma membranes from which they receive their outer envelope and leave the cytoplasm as mature virions.

Intracytoplasmic accumulation of "incomplete" particles was only observed in the thymus of Swiss mice which have a low incidence of spontaneous leukemia. To what extent the particles associated with thymic cells are responsible for the incidence of "spontaneous" murine leukemia and for the preventive antileukemic effect of thymectomy are important questions which can be raised but not answered by electron microscopy.

3. Mice are heavily contaminated by viruses (15, 32, 33). Some are latent, some are infectious, some are oncogenic, and many of them look similar under the electron microscope. Identification of a given virus by morphological methods alone is bound to be precarious and incomplete. Biological tests have to be performed, cytopathogenicity studied, and antigenicity evaluated to identify viral agents with accuracy. These tests were not done in the present study, and the very small number of particles observed in the thymic cells makes problematical any purification attempt.

However, ultrastructural data can contribute to a preliminary effort of identification. The viruses observed in the thymuses have a completely different structure from that of polyoma virus (10), of ectromelia virus (13), and of the thymic agent (34). Little is known about the ultrastructure of the LDH-elevating agent, but it seems that this widespread agent is of slightly smaller size than the viruses described here (35, 36). The morphology of an oncolytic virus described by Nelson (37) is not established clearly enough to permit comparisons.

The particles present in the thymus of conventional and germ-free mice are indistinguishable from those associated with several types of mouse leukemia (9–14) and similar to those of mammary tumors (10, 13). The fact that they have been observed in apparently healthy animals is not surprising, since "leukemic viruses presumably exist in many normal mice, without causing disease" (8). Moreover, we do not know how many of our mice would have developed "spontaneous" leukemia if they had been allowed to live longer. An extremely long latent period is well known for the mammary tumor agent. If these thymic particles are pathogenic, their association with either epithelial or lymphocytic cells may be of some importance. Epithelial cells are fixed, lymphocytes are extremely mobile and can emigrate throughout the body.

The latter can therefore carry viral infection to other lymphoid tissues, and the passage of these particles from the epithelial to the lymphoid cells might represent a crucial moment in the pathogenicity of these agents.

One should not forget, however, that similar particles have been described also in non-leukemic conditions. The viruses observed in Ehrlich ascites tumor cells (38), in an epizootic diarrhea of infant mice (39), and in cultured L cells (40) are morphologically indistinguishable from those presently under study. Whether the same agent is endowed with a large spectrum of pathogenic potentialities, or whether different viruses display very similar morphology remains a central problem. A TL (thymus-leukemia) antigen has been identified recently in the normal thymuses of certain strains of mice (41). The possible relationship between this antigen and the particles associated with thymic cells certainly merits extensive studies.

The best experimental approach to the pathogenicity of the particles associated with the thymic cells is to test the biological effects of thymic extracts. This was done several years ago by Grégoire (2) and by Metcalf (3) who concluded to the existence of a thymic lymphocytosis stimulating factor (LSF). The cell-free character of this "factor" has been recently established (42, 43) and it has not been possible to demonstrate its hormonal nature (44).

Interestingly enough, both Grégoire (2) and Osoba and Miller (43) indicate that the factor probably originates in the epithelial cells of the thymus. To our knowledge, nothing opposes the hypothetical particulate nature of this thymic factor. Obviously, a complete characterization of the thymic factor is needed, and to date we can only speculate about a hypothetical relationship between the factor and the virus particles associated with thymic cells of apparently healthy mice.

SUMMARY

Electron microscope study of thymuses of both conventional and germ-free mice has revealed the presence of typical virus particles associated with the thymic lymphocytes or with the thymic epithelial cells. The particles resemble those associated with several murine leukemias and their viral nature seems convincingly substantiated by morphological observation. Germ-free mice are therefore not virus-free. The biological significance of these particles is still unknown and we can only speculate as to the possible relationship of these particles to the incidence of "spontaneous" leukemia, to the lymphocytosis stimulating factor of Metcalf, and to the numerous latent viral infections of laboratory mice.

The skillful technical assistance of Mrs. C. Jamieson and Miss N. Lampen was greatly appreciated.

