STUDIES ON THE IN VIVO AND IN VITRO MULTIPLICATION OF THE LDH VIRUS OF MICE

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The possible role of the reticuloendothelial system (RES) in explaining several of the unusual sequelae of infection with the lactic dehydrogenase (LDH) virus has been discussed in a recent review by Notkins (1). Among these are the high plasma virus titer at 24 hr after infection, the chronic viremia, and the persistent elevation of several enzymes in the plasma of the host. More recently, additional evidence has been presented which indicates that the plasma enzyme elevation (2) and the level of viremia (3) may be caused by a virus-induced blocking of the clearance mechanism of the RES. Although it has been suggested that this blocking results from virus in RES cells, it is not clear whether the virus is replicated within or simply phagocytozed by these cells.

Several preliminary reports on the cultivation of the LDH virus in vitro have been published. Yaffe (4) has presented data on multiplication of the virus in primary mouse embryo cell cultures, but not in secondary cultures. Plagemann (5) reported that infected primary cultures of mouse spleen, lung, and embryo produce the virus for many weeks. Evans (6) stated that "no difference was observed between RV (LDH virus) growth curves in cultures prepared from whole mouse embryo, embryo liver, adult spleen, or peritoneal macrophages." More recently, Anderson et al. (7) stated that the LDH virus persisted for as long as 88 days in mouse embryo cell cultures. In contrast to these reports, others were unable to propagate the LDH virus in primary mouse embryo cell cultures (8–10). Evans and Salaman (11) have recently reported maintaining the virus for 23 passages in primary cultures of mouse macrophages.

In this paper, four series of experiments will be presented, which are compatible with LDH virus replication in the RES.

1. The LDH virus increase in the blood plasma will be compared with the increase in those organs which have the RES in common.

2. An electron microscopic study of the occurrence of the LDH virus in certain organs will be presented.

3. The failure of X-irradiation to reduce the virus titer in the plasma will be reported.

4. Studies on the in vitro replication of the LDH virus will be presented, which confirm and extend the results of Evans and Salaman (11).

Materials and Methods

Source of Virus.—The LDH virus used as inoculum was obtained from the ascitic fluid of infected CDF_1 mice (NIH strain) bearing Ehrlich ascites carcinoma (12). The method of titration of the virus has been described previously (12). Lactic dehydrogenase (LDH) activity was assayed by the method of Wroblewski and La Due (13).

In Vivo Growth Studies.—Adult female mice (20 to 25 g) of strain CDF_1 or the NIH strain of Swiss mice (G.P. mice) were inoculated intraperitoneally with $10^{6.0}$ to $10^{8.0}$ ID_{50} per dose. Plasma, ascitic fluid, or organs were collected 3 to 48 hr after inoculation. Cells were removed from blood and ascitic fluid by centrifugation at 900 RCF for 10 min. Organs were homogenized in Eagle's medium containing 20% veal infusion broth (14) to give a w/v concentration of 10 or 1%. All samples were stored at −25°C for subsequent virus assay. In some experiments (Table I) mice were perfused with a solution containing 7.5 g of sodium citrate and 8.0 g of sodium chloride/liter before harvest of the organs.

Electron Microscopy.—Samples of fresh organs of G.P. mice were fixed in 1% osmium tetroxide, and buffered to pH 7.4 with McEwen's saline (15). Dehydration was accomplished by ethanol and propylene oxide and cells were embedded in Maraglas (16). Sections, cut on a Porter-Blum microtome, were stained with lead citrate (17) and were examined in an RCA EMU 3E electron microscope.

Leukocyte and Macrophage Counts.—Total leukocyte and macrophage counts were performed with a hemocytometer, after staining with crystal violet. Differential counts were made on smears stained with Wright's solution.

Tissue Culture Methods.—14- to 16-day-old mouse embryos, and lymph nodes from adult G.P. mice, were trypsinized and cultivated in Petri dishes in various media (Table II) until monolayers were formed. The cultures were then inoculated with $10^{5.3}$ to $10^{7.0}$ ID_{50} per dose. After 1 to 5 days, the supernatant fluids were harvested and stored at −25°C for subsequent virus assay.

Macrophages.—Macrophages were obtained from adult G.P. mice which had been injected intraperitoneally 48 hr previously with 3 ml of trypticase soy broth. The peritoneal cavity was washed with 2 ml of medium 199 containing 10 units of heparin per ml. (Differential staining with Wright's stain revealed that 90% or more of the cells obtained by this method were macrophages.) After the washings were incubated for 2 hr at 37°C in 16 x 125 mm screw cap tubes or 16 x 95 mm Leighton tubes, the supernatant was replaced by 1 ml of medium 199 with 10% fetal bovine serum (FBS). The only cells adhering to the glass were, histologically, macrophages and were not removed by the usual trypsinization procedure (18). The medium was renewed 3 times per week. When the macrophage cultures were 3 to 12 days old, they were inoculated with $10^{4.0}$ to $10^{5.9}$ ID_{50} of stock virus. Supernatants of these cultures were removed for LDH virus assay 1 to 14 days after inoculation. The medium was renewed at 3-day intervals or after a sample was removed for virus assay.

RESULTS

In vivo studies.—In order to determine which organs contained an LDH virus titer comparable to that in the plasma, blood plasma, and several organs of G.P. mice and CDF_1 mice, the latter with or without Ehrlich ascitic cancer, were

TABLE I

LDH Virus Titers in Various Organs 24 Hr After Inoculation

Experiment No.	Organ	No. of Mice	Strain of mice	Perfused	Tumor* bearing	Titer inoculum	24 hr titer‡	Mean titer‡
1	Plasma	35	G.P.	−	−	6.0	9.9	
2	"	30	"	−	−	6.0	9.5	10.0
3	"	10	CDF_1	−	+	8.0	10.5	
4	Ascites fluid	5	CDF_1	−	+	8.0	10.0	
5	" "	10	"	−	+	6.0	10.3	10.3
6	" "	10	"	−	+	6.0	10.7	
7	Spleen	2	G.P.	+	−	6.0	10.1	
8	"	2	"	+	−	6.0	10.7	10.5
9	"	3	CDF_1	−	+	7.0	10.1	
10	Lymph Nodes§	2	G.P.	+	−	6.0	9.5	
11	" "	2	"	+	−	6.0	9.7	9.9
12	" "	3	CDF_1	−	+	7.0	10.5	
13	Thymus	3	CDF_1	−	+	7.0	10.5	9.8
14	"	4	"	−	−	8.0	9.1	
15	Liver	3	CDF_1	−	+	7.0	9.5	
16	"	2	"	+	−	7.0	8.9	9.0
17	"	1	G.P.	+	−	7.0	8.5	
18	Kidney	2	CDF_1	+	−	7.0	8.5	8.5
19	Pancreas	3	CDF_1	−	−	7.0	8.5	8.5
20	Brain	2	G.P.	+	−	6.0	7.5	7.5
21	Tumor cells‖	5	CDF_1	−	+	8.0	7.5	7.5

* 7-day-old Ehrlich ascites carcinoma.

