

Fig. 1

Proportions of plastic adherent cells (PA) in spleen and lymph node cell population of bat. Total mononuclear cells from spleen and lymph node. Plastic adherent cells.

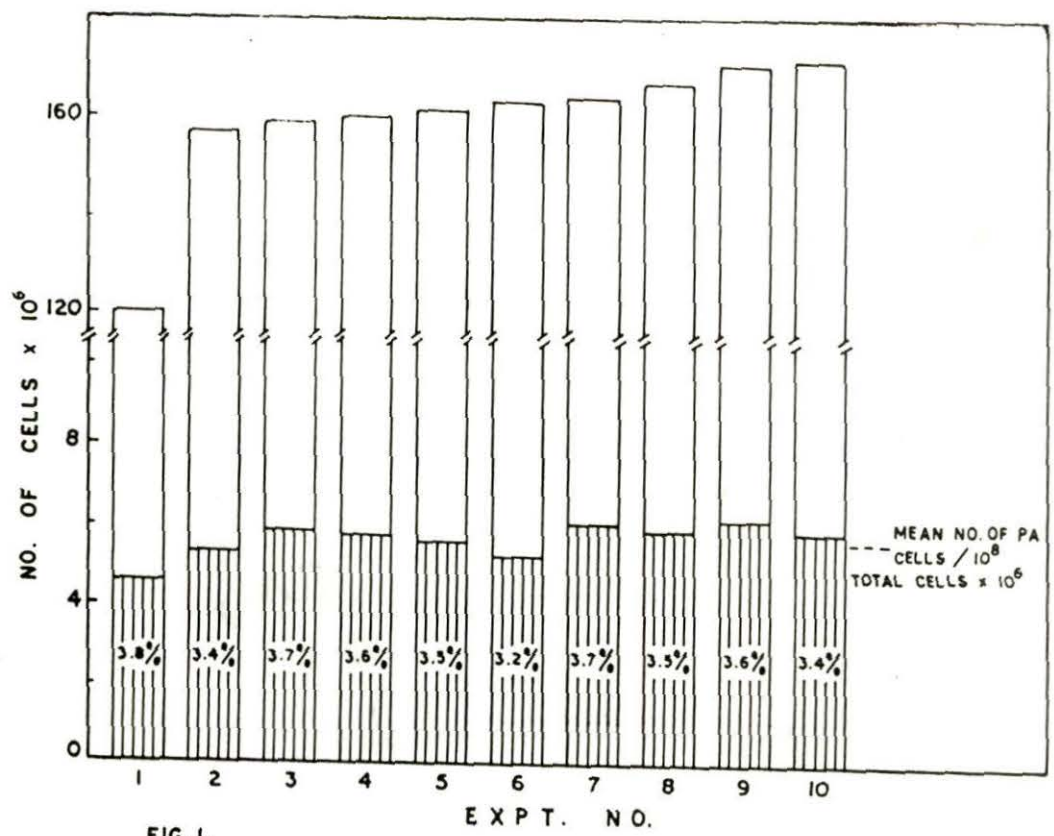


FIG. 1.

Fig. 2

Sephadex G-200 elution profile of bat immunoglobulins showing separation of two classes of Ig, one eluting in the void volume and another eluting later, almost in the same position as purified human Ig G run later under same conditions. ○—○ bat Ig, ●--● purified human Ig G.

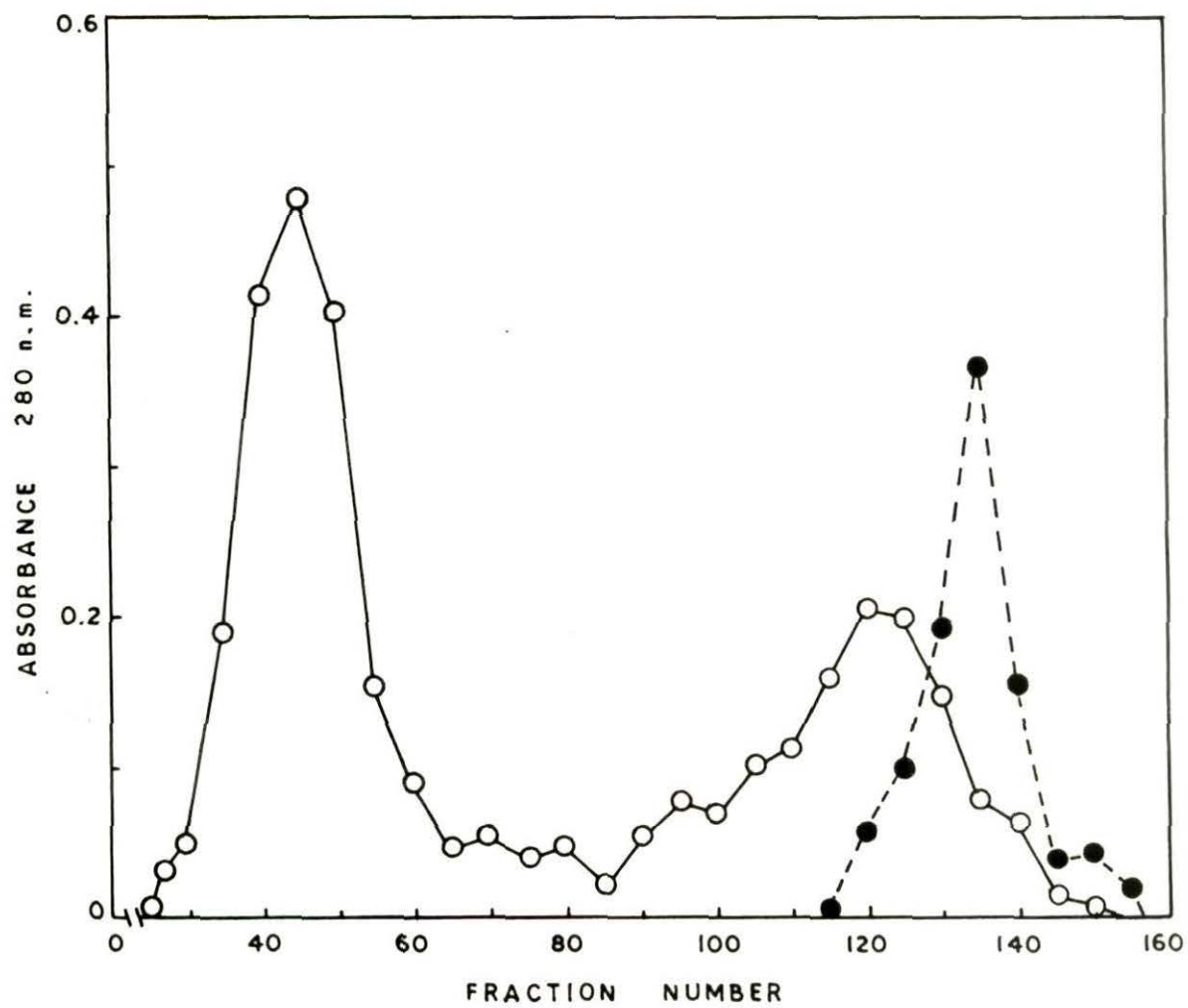


FIG. 2.