BIBLIOGRAPHY

1. McEndy, D. P., Boon, M. C., and Furth, J., On the role of thymus, spleen, and gonads in the development of leukemia in a high leukemia stock of mice, *Cancer Research*, 1944, **4**, 377.
2. Grégoire, C., and Duchateau, G., A study on lympho-epithelial symbiosis in thymus. Reactions on the lymphatic tissue to extracts and to implants of epithelial components of thymus, *Arch. Biol., Liège*, 1956, **67**, 267.
3. Metcalf, D., The thymic origin of the plasma lymphocytosis stimulating factor, *Brit. J. Cancer*, 1956, **10**, 442.
4. Metcalf, D., The effect of thymectomy on the lymphoid tissues of the mouse, *Brit. J. Haematol.*, 1960, **6**, 324.
5. Metcalf, D., The aetiology and pathogenesis of leukemia, *Australian Ann. Med.*, 1962, **11**, 211.
6. De Somer, P., Denys, P., and Leyten, R., Activity of a noncellular calf thymus extract in normal and thymectomized mice, *Life Sc.*, 1963, **11**, 810.
7. Gross, L., Serial cell-free passage in rats of the mouse leukemia virus: effect of thymectomy, *Proc. Soc. Exp. Biol. and Med.*, 1963, **112**, 939.
8. Gross, L., Oncogenic viruses, *Ann. Roy. Coll. Surg. Engl.*, 1963, **33**, 67.
9. de Harven, E., and Friend, C., Further electron microscope studies of a mouse leukemia induced by cell-free filtrates, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 747.
10. Bernhard, W., The detection and study of tumor viruses with the electron microscope, *Cancer Research*, 1960, **20**, 712.
11. de Harven, E., Ultrastructural studies on three different types of mouse leukemia; a review, *in* Tumors Induced by Viruses: Ultrastructural Studies, (A. J. Dalton and F. Haguenau, editors), New York, Academic Press, Inc., 1962, 183.
12. Dalton, A. J., Micromorphology of murine tumor viruses and of affected cells, *Fed. Proc.*, 1962, **21**, 936.
13. Dmochowski, L., The electron microscopic view of virus-host relationship in neoplasia, *Progr. Exp. Tumor Research*, 1963, **3**, 35.
14. de Harven, E., and Friend, C., Structure of virus particles partially purified from the blood of leukemic mice, *Virology*, 1964, **23**, 119.
15. Rowe, W. P., Hartley, J. W., and Huebner, R. J., Polyoma and other indigenous mouse viruses, *Lab. Animal Care*, 1963, **13**, 166.
16. Palade, G. E., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **95**, 285.
17. Caulfield, J. B., Effects of varying the vehicle for OsO_4 in tissue fixation, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 827.
18. Sabatini, D. D., Bensch, K., and Barnett, R. J., Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation, *J. Cell Biol.*, 1963, **17**, 19.
19. Luft, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
20. de Harven, E., A new technique for carbon films, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 133.

21. Karnovsky, M. J., Simple methods for "staining with lead" at high pH in electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 729.
22. Clark, S. L., Jr., The thymus in mice of strain 129/J, studied with the electron microscopy, *Am. J. Anat.*, 1963, **112,** 1.
23. Weiss, L., An electron microscopic study of thymic reticular cells in AKR and C3H mice and albino rats, *Anat. Rec.*, 1963, **145,** 297.
24. Friend, C., Darchun, V., de Harven, E., and Haddad, J., The incidence and classification of spontaneous malignant diseases of the haematopoietic system in Swiss mice, *Ciba Found. Symp., Tumour Viruses Murine Origin*, 1962, 193.
25. de Harven, E., and Friend, C., Electron microscope studies on mouse lymphomas, *in* 5th International Congress for Electron Microscopy, Philadelphia, 1962, (S. S. Breese, Jr., editor), New York, Academic Press, Inc., 1962, **2,** MM-5.
26. Howatson, A. F., and McCullogh, E. A., Virus-like particles in a transplantable mouse plasma cell tumour, *Nature*, 1958, **181,** 1213.
27. Bloom, G. D., Electron microscopy of neoplastic mast cells: a study of the mouse mastocytoma mast cell, *Ann. New York Acad. Sc.*, 1963, **103,** 53.
28. Metcalf, D., and Ishidete, M., Periodic acid–Schiff positive giant cells in the mouse thymus cortex, *Nature*, 1961, **191,** 305.
29. Haguenau, F., and Hollmann, K. H., Diagnostic différentiel entre virus et particules cytoplasmiques d'autre nature, le problème au cours de l'étude des tumeurs au microscope électronique, *Bull. Assn. Franc. Etude Cancer*, 1963, **50,** 29.
30. Downie, A. W., Pathways of virus infection, *in* Mechanisms of Virus Infection, (W. Smith, editor), New York, Academic Press, Inc., 1963, 101.
31. Burmester, B. R., The vertical and horizontal transmission of avian visceral lymphomatosis, *in* Basic Mechanisms in Animal Virus Biology, *Cold Spring Harbor Symp. Quant. Biol.*, 1962, **27,** 471.
32. Stewart, S. E., and Haas, V. H., Lymphocytic choriomeningitis virus in mouse neoplasms, *J. Nat. Cancer Inst.*, 1956, **17,** 233.
33. Pearson, H. E., and Baker, R. F., Persistent Theiler's virus in ependymoma tissue culture and the problem of virus-like bodies seen by electron microscope, *J. Nat. Cancer Inst.*, 1961, **27,** 793.
34. Rowe, W. P., and Capps, W. I., A new mouse virus causing necrosis of the thymus in newborn mice, *J. Exp. Med.*, 1961, **113,** 831.
35. Bladen, H. A., and Notkins, A. L., Electron microscope demonstration of the lactic dehydrogenase agent, *Virology*, 1963, **21,** 269.
36. Rowson, K. E. K., Mahy, B. W. J., and Salaman, M. H., Size estimation by filtration of the enzyme-elevating virus of Riley, *Life Sc.*, 1963, **7,** 479.
37. Nelson, J. B., Recovery and behavior of hepatitis virus from Swiss mice infected with ascites tumor, *Proc. Soc. Exp. Biol. and Med.*, 1963, **113,** 909.
38. Adams, W. R., and Prince, A. M., An electron microscope study of the morphology and distribution of the intracytoplasmic "virus-like" particles of Ehrlich ascites tumor cells, *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 161.
39. Adams, W. R., and Kraft, L. M., Epizootic diarrhea of infant mice: identification of the etiologic agent, *Science*, 1963, **141,** 359.