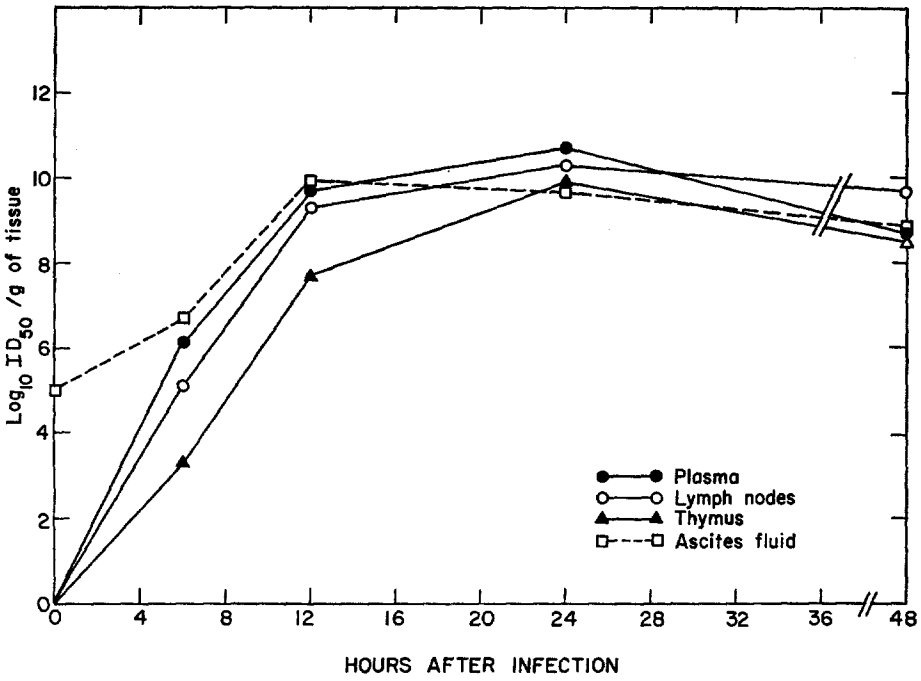
‡ Titers expressed as ID_{50}/ml (plasma, ascites fluid) or gram wet weight (organs, cells).

§ Superficial lymph nodes.

‖ 3 × washed with Eagle's medium plus 20% veal infusion broth.

assayed for virus content 24 hr after inoculation. The results are presented in Table I. It can be seen that the spleen, superficial lymph nodes (submaxillary, axillary, cubital, and peritoneal), and thymus showed titers approaching that in the plasma and the ascitic fluid, whereas the liver titer was somewhat lower. Kidney, pancreas, brain, and washed tumor cells had still lower titers. It can further be seen (column 5, Table I) that the amount of plasma retained by the organs did not significantly affect the virus titers.

Next, it was determined whether the rise in titer in the organs containing the greatest amount of virus coincided with the increase in titer in the plasma. Text-fig. 1 presents the increase in virus titer in plasma, ascitic fluid, superficial lymph nodes, and thymus of CDF_1 mice having Ehrlich ascitic carcinoma. In lymph nodes, the rise in titer coincided with that in the plasma; the titers in the thymus, at 6 and 12 hr after infection, were lower than those in the plasma at similar times. Text-fig. 2 presents the rise in virus titer in plasma and superficial



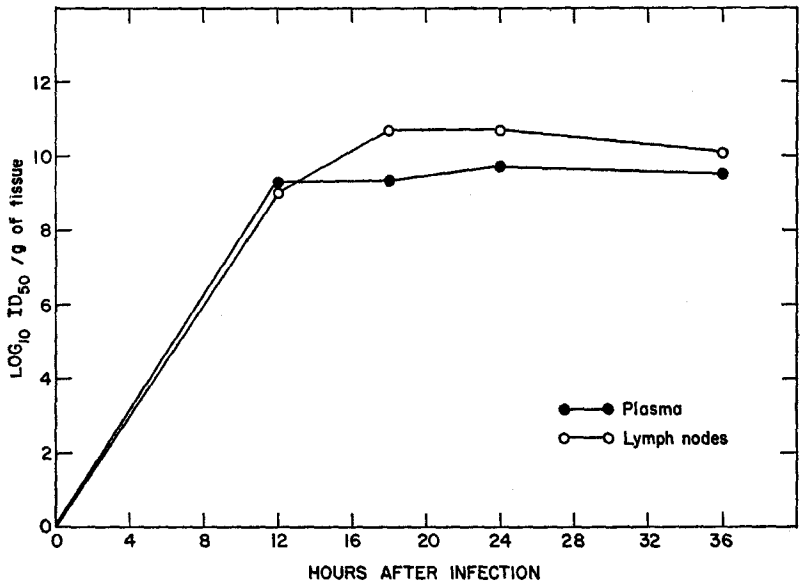
TEXT-FIG. 1. Increase in LDH virus titers, at various times after infection, in plasma, lymph nodes, thymus, and ascites fluid of CDF_1 mice with Ehrlich ascites carcinoma.

lymph nodes of infected normal CDF_1 mice. The titer in the lymph nodes is higher than in the plasma at 18, 24, and 36 hr after infection. Text-fig. 3 presents the increasing titers of virus in plasma, spleen, superficial and deep lymph nodes (mesenteric lymph nodes and Peyer's patches) of normal G.P. mice. Early after infection, the titers of the deep lymph nodes paralleled those in the plasma. Until 20 hr after infection, the titers in the spleen and superficial nodes remained somewhat lower than that in the plasma.

These data prompted the electron microscopic examination of lymph nodes and spleens at various stages of infection up to 24 hr. To date, particles characteristic of the LDH virus, as seen in virus preparations purified from ascitic

fluid (12) (Fig. 1), have been found in large numbers only in the cytoplasm of certain lymph node cells (Figs. 2 to 7), never in the nuclei. They have been rarely observed, in small number, in germinal center cells of infected spleens.

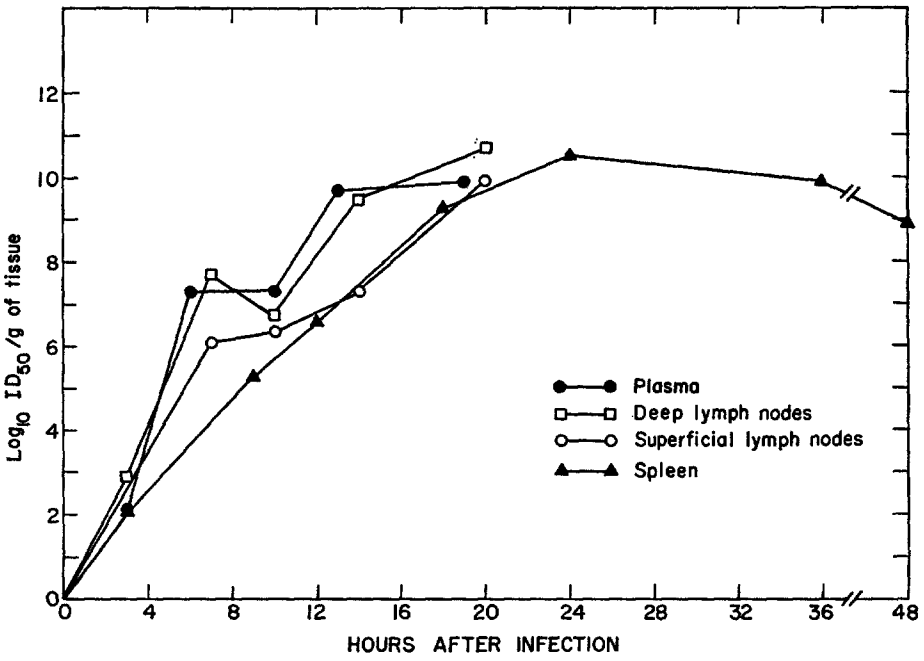
Within lymph nodes in animals infected for 18 to 24 hr, the cytoplasm of a small number of cells was observed at low power to have an overall increase in density over that of adjacent cells (Fig. 2). On higher magnification (Fig. 3), the details of the cytoplasm of such a cell may be seen. Two types of particles are apparent. The first consists of a small densely staining annulus about 25 mμ in