Fig. 3

Cytotoxicity of rabbit anti-bat brain serum at different dilutions against different immunocompetent cell populations of bat. O—O nylon wool non adherent cells, ●—● nylon wool adherent cells, ○—○ plastic adherent cells, ●—● nylon wool non adherent cells but with antiserum preabsorbed with the same type of cells.

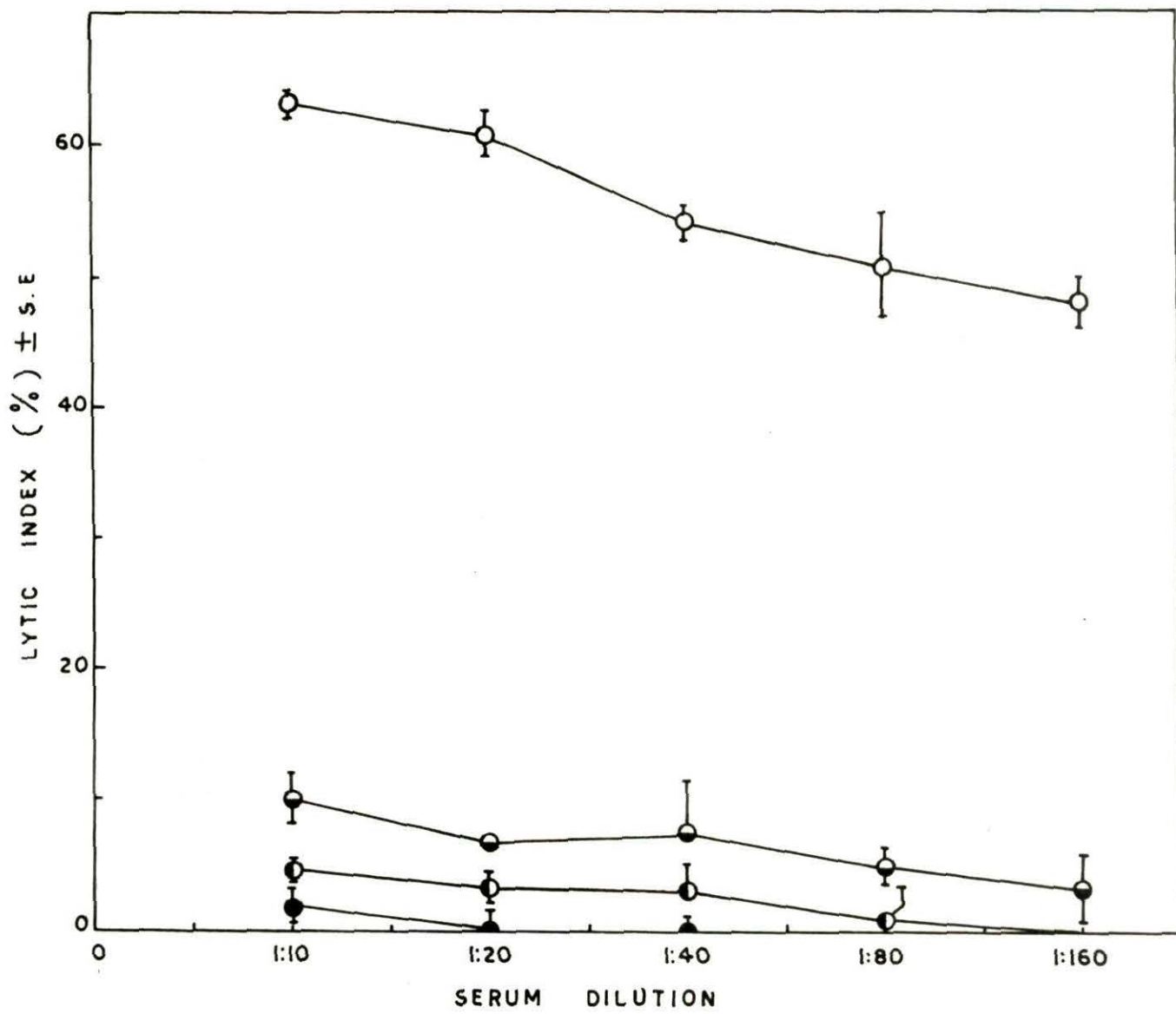




FIG. 3.

Fig. 4

Histogram showing density of lymphocytes per  $0.001 \text{ mm}^2$  area in different regions of splenic white pulp in normal bats and bats after the course of anti-brain serum treatment.  region adjacent to central arteriole,  region distant to central arteriole.