40. Dales, S., and Howatson, A. F., Virus-like particles in association with L strain cells, *Cancer Research*, 1961, **21**, 193.
41. Old, L. J., Boyse, E. A., and Stockert, E., Antigenic properties of experimental leukemias. I. Serological studies *in vitro* with spontaneous and radiation-induced leukemias, *J. Nat. Cancer Inst.*, 1963, **31**, 977.
42. Levey, R. H., Trainin, N., and Law, L. W., Evidence for function of thymic tissue in diffusion chambers implanted in neonatally thymectomized mice, preliminary report, *J. Nat. Cancer Inst.*, 1963, **31**, 199.
43. Osoba, D., and Miller, J. F. A. P., The lymphoid tissues and immune responses of neonatally thymectomized mice bearing thymus tissue in Millipore diffusion chambers, *J. Exp. Med.*, 1964, **119**, 177.
44. Rudali, G., Research of a "thymic hormone" in the etiology of lymphoid leukoses of AKR mice, *Acta, Unio Internat. Contra Cancrum*, 1963, **19**, 252.

EXPLANATION OF PLATES

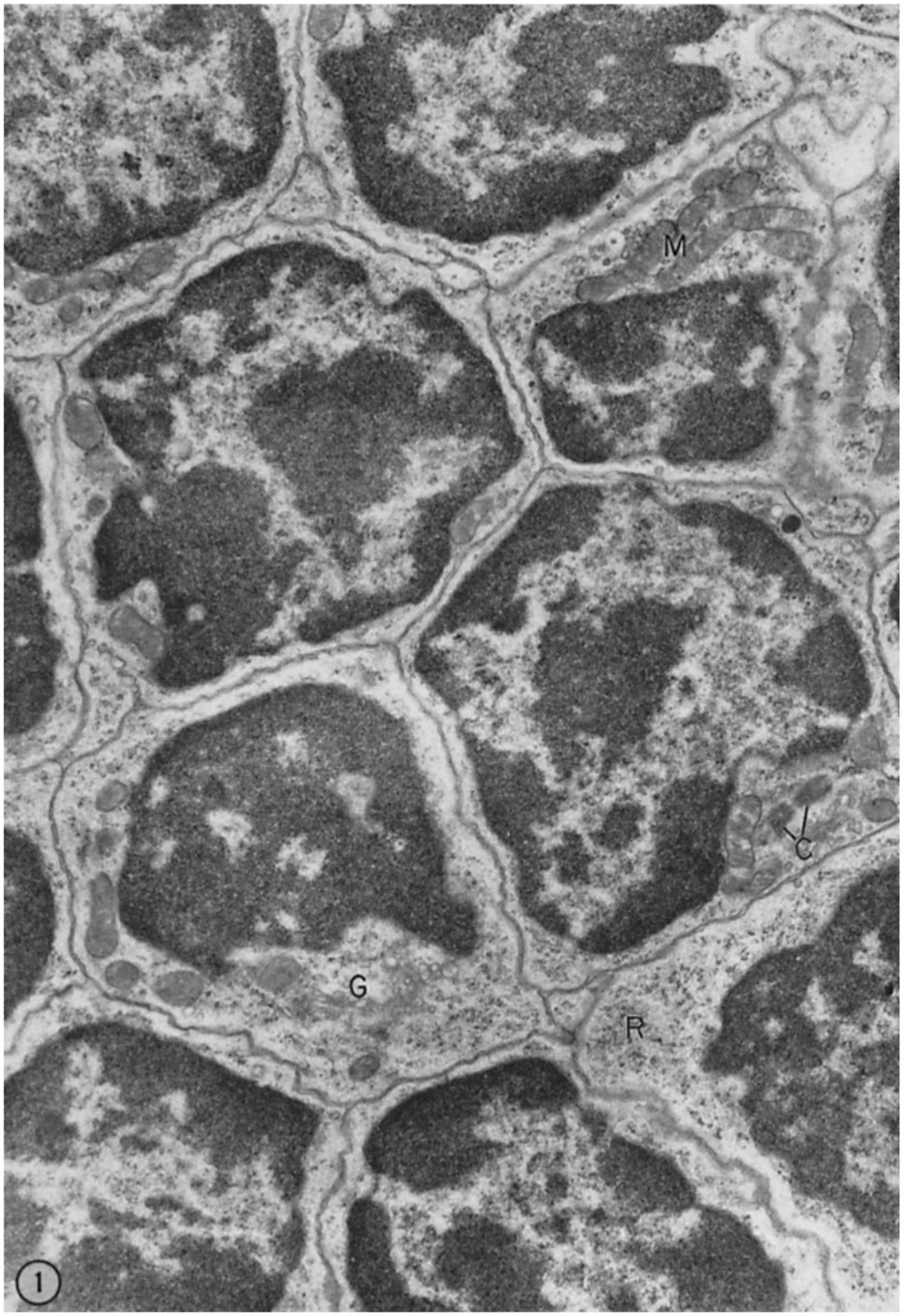
Abbreviations for Figures

C, centriole
D, dense bodies
F, microfibrils
G, Golgi complex
M, mitochondria
MV, microvilli
N, nucleus
NM, nuclear membrane
P, viral pedicle
PM, plasma membrane
R, ribosomes
Va, intracytoplasmic vacuole
VP, virus particle

Figs. 1 to 9 represent electron micrographs of thin sections prepared from osmium tetroxide–fixed tissues embedded in methacrylate. Figs. 10 to 14 are from specimens fixed first in glutaraldehyde and postfixed with osmium tetroxide and embedded in epon 812. All sections were stained with uranyl acetate for 60 minutes, followed by lead hydroxide for 30 minutes.