TEXT-FIG. 2. Increase in LDH virus titer, at various times after infection, in plasma and lymph nodes of normal CDF_1 mice.

diameter. A second particle appears to consist of a similar dense annulus with a poorly defined less dense halo extending the diameter to about 50 mμ. A third structure is frequently observed as a loosely fitting single membrane of extremely variable size, shape, and density surrounding any number of 25 or 50 mμ particles. These three structures are indistinguishable in size and morphology from those particles observed in pellets of partially purified virus from infected ascitic fluid (Fig. 1). Neither the 25 nor the 50 mμ particles have been observed in uninfected cells, nor, as yet, in cells infected for less than 18 hr. The variability of the various membrane-bounded structures associated with the LDH virus particles is further illustrated in Figs. 4 and 5, which are portions of the same cell 24 hr after infection. In Fig. 4, small, double membraned vesicles are predominant, some in a figure-8 configuration surrounded by, but not con-

taining, a large number of 25 mμ particles. In Fig. 5, most particles are located within single membraned structures, and are either of the 25 or 50 mμ type. Occasionally, as in Fig. 6, 25 or 50 mμ particles appear to fill small, single membraned vesicles. These vesicles may contain an amorphous material of a density sufficiently similar to that of the outer halo as to render impossible the differentiation of the type of the particles.



TEXT-FIG. 3. Increase in LDH virus titer, at various times after infection, in plasma, superficial and deep lymph nodes, and spleen of normal G.P. mice.

Fig. 7 shows a lymph node cell 18 hr after infection of the animal. Most of the above described structures and variations may be seen in a small portion of this single cell.

In order to evaluate further the target cells for the LDH virus, multiplication of the virus in X-irradiated mice was studied. Normal CDF_1 mice were irradiated with 900 R (total body X-irradiation). 48 hr after irradiation the mice were bled. The lymphocyte count in the blood was reduced by 99%, but the number of macrophages in the peritoneal fluid was not reduced significantly. Similar results have been reported by Kornfield and Green (19). The mice were then inoculated with the LDH virus, $10^{4.0}$ ID_{50} per dose in the first experiment, $10^{7.0}$ ID_{50} per dose in the second. 72 hr after irradiation the mice were again bled and

their organs harvested. The average wet weight of the thymus, lymph nodes, and spleen was reduced (48, 77, and 78 % respectively). The virus titer of the plasma of the irradiated mice, 24 hr after inoculation, was $10^{10.6}$ ID_{50} per ml, and that of the nonirradiated controls was lower, i.e. $10^{9.9}$ ID_{50} per ml (average of two experiments). These results indicated that virus replication was not affected by a reduction of the radiation-sensitive cells, thus leaving the nonsensitive macrophages as probable target cells for the virus.

TABLE II

In vitro Cultivation of LDH Virus

Experiment No.	Cell type	Titers of supernatants (ID_{50}/ml log 10) at various hr after inoculation of virus					
		0	24	48	72	96	120
1*	Mouse embryo	5.3	3.5	—	<3.0	—	—
2	" "	5.3	3.5	—	<3.0	—	—
3	" "	5.3	3.1	—	<3.0	—	—
4	Lymph node	6.0	3.5	—	<3.0	—	<3.0
5	" "	7.0	4.3	2.7	—	<1.0	—
6	Peritoneal macrophages	5.1	6.7	7.5	6.9	—	—
7	" "	4.1	6.5	6.7	6.1	—	—
8	" "	4.0	5.7	6.7	6.7	—	—
9	" "	5.9	6.9	6.7	6.3	—	4.1
10	None	7.0	3.5	<2.0	—	—	—

* Media used throughout the above experiments: experiment 1, Eagle's plus 5% fetal bovine serum; experiment 2, Eagle's plus 10% skimmed milk; experiment 3, Eagle's plus 20% veal infusion broth; experiment 4, medium 199 plus 5% fetal bovine serum; experiments 5 6, 7, 8.9, and 10, medium 199 plus 10% fetal bovine serum. Media were renewed only when samples were taken. All media contained 100 units of streptomycin and 100 units of penicillin per ml.

In vitro studies. Based on these results, cultivation of the LDH virus was attempted on cultures of lymph nodes and macrophages using medium 199 and 10% FBS. The titers in mouse embryo cell cultures and in cell-free suspensions of the virus, kept under the same external conditions, were determined for comparison. Routinely, the titers of the supernatants were determined since the quantitative recovery of macrophages from glass surfaces is cumbersome. However, a few preliminary experiments showed that the titers of washed and homogenized cells, when corrected for volume, were of the same order as those of the supernatants. The results of several experiments are presented in Table II. A gradual decrease in titer was found in the supernatant of infected mouse embryo and lymph node cultures. The rate of this decrease in titer was less than that in control LDH virus suspensions without cells (also incubated at 37° C). An increase in LDH virus titer was found only in the supernatant of

peritoneal macrophage cultures: the maximum titer, of about $10^{7.0}$ ID_{50} per ml., was reached 48 hr after inoculation. In experiment 9, Table II, additional virus titers of a macrophage culture, 7 and 10 days after inoculation, were determined. After 7 days, the titer had decreased to $10^{1.7}$ ID_{50} per ml, and after 10 days no virus could be detected.

The infected macrophage culture supernatants did not show an increase in LDH *enzyme* concentration. In fact, the LDH enzyme activity of the supernatant of both infected and uninfected cells was less than 200 units/ml, which is lower than the concentration found in the plasma of normal mice (12). No cytopathic effect in the cultures was observed, which is consistent with the absence of histopathological lesions in vivo. In order to establish that this increase

TABLE III

Serial Passage of LDH Virus in Primary Mouse Macrophage Cultures

Experiment No.	Passage time	Medium dilution factor	Original inoculation	Virus titers of supernatants (Log 10 ID_{50}/ml)										
				Passage										
				1	2	3	4	5	6	7	8	9	10	11
	days													
1*	1	1:1000	6.1	6.7	7.0	3.7	<1.0	—	—	—	—	—	—	—
2	3	1:1000	5.9	6.3	5.5	<1.0	<1.0	—	—	—	—	—	—	—
3	5	1:10	5.9	4.1	<1.0	<1.0	—	—	—	—	—	—	—	—
4	3	1:10	4.5	6.5	6.7	5.5	5.1	5.5	4.5	4.9	4.7	5.7	5.3	5.3

* Medium used throughout the above experiments: medium 199 plus 10% fetal bovine serum, with 100 units of streptomycin and 100 units of penicillin per ml.

of LDH virus titer in the peritoneal macrophage culture did, indeed, represent multiplication, and not disaggregation of LDH virus clumps, serial passage experiments were performed. The results are presented in Table III. In experiments 1 to 3, serial passages were performed by harvesting the supernatants at the time intervals given in column 2. An aliquot was diluted, as indicated in column 3, and 1 ml of this dilution used to replace the medium of each of 5 fresh cultures per transfer. A second aliquot was used to determine the LDH virus titer at the time of transfer. These titers are given in columns 4 to 7. It can be seen that in these 3 experiments no demonstrable infectivity remained after 2 to 4 passages.