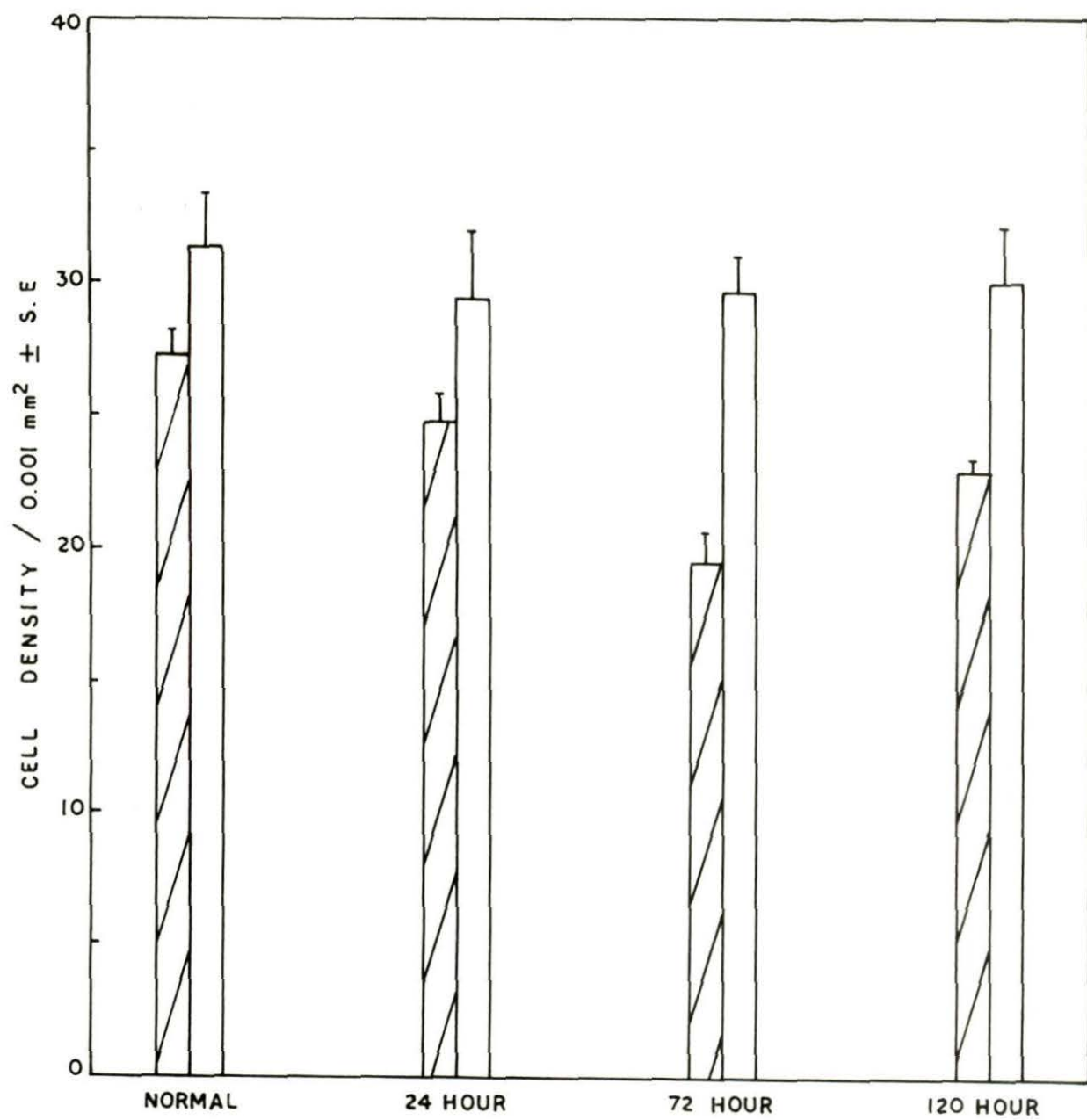


FIG. 4

Fig. 5 Histogram showing density of lymphocytes per  $0.001 \text{ mm}^2$  area in different regions of lymph node from normal bats and bats after the course of anti-brain serum treatment  paracortical area,  follicular area.

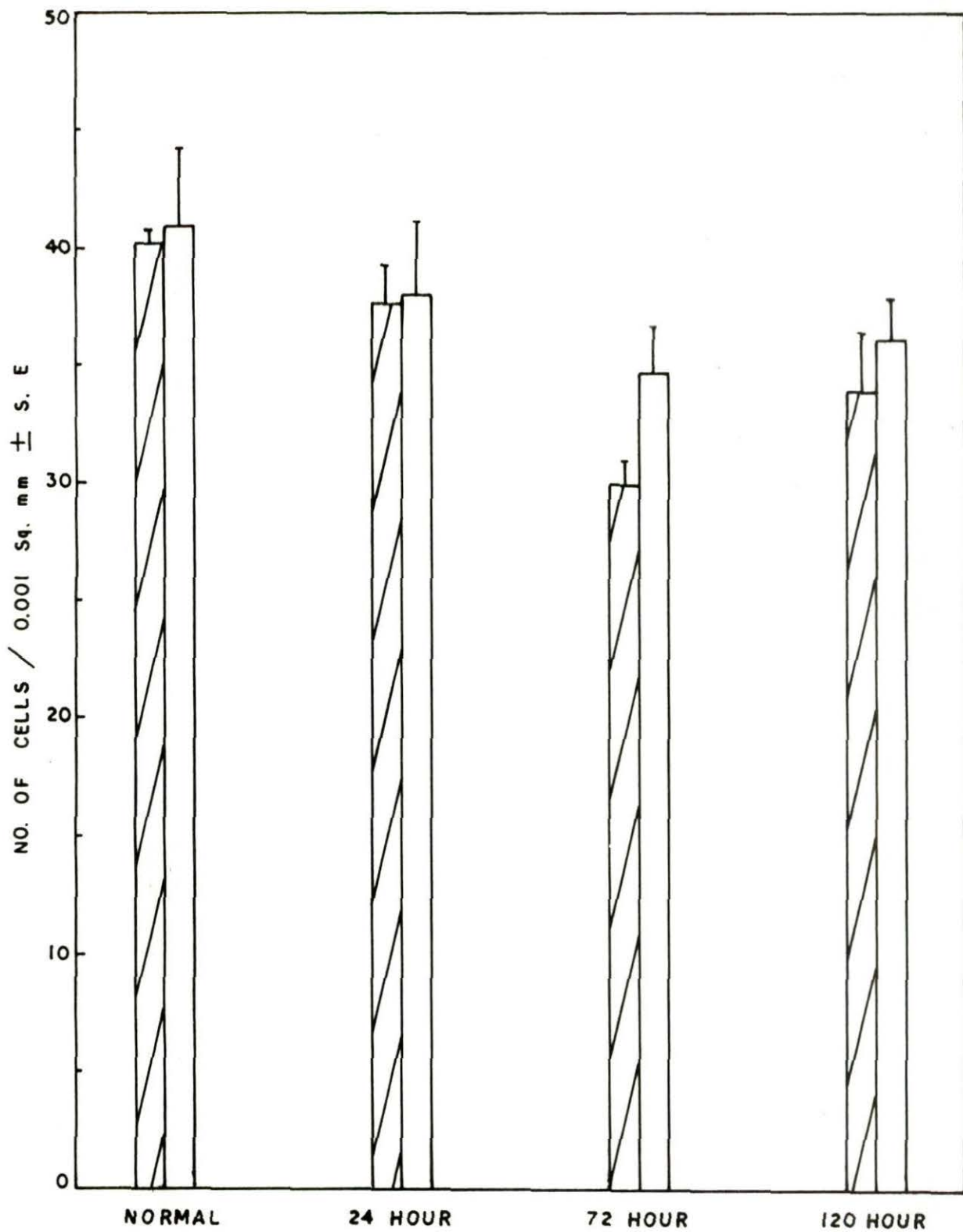


FIG. 5.