PLATE 81

FIG. 1. Thymus cortex of a young Swiss mouse showing several thymic lymphocytes. The cells are slightly polyhedral in shape. The nucleocytoplasmic ratio is very high. Within the cytoplasmic rim, clusters of ribosomes (*R*), mitochondria (*M*), Golgi complex (*G*), and centrioles (*C*) are seen. The chromatin is marginated. No virus particles are seen within these cells. Magnification 18,000.

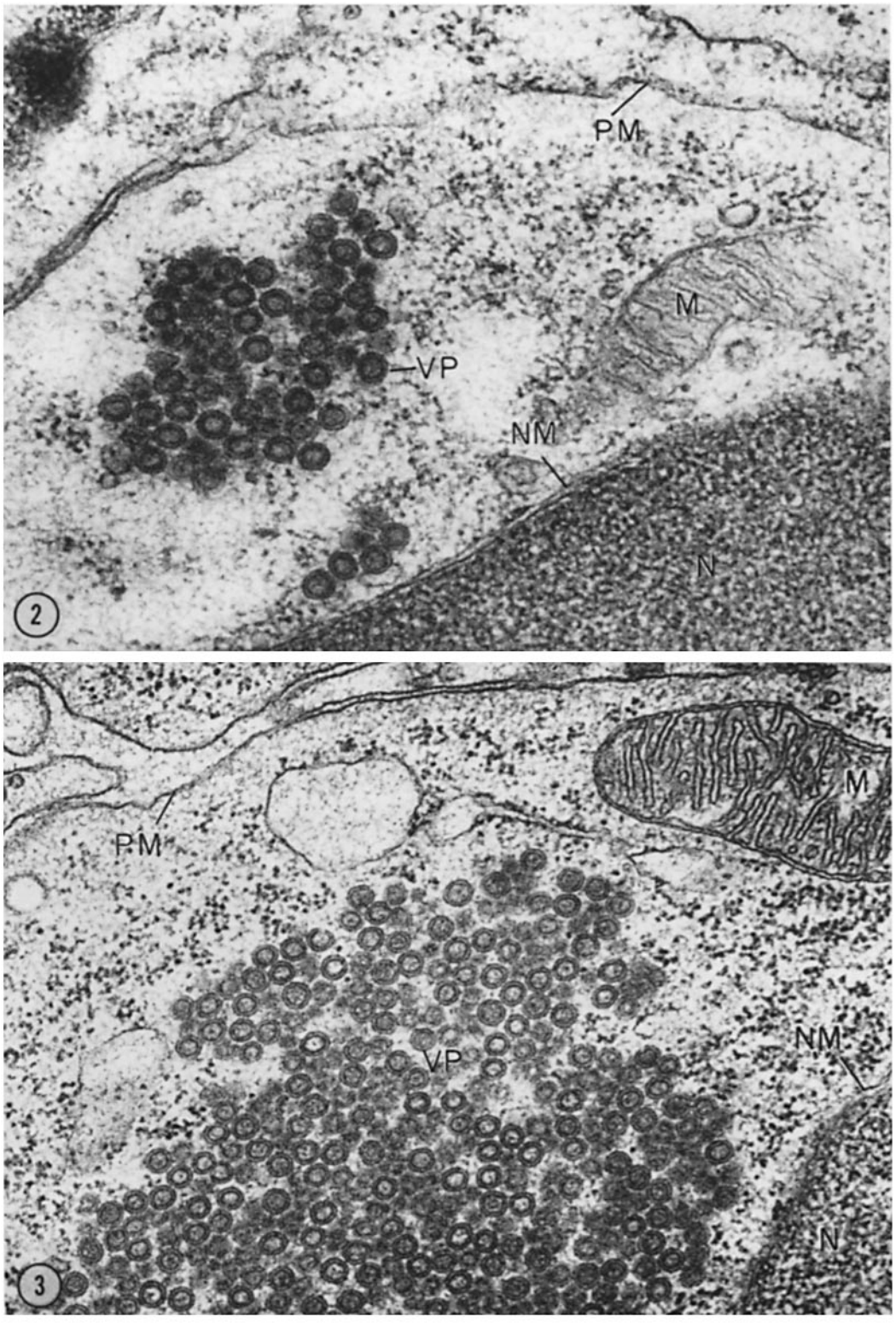


(de Harven: Virus particles in thymus)

PLATE 82

FIG. 2. Thymic lymphocyte in a young male Swiss mouse. Within the cytoplasmic matrix a group of round particles (*VP*), approximately 70 mμ in diameter, is seen. These particles consist of two concentric shells and have a relatively clear center. They are not associated with any cytoplasmic organelle. Four particles are seen near the nuclear membrane (*NM*). Magnification 60,000.