Several possibilities were considered to account for the gradual loss of titer and failure of the virus to grow after 2 to 4 serial passages. Changes in either the virus or the host might be involved. Production of interferon, or inhibitory metabolic products by the infected host cells might inhibit subsequent replication. Interferon production was unlikely in view of its demonstrated low titer in in-

fected animals (20, 12). Indeed, the supernatants of the culture passages, tested for interferon, proved negative. However, nonspecific virus inhibitors are known to be present in a variety of biological materials. If the inhibitory factor was proteinaceous, digestion with a proteinase might eliminate its effect.

Preliminary experiments had shown that LDH virus preparations, digested with trypsin at room temperature, did not lose their infectivity titer when tested in mice. Therefore, serial tissue culture passage experiments were performed in which the undiluted supernatants were digested with 0.2 mg of trypsin per ml for 1 hr at 37° C. Under these conditions a decrease in titer of about 0.5 log occurred. Experiment 4 presents the results. It can be seen that, using this method, the titer in the supernatants could be maintained for at least 33 days, during 11 serial passages, involving a ten fold dilution at each passage. Therefore, the ultimate dilution factor in 11 transfers, without considering thermal or trypsin inactivation, was 10^{11}. This eliminated the possibility that the rise in titer after each passage was attributable to a gradual disaggregation of virus clumps in the original inoculum.

DISCUSSION

The in vivo studies indicated that the increase in LDH virus titer preceded or paralleled the virus increase in blood plasma only in those loci which contain an appreciable number of macrophages, namely the lymph nodes and the ascitic fluid of tumor-bearing mice. The lymphocytes probably were not the target cells for virus multiplication, because on X-irradiation the lymphocytes were materially reduced without a concomitant reduction in virus titer in the plasma, whereas the number of peritoneal macrophages was not reduced. (Also, the virus did not multiply in lymph node culture in vitro). The Ehrlich ascitic cells as probable target cells were eliminated because isolated and washed Ehrlich ascites cells, pelleted by slow centrifugation, contained 24 hr after host infection only $10^{7.5}$ ID_{50} per ml cells, compared with an ID_{50} of $10^{10.3}$ per ml of ascitic fluid (See Table I). Further attempts to demonstrate virus in ascites cells from infected mice by electron microscopy failed.

The electron microscopic demonstration of the LDH virus in infected lymph nodes and in ascitic fluid, often in aggregates of virus particles bounded by single or double membranes, also implicates the macrophages as target cells. Macrophages in uninfected nodes and those infected less than 18 hr, not infrequently contain within their cytoplasm single membraned vesicles containing amorphous dense material. These vesicles are not readily differentiated from similar vesicles in infected cells containing the LDH virus. However, neither the 25 nor the 50 mμ particle has been observed in such cells. It seems probable that these two types of particles represent two developmental forms of the LDH virus. However, a more detailed study of the early phases of replication will likely have to await a virus-host system more conducive to the recognition of cells infected

for only a few hours. De-Thé and Notkins (21), who have reported that peritoneal macrophages from infected mice contained LDH virus particles, found only complete virus particles in their preparations.

In regard to the in vitro studies it is interesting to note that Evans and Salaman (11) cultivated the LDH virus in mouse macrophages without having to resort to trypsinization of the material before reinoculation. They, however, found a loss in virus titer, similar to that reported here, when serial passage without trypsinization was attempted in primary mouse embryo cell cultures. There are at least three possibilities to account for the observation that we could cultivate the virus only following trypsinization of the inoculum:

1. Our LDH virus strain might be different from the one used by Evans and Salaman.

2. Medium 199 with 10% FBS is less favorable than Eagle's basal medium with 5 to 10% lamb serum, used by them.

3. In our case the virus used for inoculum and the macrophages for in vitro culture were obtained from different strains of mice, which may have led to the formation of a virus inhibitor.

Alternative 1 has not been studied. Alternative 2 is not supported by the finding that the macrophages can be maintained in good condition in medium 199 and 10% FBS for at least 60 days (22), whereas Evans and Salaman reported that they could maintain their culture in a healthy state for not longer than 14 to 21 days. They also reported that 1 day-old macrophage cultures supported virus replication better than 8 day-old cultures. We found that virus replication in cultures inoculated when 12 days old (Table II, experiment 6) was better than in cultures inoculated when 3 days old. Alternative 3 seems more probable for the following reasons. The presence of apparent host material in LDH virus preparations was demonstrated by electron microscopic observation of purified virus preparations (12). Virus particles occurred frequently in single membraned vesicles, and occasionally in double membraned bodies, similar to those occurring in the macrophages, as reported above. This occurrence of membranes of host origin around viruses and other intracellular parasites has previously been shown by the tetracycline-staining method (23). The presence of this material, when derived from a heterologous mouse strain might have led to the production of a trypsin-sensitive inhibitor of virus multiplication. This inhibitor continued to appear in subsequent cultures unless the inocula were trypsinized. Evans and Salaman also used two mouse strains, but they do not mention whether one strain was used both as a source of LDH virus and of macrophages in their culture experiments, thus accidentally avoiding the need of trypsinization. Studies are in progress to determine whether trypsinization of the LDH virus, and other viruses which do not lose their infectivity following trypsinization, will allow their multiplication in other cell lines, heretofore found unsuitable.

The degree to which virus-containing vesicles occurred might well underlie the contrary reports regarding the occurrence of two types of LDH virus (1). Also, the nature of the vesicles and the source of host cell cultures might well explain the contradictory results regarding the in vitro virus culture as mentioned in the introduction.

SUMMARY

In vivo analysis of the virus titer in various loci, 24 hr after infection, showed that a titer similar to that in the blood plasma was found in the ascitic fluid of Erlich ascites cancer-bearing mice, and in lymph nodes, spleen, and thymus, i.e. loci which contain macrophages as a common cell type. However, only in the lymph nodes and in the ascitic fluid did the increase in virus titer precede or parallel the increase in the plasma.

The LDH virus titer in the plasma of X-irradiated mice was similar to that of control mice, eliminating radiation-sensitive cells but not macrophages as target cells of the virus.

Electron microscopic observation of infected lymph node cells revealed the presence of two types of particles: one consisting of small densely stained annuli, about 25 mμ in diameter and one of similar dense annuli with a halo extending the diameter to about 50 mμ. Such particles were repeatedly observed within single or double membraned vesicles.

In vitro, the LDH virus multiplied only in cultures of mouse peritoneal macrophages, maintained in medium 199 with 10% FBS. The virus titer could be maintained for at least 33 days, during eleven serial passages, involving an overall dilution factor of 10^{11}. These results corroborate the findings of Evans and Salaman, who used peritoneal macrophages maintained in Eagle's medium and 5 to 10% lamb serum. However, in the serial passage experiments reported here, the virus titer could only be maintained following trypsinization of each successive inoculum. The role of macrophages as the target cell for LDH virus multiplication in vivo is discussed.