Table 1 . Lymphocytic sub populations of bat separated on untreated nylon wool column

Expt. No.	Total No. of cells incubated x 10 <sup>6</sup>	No. of cells (NA + NNA) <sup>a</sup> recovered x10 <sup>6</sup> (% recovery)	Mean of % recovery ( $\pm$ SE)	No. of NA cells recovered x10 <sup>6</sup>	Mean ( $\pm$ SE)	No. of NNA cells recovered x 10 <sup>6</sup>	Mean ( $\pm$ SE)	Ratio of NA : NNA
1	150	4.81 (3.21)		2.31		2.50		
2	150	5.26 (3.51)		2.42		2.84		
3	150	6.35 (4.23)	3.840 (0.224)	2.43	2.51 (0.143)	3.92	3.248 (0.255)	1:1.4
4	150	6.37 (4.25)		2.42		3.95		
5	150	4.85 (3.23)		2.28		2.57		
6	150	6.93 (4.62)		3.22		3.71		

<sup>a</sup> NA and NNA stand for nylon wool adherent and nylon wool non adherent cells respectively in all tables.

Table 2. Lymphocytic sub populations of bat separated on nylon wool column pretreated with EDTA and  $\text{NaHCO}_3$

Expt. No.	Total cells incubated $\times 10^6$	No. of cells (NA + NNA) <sup>a</sup> recovered $\times 10^6$ (% recovery)	Mean of % recovery ( $\pm$ SE)	No. of NA cells recovered $\times 10^6$	Mean ( $\pm$ SE)	No. of NNA cells recovered $\times 10^6$	Mean ( $\pm$ SE)	Ratio of NA : NNA
1	150	67.92 (45.28)		15.04		52.88		
2	150	72.45 (48.29)	40.60 (3.760)	15.44	13.15 (1.208)	57.01	47.75 (4.484)	1:3.63
3	150	55.29 (36.86)		11.15		44.14		
4	150	47.95 (31.97)		10.98		36.97		

<sup>a</sup> Abbreviations as in Table 1

Table 3 . Proportion of three different immunocompetent cell types of bat separated  
adhesibility to plastic and nylon wool<sup>a</sup>

Expt. No.	No. of PA cells <sup>b</sup> recovered x 10 <sup>6</sup>	No. of NA cells recovered x 10 <sup>6</sup>	No. of NNA cells recovered x 10 <sup>6</sup>	Ratio of three cell types PA:NA:NNA	Mean ratio PA:NA:NNA
1	4.60	11.58	40.82	1.00:2.52:8.87	
2	5.84	16.42	61.44	1.00:2.81:10.52	1.00:2.35:8.83 (1:2:9 approx.)
3	6.20	12.30	48.62	1.00:1.98:7.84	
4	5.80	12.18	46.94	1.00:2.10:8.09	

<sup>a</sup> Each experiment was done with the total cells obtained from secondary lymphoid organs of an individual bat.

<sup>b</sup> PA stands for plastic adherent cells. Other abbreviations as in Table 1.

Table 4. Neutral red positive cells in three different immunocompetent cell populations of bat separated by adhesibility.

Cell type	Expt. No.	No. of cells $\times 10^6$	Neutral red positive cells	
			No. of cells $\times 10^6$	% of cells
Plastic adherent	1	4.60	4.05	88.04
	2	5.80	5.30	91.38
	3	5.84	5.22	89.38
Nylon wool adherent	1	11.60	1.25	10.78
	2	12.16	1.50	12.34
	3	16.64	2.31	13.88
Nylon wool non adherent	1	40.80	2.61	6.40
	2	46.96	3.66	7.79
	3	61.44	3.81	6.20

Table 5 . Enumeration of surface Ig M and Ig G bearing cells in the plastic adherent cell population of bats.

Expt. No.	Percentage of surface Ig bearing cells			
	Ig M <sup>+</sup> cells	Mean ( <u>±</u> SE)	Ig G <sup>+</sup> cells	Mean ( <u>±</u> SE)
1(a)*	4.00	8.25 (2.63)	4.17	7.43  (3.43)
	13.04		3.85	
	7.69		14.29	
1(b)*	13.64	7.45 (3.12)	8.00	7.08 (1.82)
	3.70		3.00	
	5.00		9.68	
2(a)	11.76	14.51 (3.28)	10.53	7.82 (2.03)
	21.05		3.85	
	10.71		9.09	
2(b)	20.00	12.33 (4.12)	4.17	12.13 (4.34)
	5.88		22.22	
	11.11		10.00	

\* (a) and (b) represents two separate experiments with cells from an animal. In each set three tubes containing  $10^6$  cells were used.

Table 6 . Enumeration of surface Ig M and Ig G bearing cells in the nylon wool non adherent cells of bats.

Expt. No.	Percentage of surface Ig bearing cells			
	Ig M positive cells	Mean (+ SE)	Ig G positive cells	Mean (+ SE)
1(a)*	4.17	6.08 (0.98)	8.69	8.19 (1.85)
	7.41		11.11	
	6.67		4.76	
1(b)*	8.00	7.17 (1.73)	4.17	7.44 (3.43)
	9.68		3.85	
	3.85		14.29	
2(a)	19.05	10.32 (4.64)	5.56	9.43 (2.34)
	3.23		13.64	
	8.69		9.09	
2(b)	8.00	9.57 (0.81)	7.69	6.42 (1.13)
	10.71		4.17	
	10.00		7.41	
3	3.13	7.91 (4.72)	3.57	2.17 (0.89)
	3.45		0.00	
	26.32		3.45	
	6.67		0.00	
			3.85	

\* As in Table 5

Table 7. Enumeration of surface Ig M and Ig G bearing cells in the nylon wool adherent cells or bats.