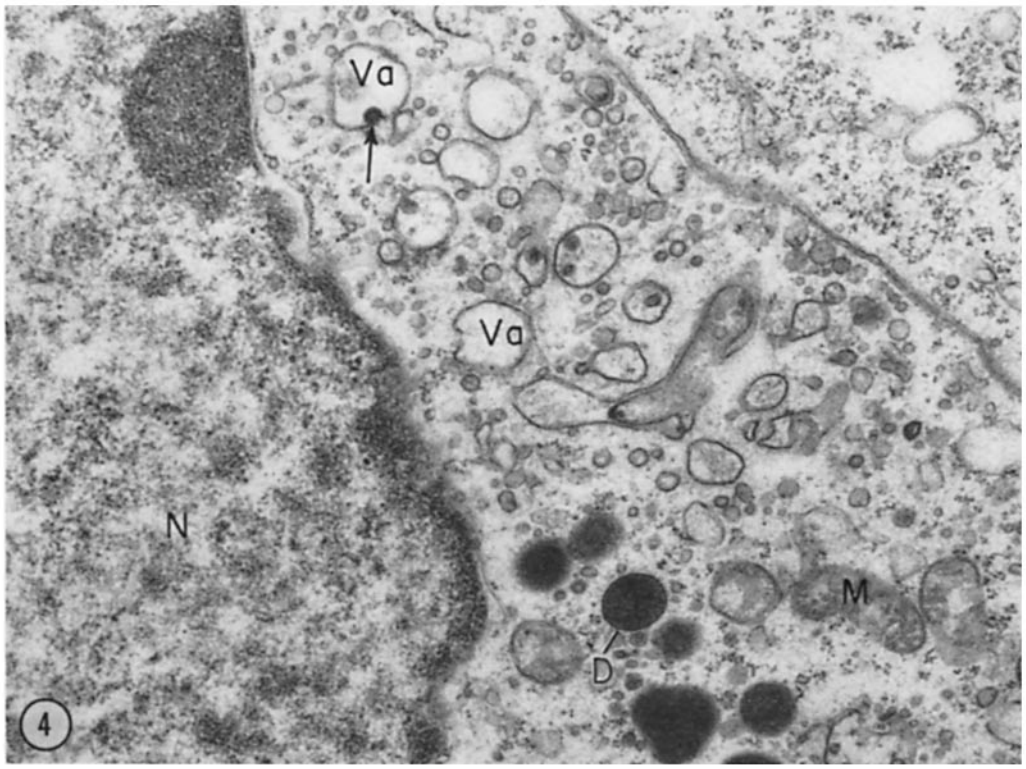
FIG. 3. Transplanted malignant lymphoma in a DBA/2 male mouse. A large intracytoplasmic inclusion body consisting of many spherical particles is seen. The fine structure of these particles is identical with that of the particles seen in Fig. 2. Magnification 48,000.

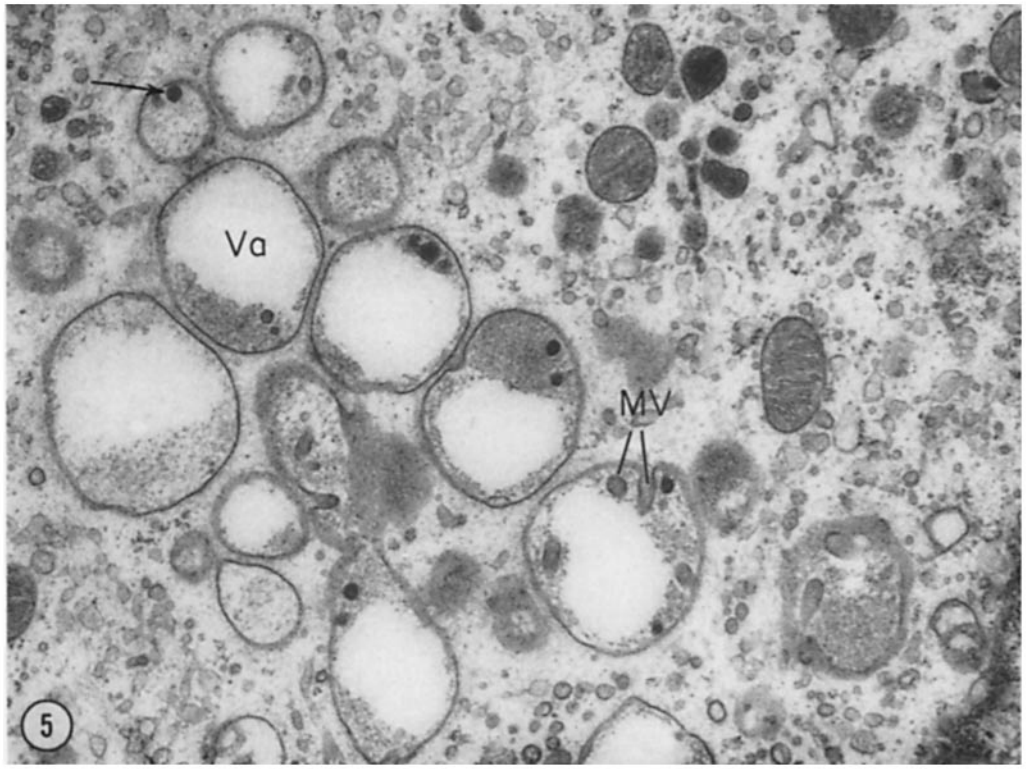


(de Harven: Virus particles in thymus)