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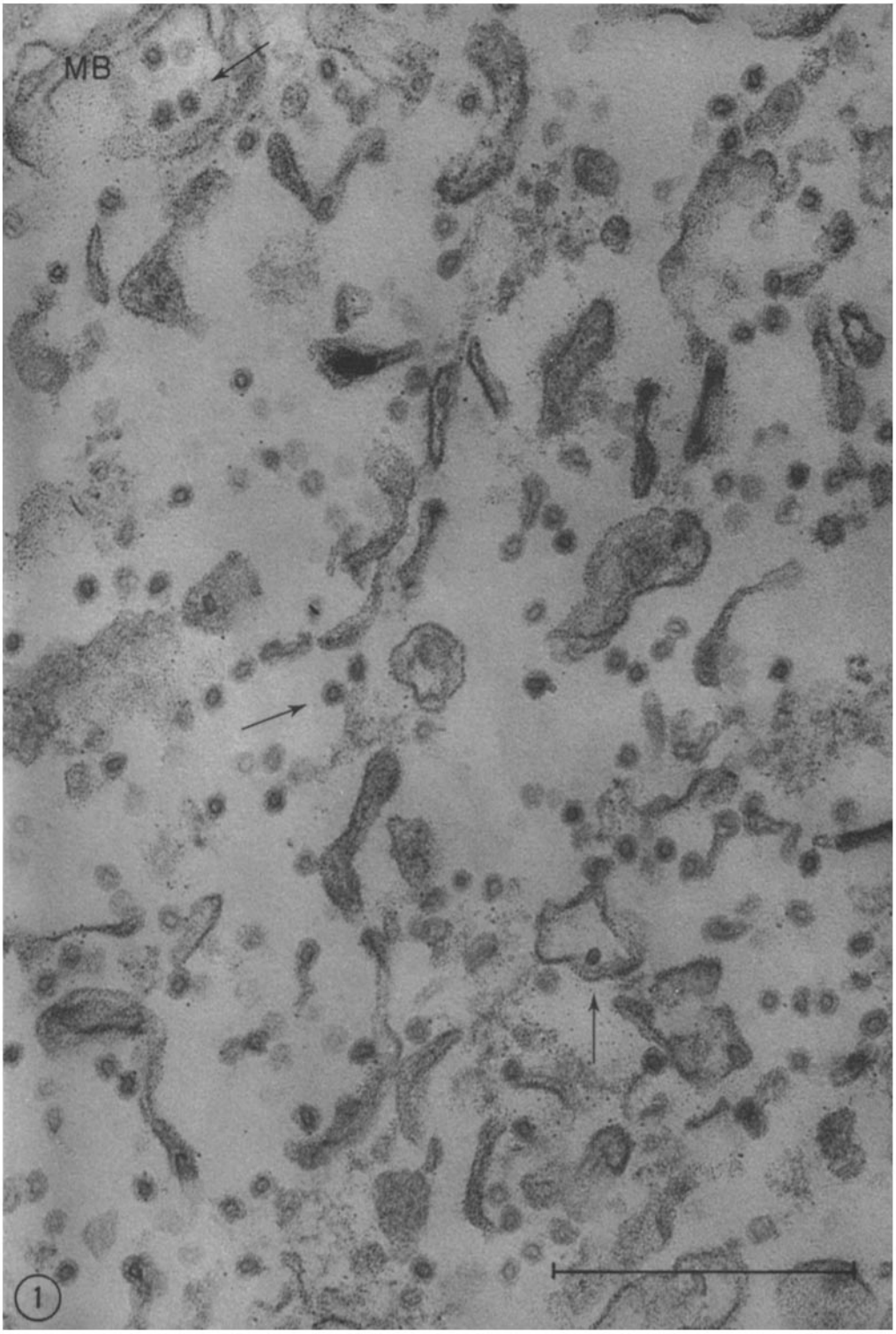
EXPLANATION OF PLATES

PLATE 91

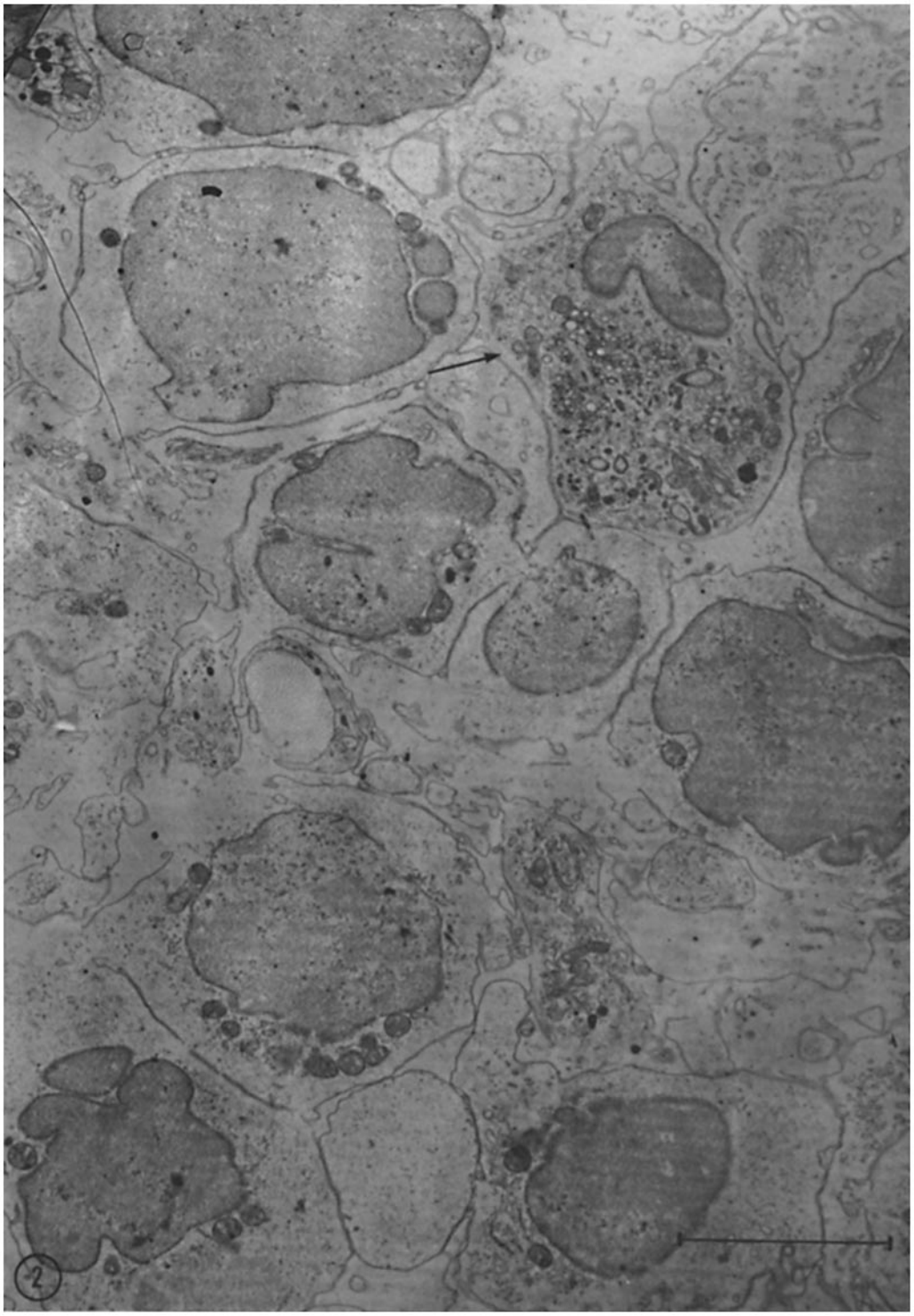
FIG. 1. A section of a pellet of partially purified virus obtained from Ehrlich ascites fluid. The LDH virus consists of a dense inner annulus of 25 mμ diameter and the less distinct outer halo of 50 mμ in diameter (arrows). One or more of these particles may be bounded by an additional membrane (*MB*). The preparation of this material has been described (12). The bar represents 500 mμ. × 90,000.