Expt. NO.*	Percentage of surface Ig bearing cells			
	Ig M <sup>+</sup> cells	Mean ( <u>±</u> SE)	Ig G <sup>+</sup> cells	Mean ( <u>±</u> SE)
1(a)	58.33	57.48 (1.35)	33.33	36.51 (3.17)
	54.84		42.86	
	59.26		33.33	
(b)	61.54	58.46 (6.58)	40.74	39.35 (5.59)
	45.83		48.28	
	68.00		29.03	
2(a)	50.00	50.86 (4.37)	28.57	30.26 (2.19)
	58.82		34.62	
	43.75		27.59	
(b)	57.89	53.04 (2.78)	36.84	33.18 (4.72)
	48.28		23.81	
	52.94		38.89	
3	58.33	52.91 (5.93)	30.43	34.61 (1.98)
	33.33		36.84	
	64.29		33.33	
	63.16		31.25	
	45.45		41.18	

\* As in Table 5

Table 8. Surface Ig M and Ig G bearing lymphocytes from different lymphoid organs & peripheral blood of bats.

Source of cell	Expt. No.	Percentage of surface Ig bearing cells				
		Ig M <sup>+</sup> cells	Mean ( $\pm$ SE)	Ig G <sup>+</sup> cells	Mean ( $\pm$ Se)	
Bone marrow	1a)	24.00	20.16 (2.38)	23.53	13.34 (5.12)	
		20.69		7.41		
		15.79		9.09		
	b)	25.00	22.36 (1.64)	10.71	9.64 (0.98)	
		22.73		10.53		
		19.35		7.69		
	2a)	20.69	24.47 (1.92)	28.57	20.04 (4.55)	
		25.81		18.52		
		26.92		13.04		
		b)	24.24	23.40 (2.31)	22.73	18.93 (1.91)
			19.05		16.67	
			26.92		17.39	
Spleen	1a)	44.44	42.99 (0.80)	27.27	26.95 (1.04)	
		41.67		28.57		
		42.86		25.00		
	b)	45.45	41.19 (5.24)	27.78	24.05 (4.13)	
		30.77		28.57		
		47.37		15.79		
	2a)	45.83	41.99 (4.96)	36.00	30.80 (3.94)	
		48.00		23.08		
		32.14		33.33		

Contd..

Table 8 (Contd..)

Source of cell	Expt. No.	Percentage of surface Ig bearing cells			
		Ig M <sup>+</sup> cells	Mean ( $\pm$ SE)	Ig G <sup>+</sup> cells	Mean ( $\pm$ SE)
Spleen	2b)	40.91	40.41 (3.29)	30.77	30.32 (6.67)
		34.48		18.52	
		45.83		41.67	
	1a)	18.18	19.38 (1.03)	9.52	9.66 (1.18)
		21.43		11.76	
		18.52		7.69	
Mesenteric lymph node	1b)	30.77	21.27 (4.77)	4.17	13.24 (5.21)
		15.79		22.22	
		17.24		13.33	
	2a)	17.39	22.77 (2.70)	7.41	11.20 (1.89)
		25.93		13.16	
		25.00		13.04	
	2b)	19.23	20.81 (1.67)	12.50	10.28 (1.29)
		24.14		8.00	
		19.05		10.34	
	1a)	46.67	44.34 (4.10)	10.00	24.92 (8.02)
		36.36		37.50	
		50.00		27.27	
Peripheral blood	1b)	33.33	51.85 (9.79)	44.44	34.74 (5.61)
		66.67		25.00	
		55.56		34.78	

Contd..

Table 8 (Contd..)

Source of cell	Expt. NO.	Percentage of surface Ig bearing cells			
		Ig M <sup>+</sup> cells	Mean ( <u>±</u> SE)	Ig G <sup>+</sup> cells	Mean ( <u>±</u> SE)
Peripheral blood	2a)	43.75	49.58 (4.68)	38.89	36.57 (1.67)
		46.15		33.33	
		58.82		37.50	
	2b)	57.14	53.59 (7.05)	35.29	35.93 (0.79)
		40.00		35.00	
		63.64		37.50	

Table 9. Proportion of three different immunocompetent cell types of bat after in vivo administration of rabbit anti-bat brain serum

Time after antiserum treatment	No. of PA <sup>a</sup> cells x10 <sup>6</sup>	No. of NA cells x 10 <sup>6</sup>	No. of NNA cells x 10 <sup>6</sup>	Ratio of three cell types PA:NA:NNA
24 hrs	6.8	10.4	48.2	1:1.53:7.09
72 hrs	5.6	10.7	24.18	1:1.91:4.32
120 hrs	8.6	8.8	38.4	1:1.02:4.47

Plate - I

Fig. 1 &amp; 2

Scanning electron micrographs of plastic adherent cells, i.e. macrophages of normal bat.

1) One with flattened pseudopodial projections (arrow) and (2) another with finger like filopodial projections.

X 11,000,            X 10,500

[ Bar equals to 2  $\mu$ m in these and subsequent SEM photographs. ]

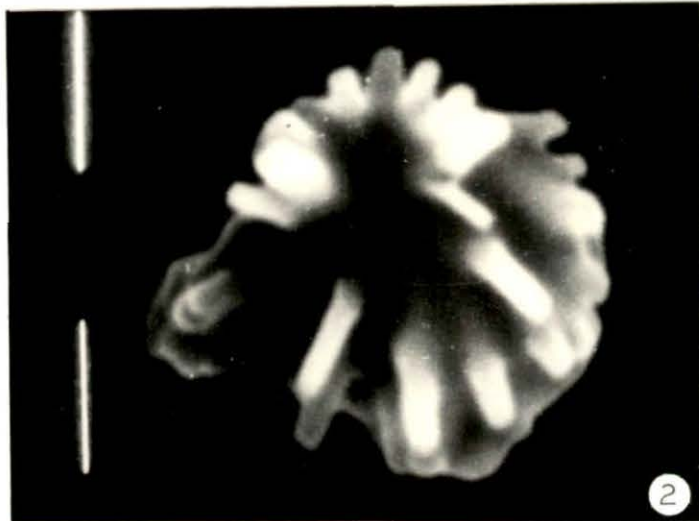
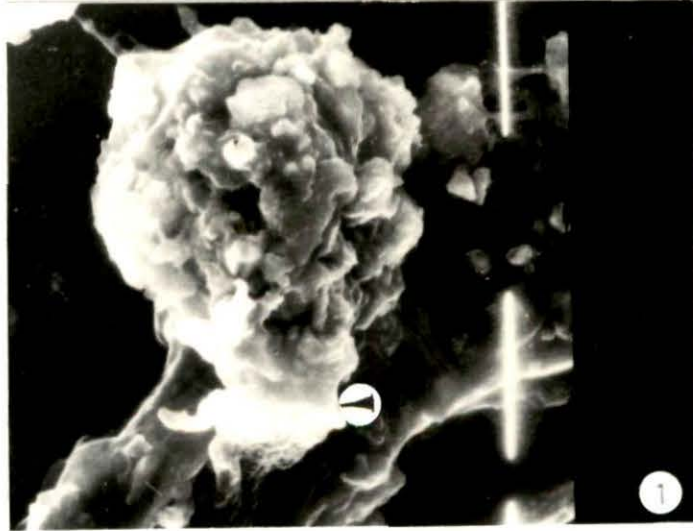