PLATE 83

FIGS. 4 and 5. Portions of epithelial cells of the thymus of a Swiss mouse. The cytoplasm contains large vacuoles (*Va*), mitochondria (*M*), scattered ribosomes, vesicular elements of the endoplasmic reticulum, and dense bodies (*D*) of possible lysosome nature. Dense particles of approximately 100 mμ in diameter are present within the large vacuoles (arrows). Only a higher magnification study would permit the demonstration of the viral nature of these particles. Cross-sections of microvilli (*MV*) have approximately the same diameter but completely lack the density and the inner structure of the viral particles. Magnifications: Fig. 4, 24,000; Fig. 5, 21,000.





(de Harven: Virus particles in thymus)

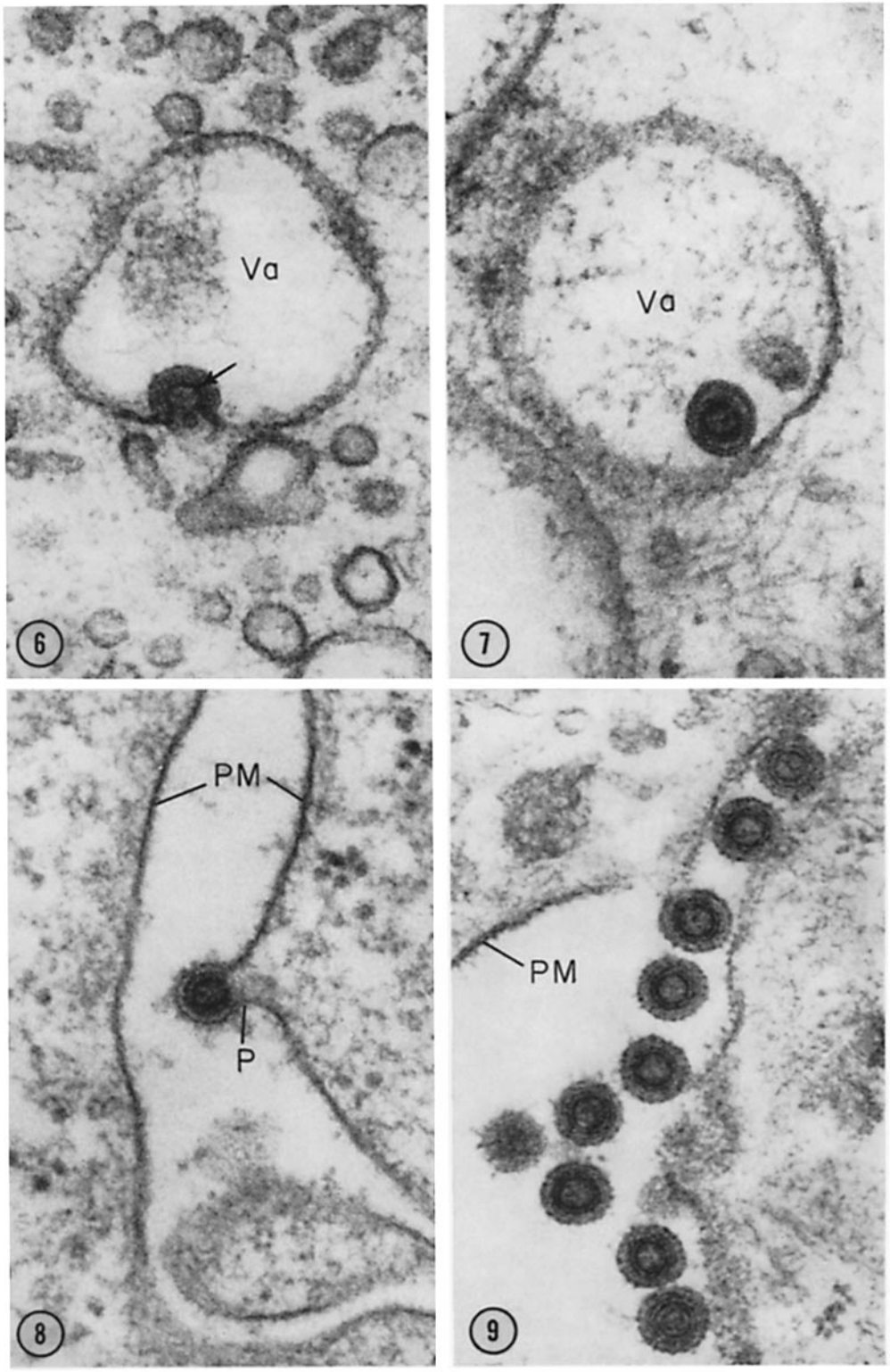
PLATE 84

FIG. 6. Higher magnification of the vacuole seen on top of Fig. 4. The particle is in the process of budding out from the limiting membrane. The inner components of the particle show the high density and characteristic structure of this phase of virus maturation. Magnification 95,000.