(du Buy and Johnson: Multiplication of the LDH virus)

PLATE 92

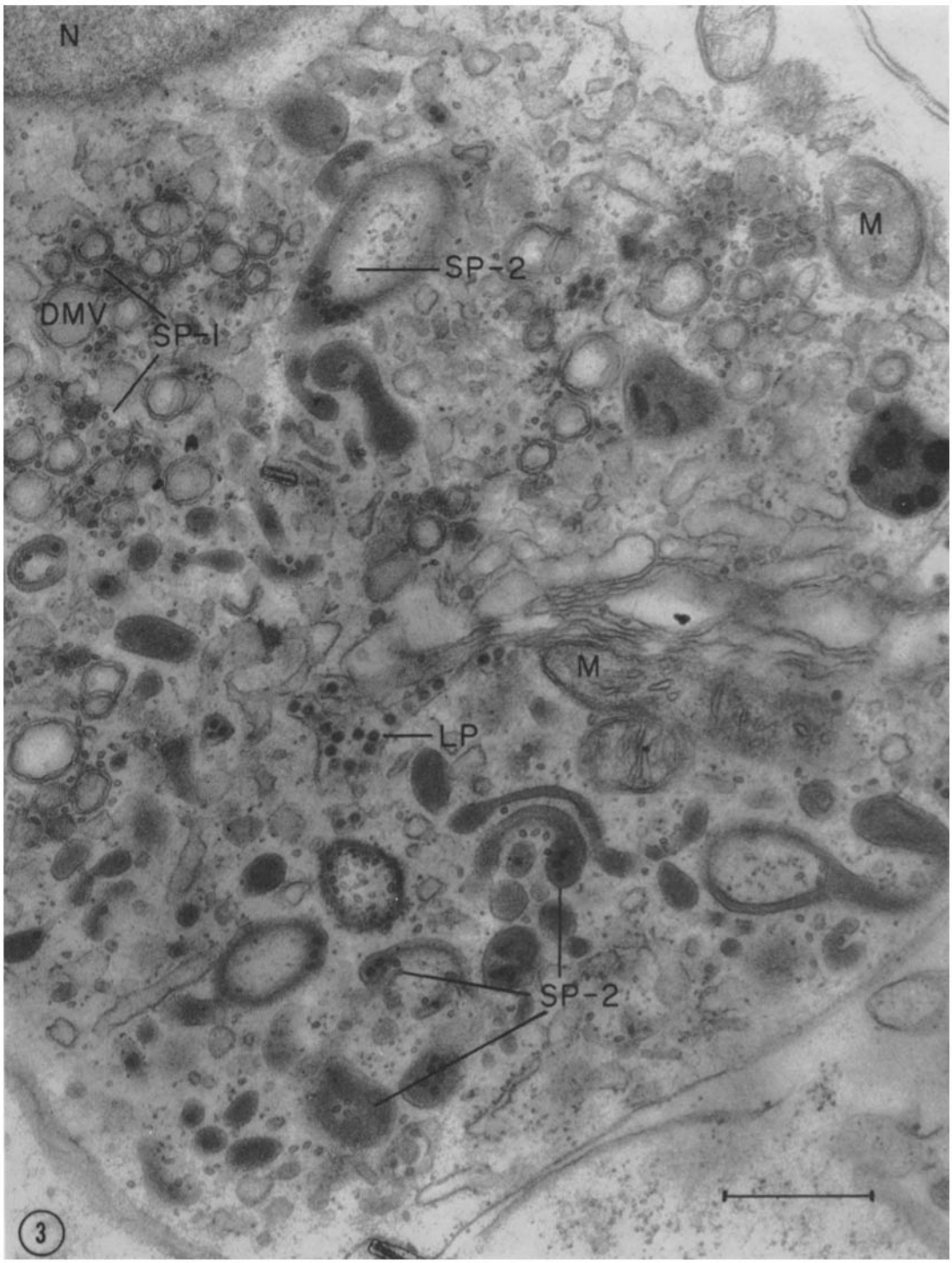
FIG. 2. A section of lymph node cells, 24 hr after infection, at low magnification. A cell with dense cytoplasmic material is easily discerned (arrow). The bar represents 5 μ. $\times$ 6100.



(du Buy and Johnson: Multiplication of the LDH virus)

PLATE 93

FIG. 3. An enlargement of the dense cytoplasmic material of Fig. 2. Small, uniform, annular particles of 25 mμ diameter (*SP-1*) are seen scattered among double membraned vesicles (*DMV*) and occasionally within single membraned dense particles (*SP-2*). The larger particles (*LP*) of 50 mμ diameter with a halo are usually associated in varying number with irregular single membranes. Apparently normal mitochondria (*M*) and part of the nucleus (*N*) are identified. The bar represents 500 mμ. × 44,650.