Plate - II

Fig. 1 & 2      SEM photographs of two plastic adherent cells from a normal bat with bulbous protrusions, similar to follicular dendritic cells; size of the cells is bigger than most other plastic adherent cells. X 8,500      X 5,000

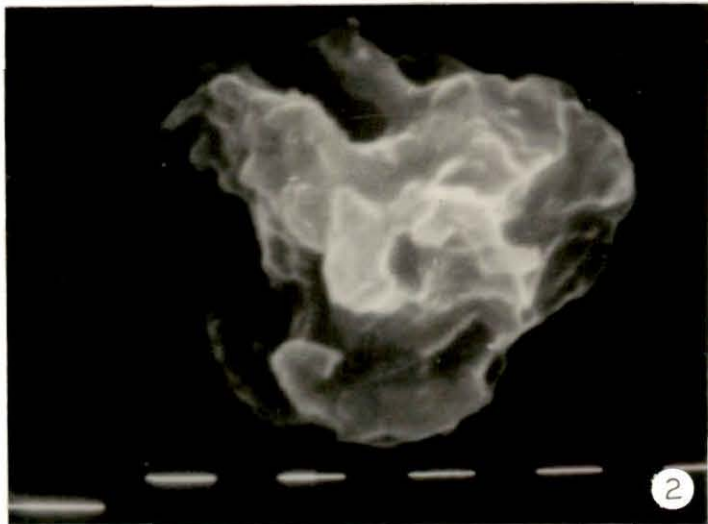
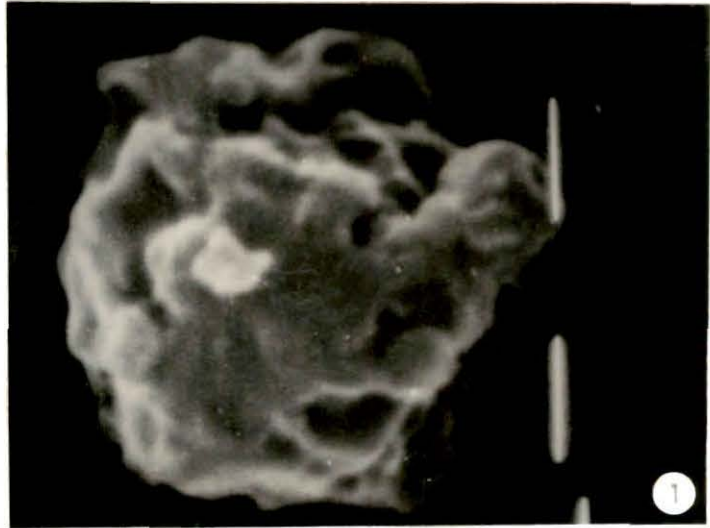


Plate - III

Fig. 1 & 2 SEM photographs of plastic adherent cells from bat immunized with SRBC showing that pseudopodial projections are not as prominent as in normal cells. X 6,000, X 6000.

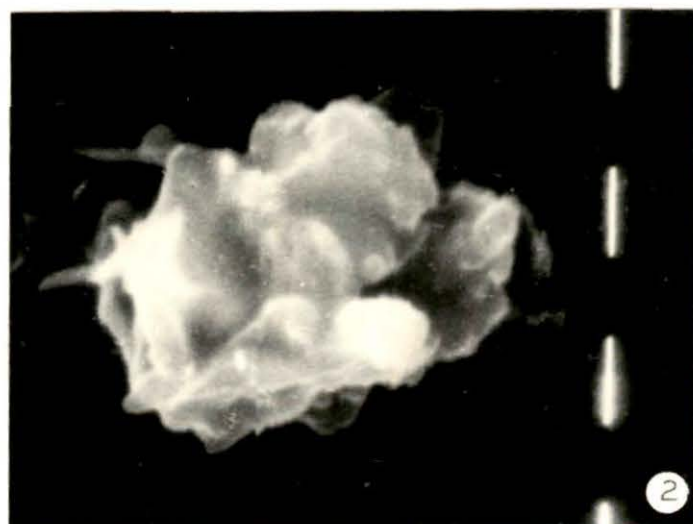
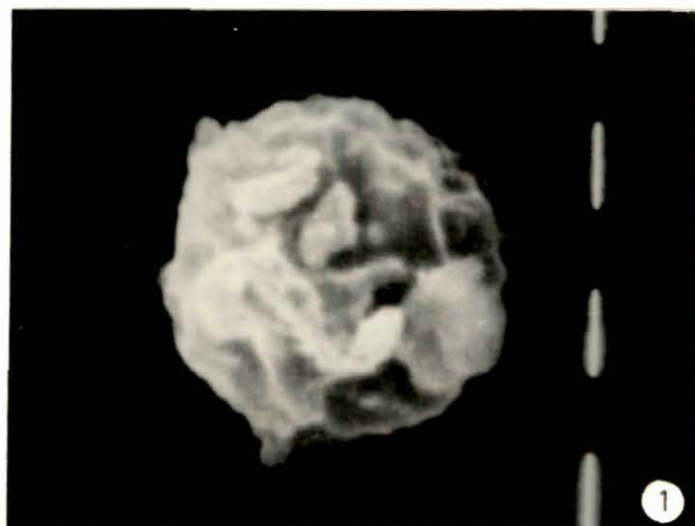


Plate - IV

Fig. 1 & 2      Typical nylon wool non adherent cells (equivalent to T cells) from a normal bat showing smooth cell surface devoid of any projections but occasional surface ridges (arrow). Diameter of the cells is about 7  $\mu\text{m}$  in Fig. 1 and about 8  $\mu\text{m}$  in Fig. 2. X 5,500, X 4,250.