FIG. 7. Higher magnification of the cytoplasmic vacuole seen on top of Fig. 5. This vacuole apparently contains a mature particle which is no longer attached to the limiting membrane. The envelope of the particle has the typical unit membrane structure, and the inner components are very similar in shape, contrast, and proportion to the intracytoplasmic particles seen in Figs. 2 and 3. The lumen of these large vacuoles can be considered as an equivalent of the extracellular space, and the mature particle seen in this figure as an extracellular particle. The viruses present within cytoplasmic vacuoles of epithelial cells cannot be interpreted as phagocyted particles since virus differentiation is frequently observed along the membrane limiting these vacuoles. This situation closely parallels that observed in the megakaryocytes of leukemic mice (9). Magnification 105,000.

FIG. 8. Lymphoma cells infiltrating the liver of a Swiss mouse, 53 days after intraperitoneal inoculation of a filtrate prepared from DBA/2 transplanted lymphoma tissue. Typical budding of a particle is seen along the plasma membrane of this infiltrating cell. The process is more advanced than in the case of Fig. 6 and a pedicle (*P*) is formed in direct continuity with the future envelope of the particle. Magnification 108,000.

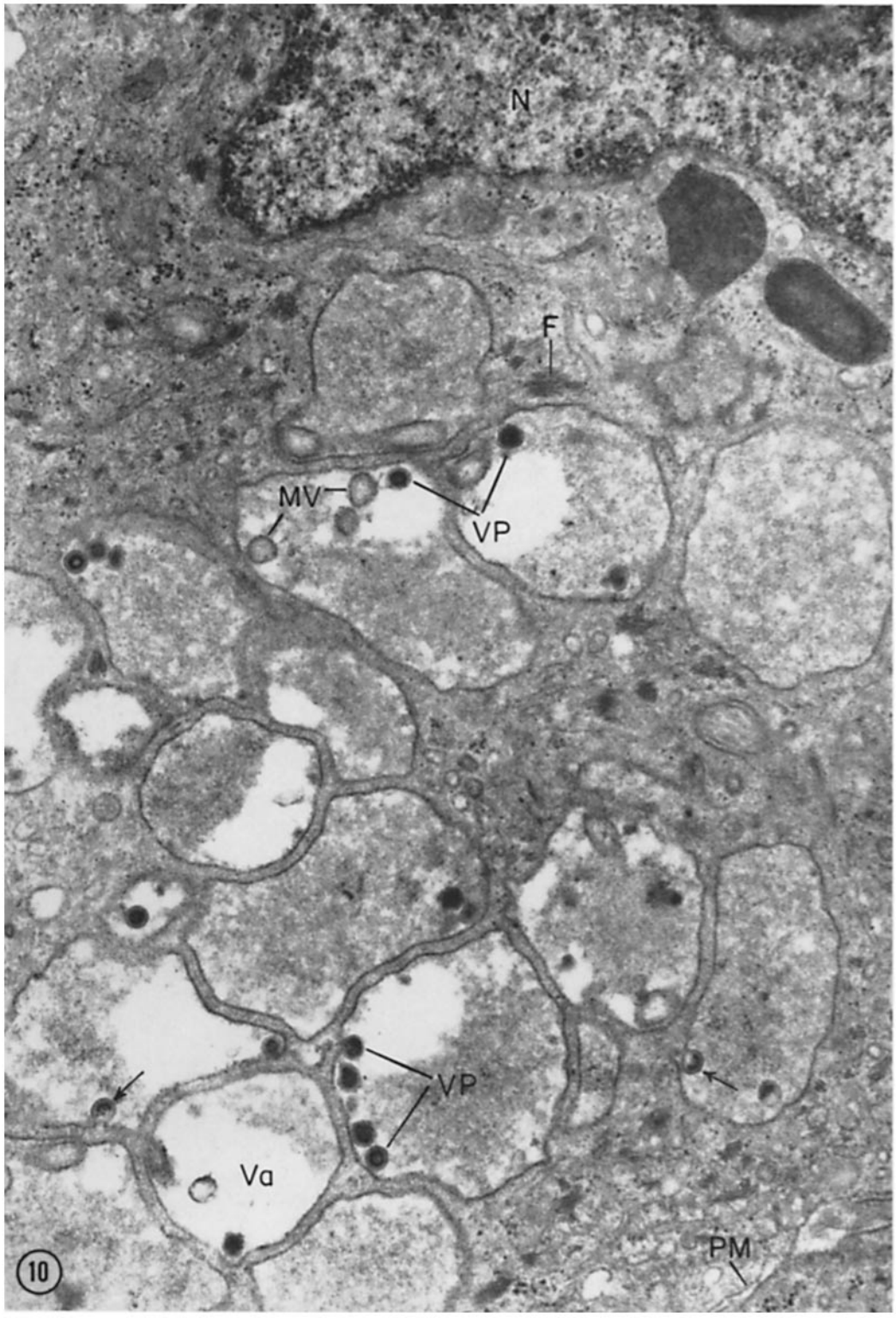
FIG. 9. Spontaneous mammary carcinoma of a C3H mouse. A group of extracellular particles is seen which all clearly show inner structures indistinguishable from the particles seen in Figs. 2 and 3. Note also the great similarity between these particles and those present in normal thymuses (Fig. 7). Magnification 80,000.