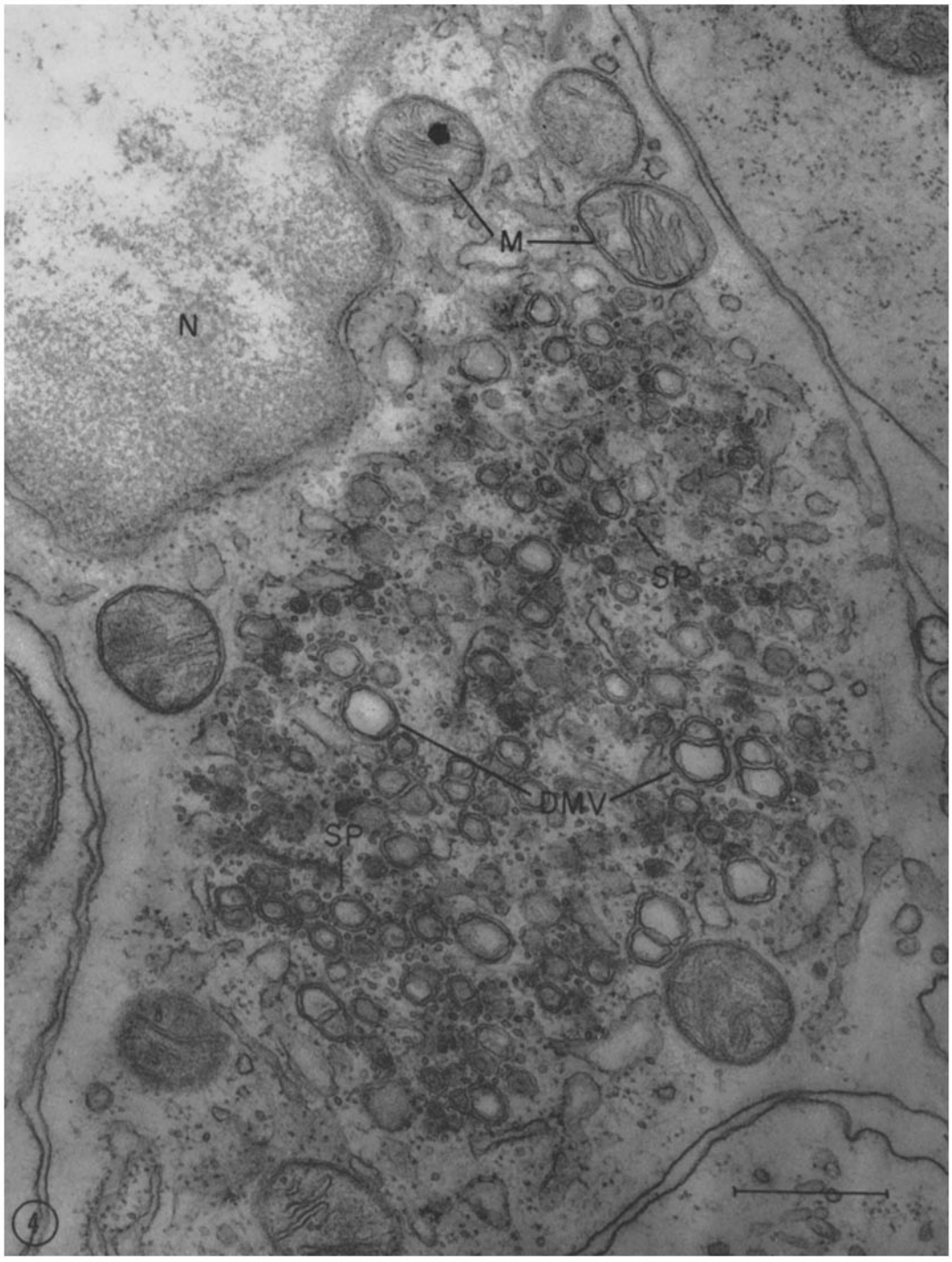


(du Buy and Johnson: Multiplication of the LDH virus)

PLATE 94

FIG. 4. Section of a cytoplasmic region of a cell of a lymph node, 24 hr after infection. The predominant picture is that of small 25 mμ annular particles interspersed between double membraned vesicles, some of which have a figure-8 appearance.

M, mitochondria; *DMV*, double membraned vesicles; *N*, nucleus; *SP*, small particles. The bar represents 500 mμ. × 42,750.

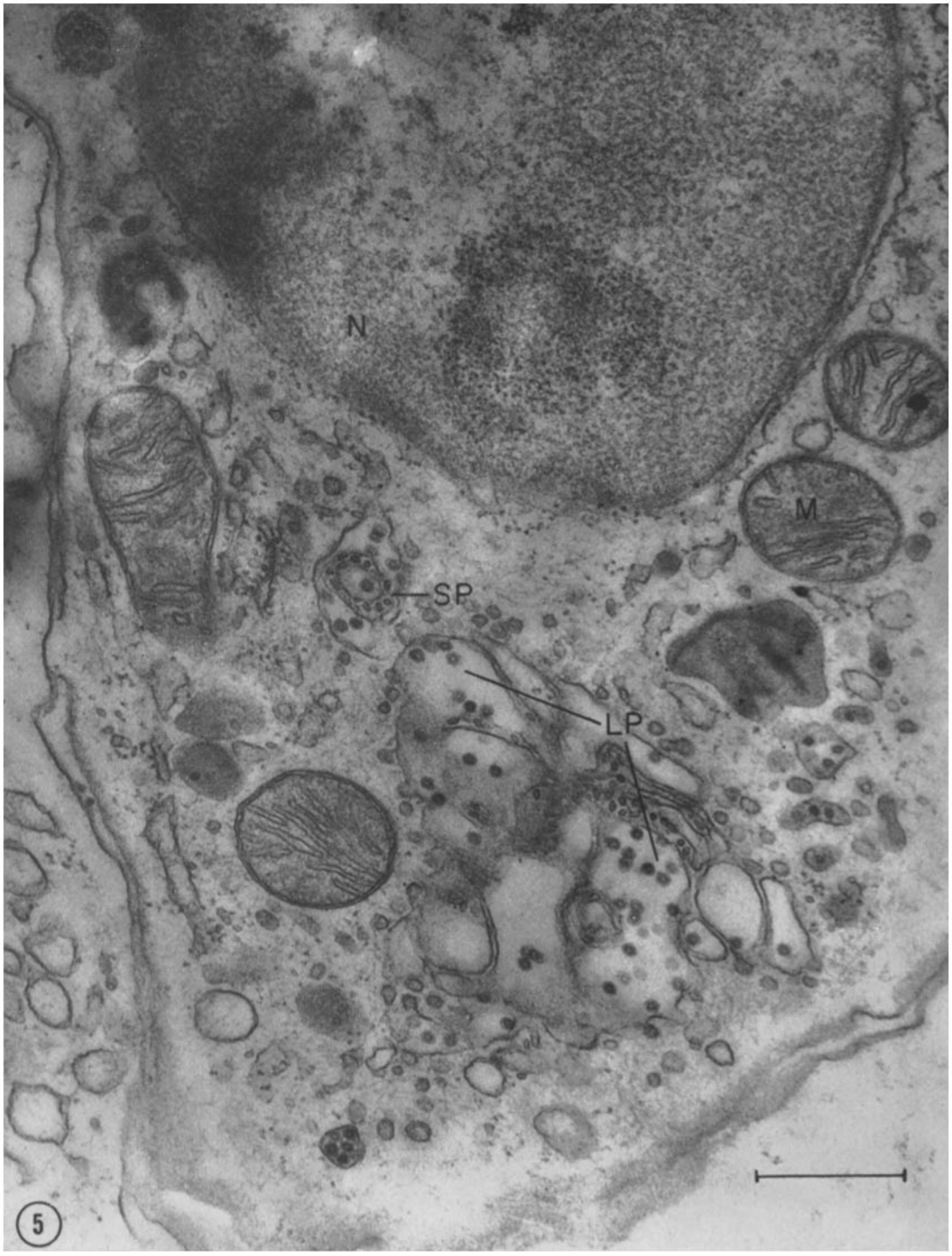


(du Buy and Johnson: Multiplication of the LDH virus)

PLATE 95

FIG. 5. Different region of the same cell, shown in Fig. 4. Small and large particles can be seen, associated with single membraned vesicles.