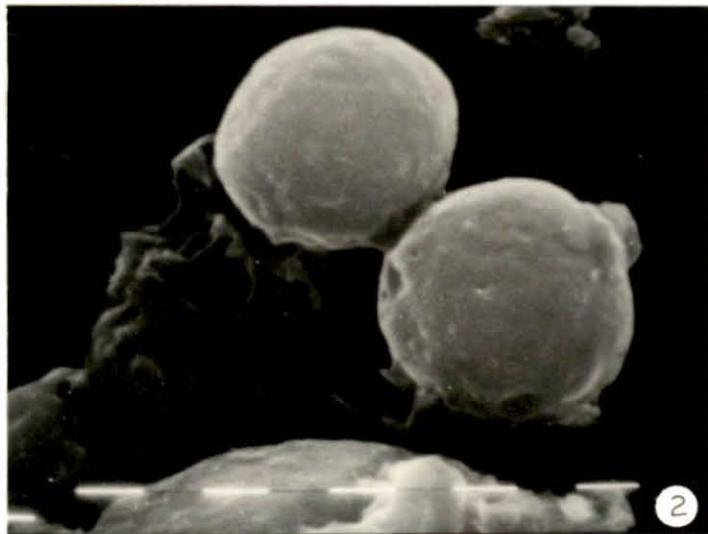
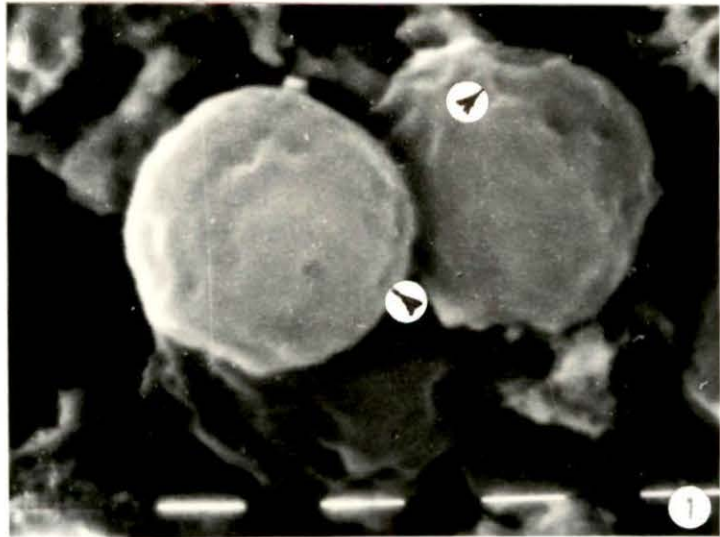


Plate - V

Fig. 1 & 2 SEM photographs of nylon wool non adherent cells from immunized bat showing some surface rufflings (arrow). Size of cell is comparatively bigger and about 7  $\mu\text{m}$  in Fig. 1 and about 10  $\mu\text{m}$  in Fig. 2.  
X 6000, X 5,250

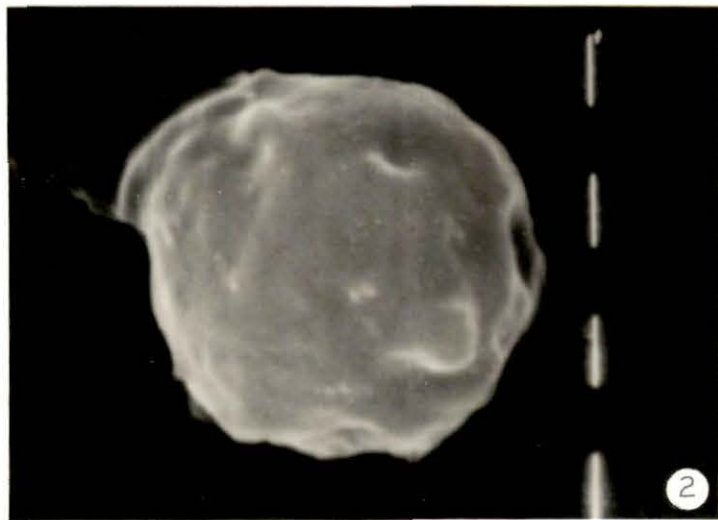
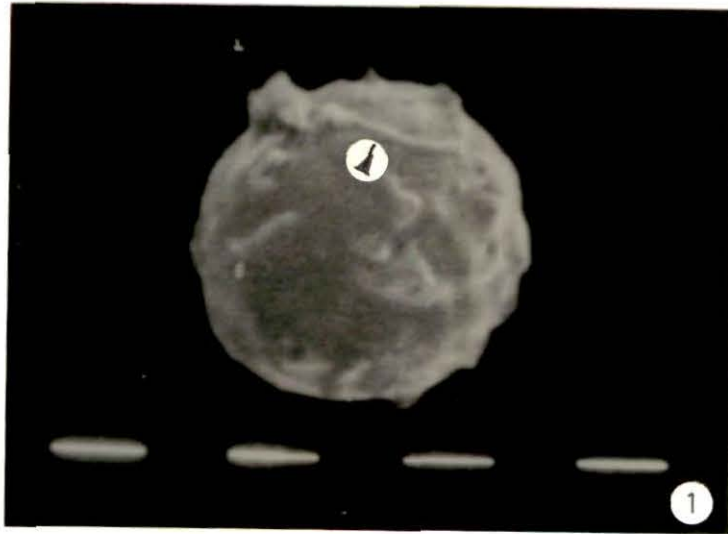


Plate - VI

Fig. 1 & 2 SEM photographs of nylon wool adherent cells from normal bat. Some small microvilli ( MV ) and pits ( P ) are seen on the surface. Size of cell is about 9  $\mu$ m.  
X 8000, X 5500