(de Harven: Virus particles in thymus)

PLATE 85

FIG. 10. Epithelial cell in the thymus of a 45-day-old germ-free C_3H mouse. Despite the difference in fixation (glutaraldehyde has been used here), the similarity between this cell and those illustrated in Figs. 4 and 5 is striking. Cytoplasmic microfibrils (*F*) are preserved, and the cytoplasmic matrix has a great electron density. Large cytoplasmic vacuoles (*Va*) contain a few typical mature virus particles (*VP*) easily distinguished from cross-sections of microvilli (*MV*). The arrows point to a thickening of the limiting membrane presumably corresponding to an early phase of virus differentiation. Magnification 35,000.

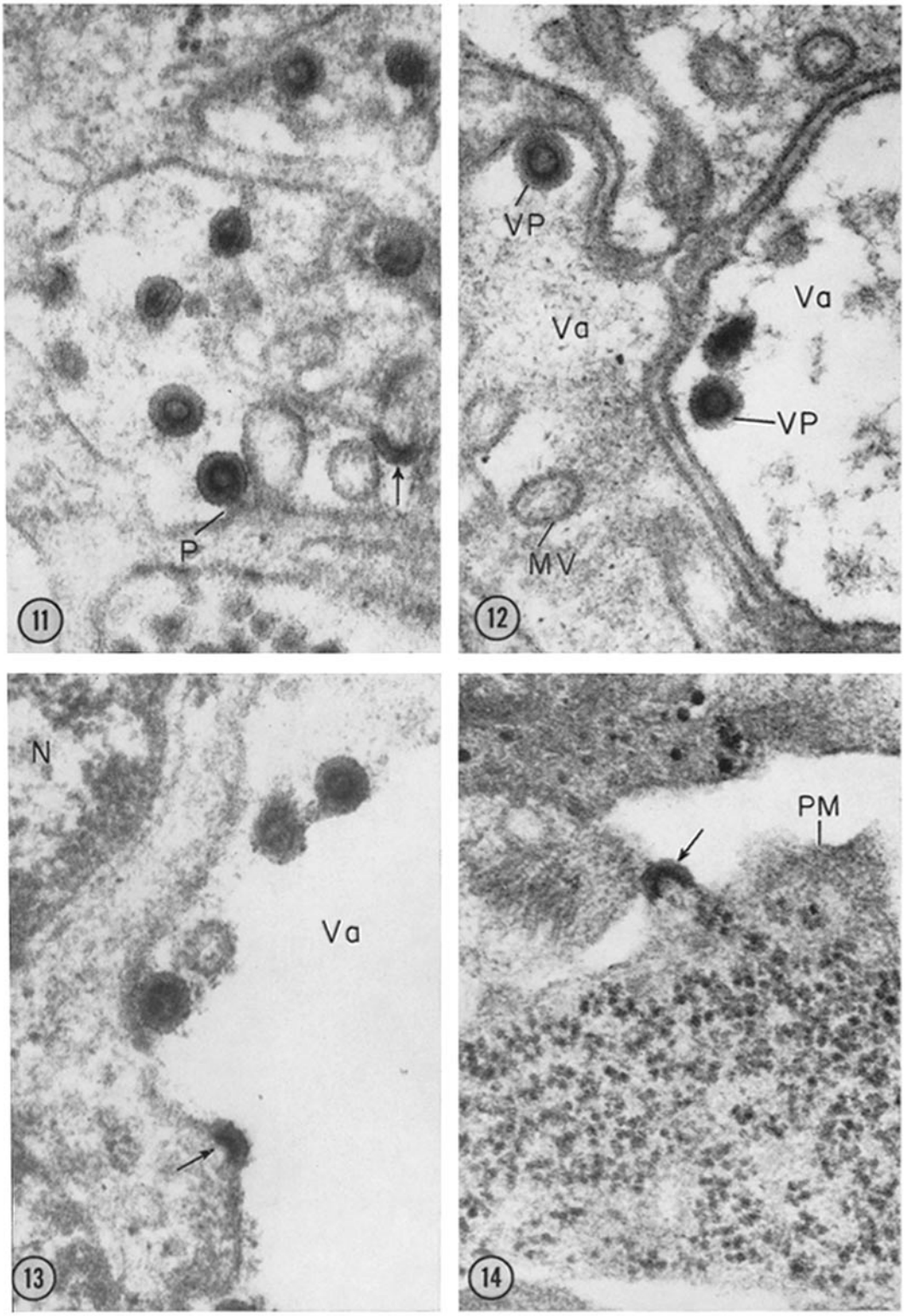


(de Harven: Virus particles in thymus)

PLATE 86

FIGS. 11 to 13. Higher magnification study of several vacuoles of epithelial cells observed in the thymus of the same germ-free mouse as in Fig. 10. Several virus particles are seen (*VP*). Most of them are mature particles, but some are still attached to the limiting membrane by a pedicle (*P*). The arrow points to an early phase in the virus differentiation process. Magnifications: Fig. 11, 65,000; Fig. 12, 72,000; Fig. 13, 72,000.

FIG. 14. Portion of the cytoplasm of a thymic lymphocyte. The plasma membrane is poorly outlined (*PM*), but shows however a viral bud protruding toward the extracellular space (arrow). Magnification 64,000.



(de Harven: Virus particles in thymus)