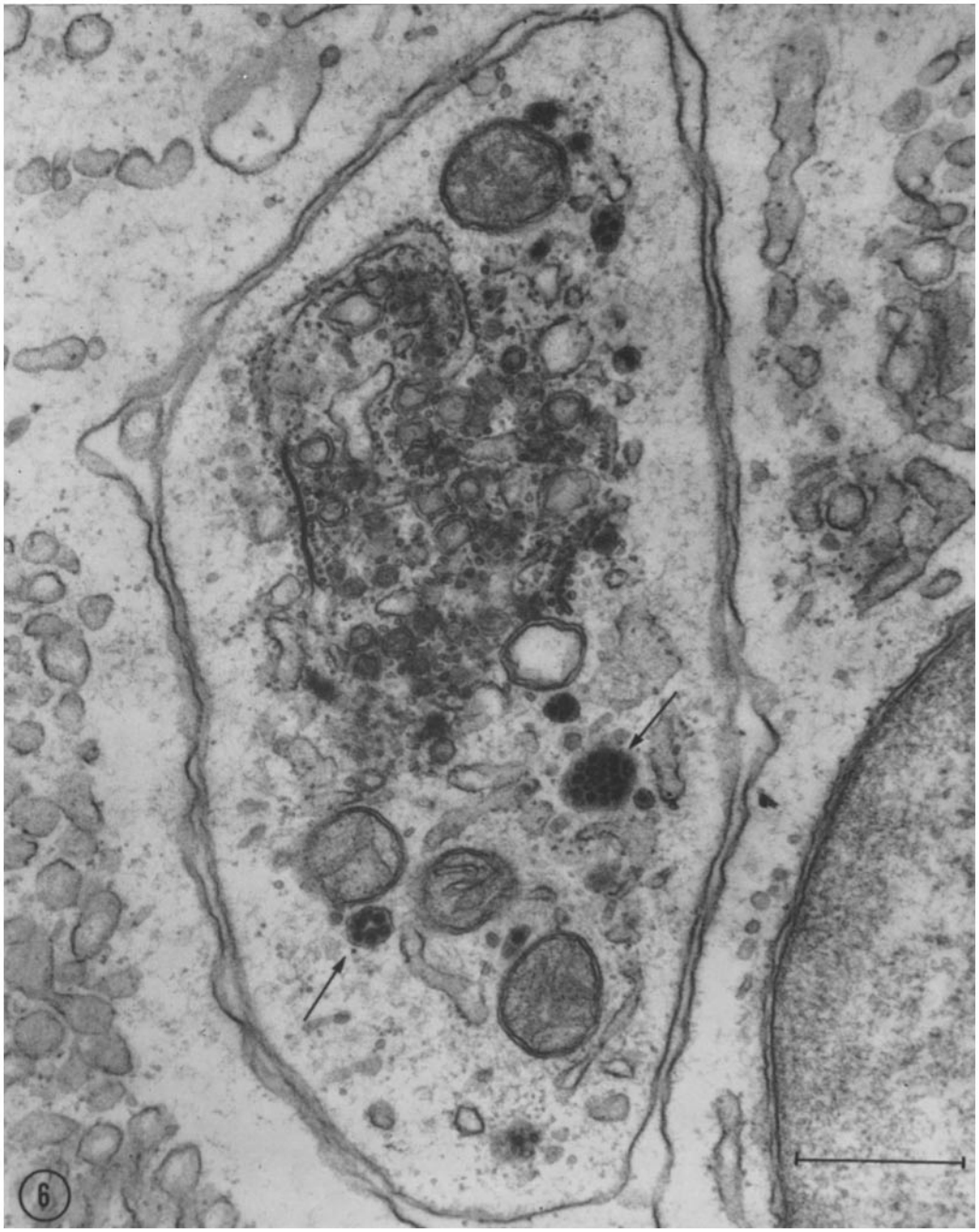
M, mitochondria; *N*, nucleus; *SP*, small particles; *LP*, large particles. The bar represents 500 mμ. $\times$ 44,650.



(du Buy and Johnson: Multiplication of the LDH virus)

PLATE 96

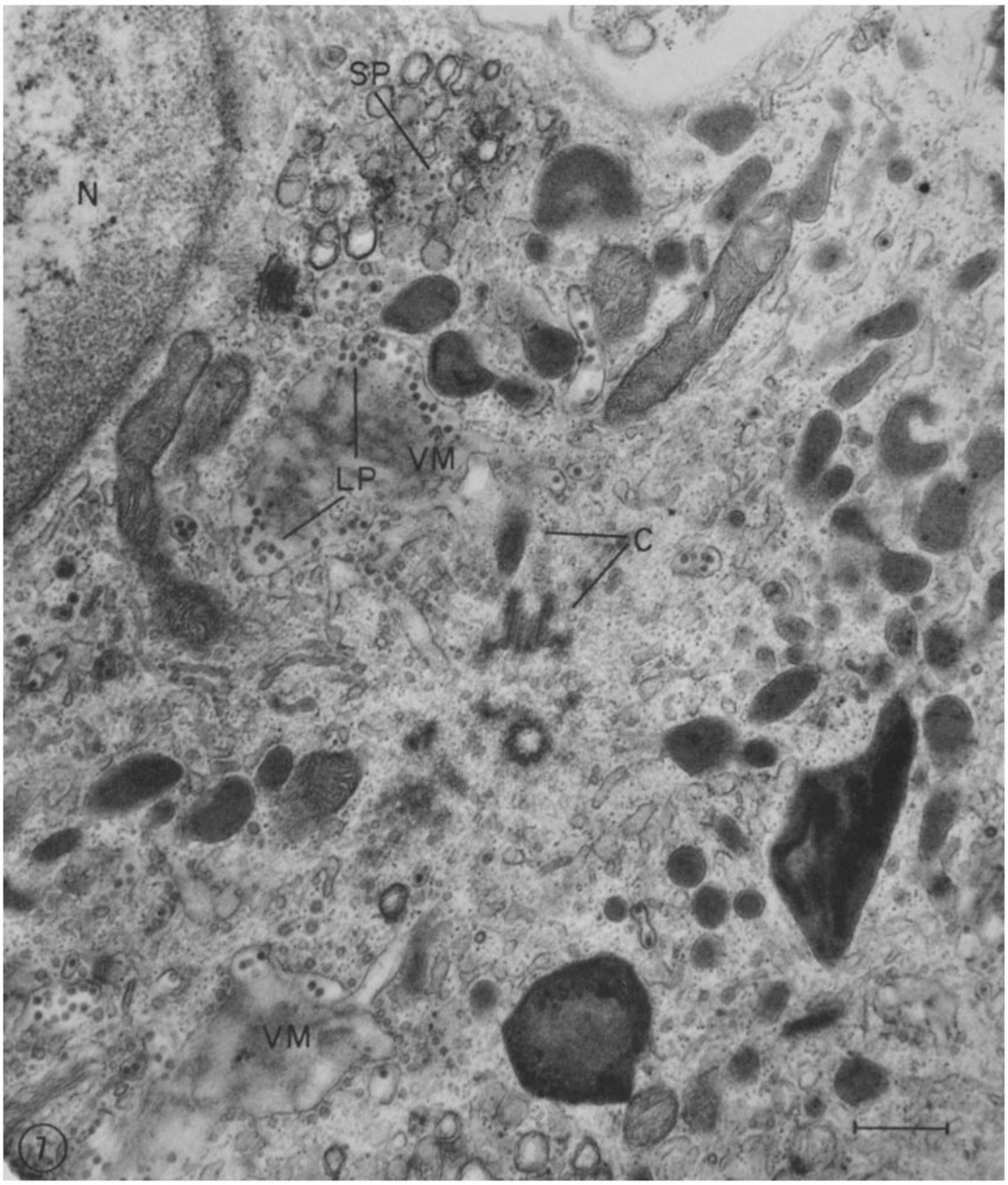
FIG. 6. A section through the cytoplasm of a lymph node cell, 24 hr after infection. Small particles and intravesicular aggregates (arrows) predominate. The bar represents 500 mμ. × 47,000.



(du Buy and Johnson: Multiplication of the LDH virus)

PLATE 97

FIG. 7. A lymph node cell, 18 hr after infection, in which small extravesicular particles (*SP*) and large intravesicular particles (*LP*) predominate. It is possible that the halo is derived from some of the vesicular material (*VM*). Just to the lower right of center a pair of centrioles (*C*) is visible. N, nucleus. The bar represents 500 mμ. $\times$ 26,800.



(du Buy and Johnson: Multiplication of the LDH virus)