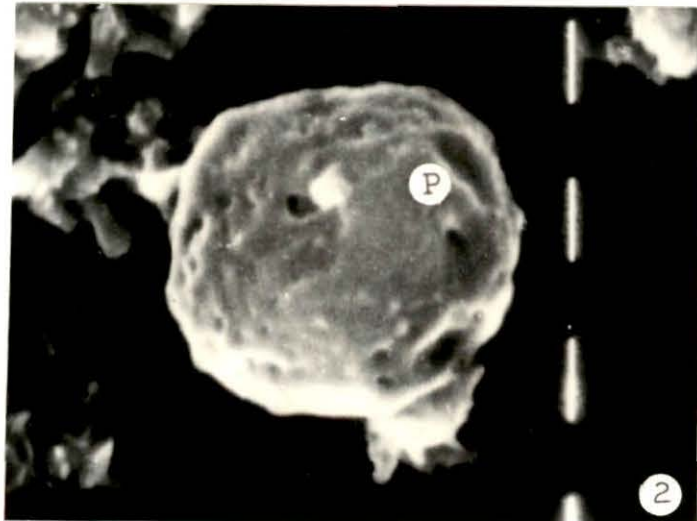
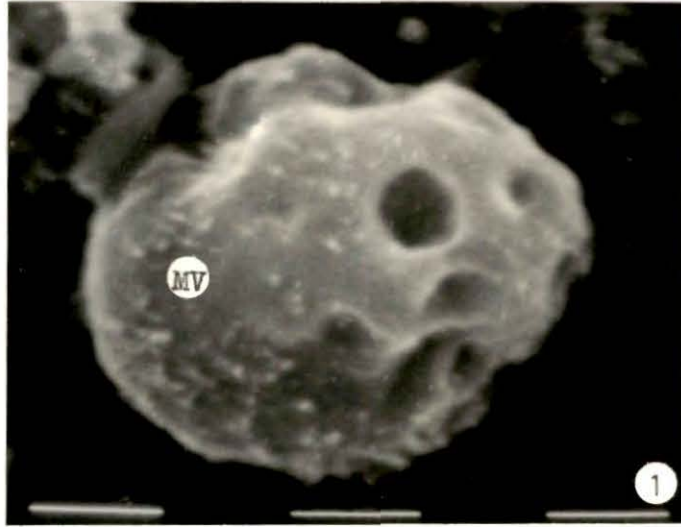


Plate - VII

Fig. 1,2 & 3 SEM photographs of nylon wool adherent cells from an immunized bat. The cell surface is highly ruffled. Long filamentous surface projections can be seen in Fig. 1 and 2 (arrow). Size of the cells about 9  $\mu\text{m}$  in Fig. 1 and 2, and about 12  $\mu\text{m}$  in Fig. 3. X 6000, X5250, X 5000.

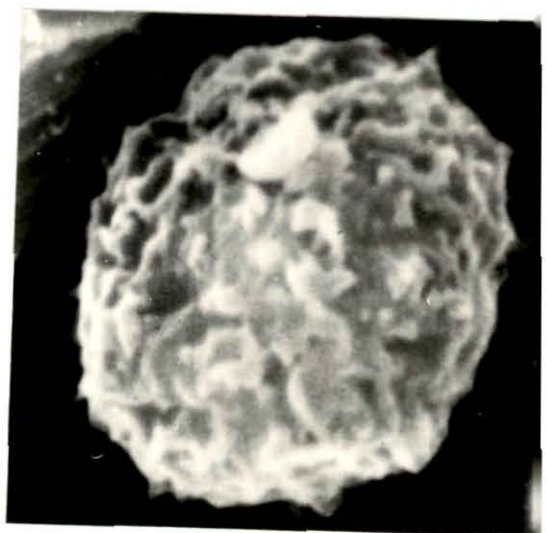
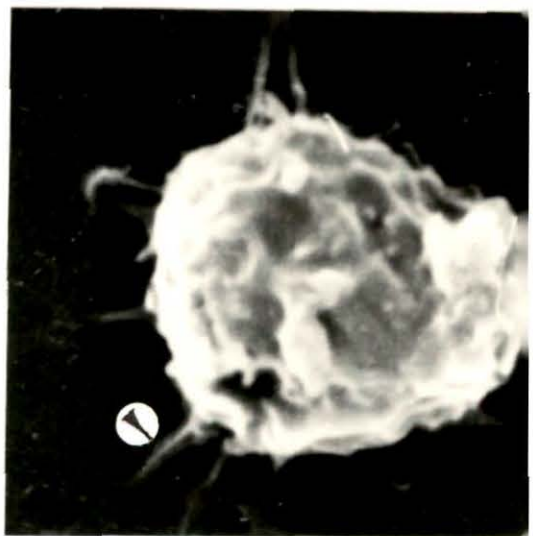
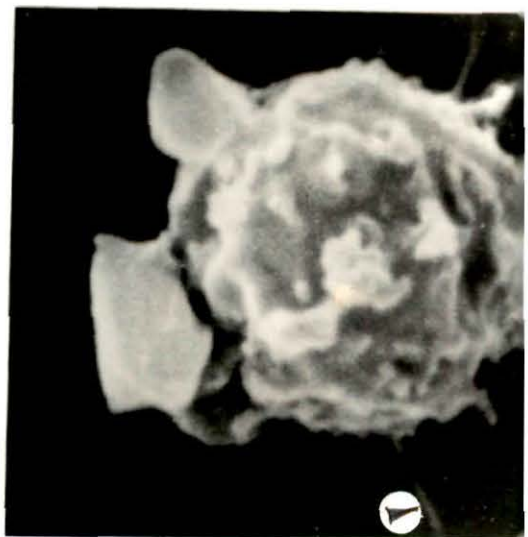


Plate - VIII

Fig. 1

Photograph showing the separation of major immunoglobulin classes of bat by polyacrylamide gel electrophoresis. Lane 3 contains bat Ig obtained by affinity chromatography, lane 2 contains purified human Ig G and lane 1 contains a mixture of bat Ig, human Ig G and BSA.

1 2 3

IgG

BSA

IgM

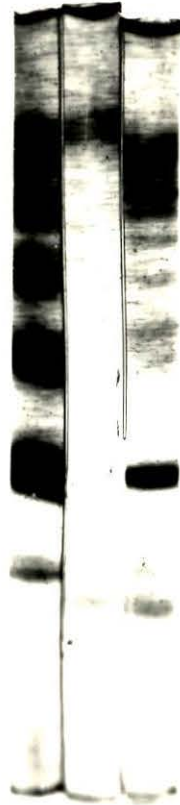


Plate - IX

- Fig. 1                    Photomicrograph showing indirect immunofluorescence of spleen cells of bat, treated with rabbit anti-bat Ig M (anti-B Ig M) and then fluoresceinated goat anti-rabbit Ig (Fl-anti-R Ig). Fluorescence on the cells is in the form of ring and patches. X
- Fig. 2 & 3                Photomicrographs of spleen cells treated with rabbit anti-bat Ig G (anti-B Ig G) and then Fl-anti-R Ig showing a distinct ring type of immunofluorescence on the central cell, which is shown in magnified view in Fig. 3. X 700, X 750, X 900.

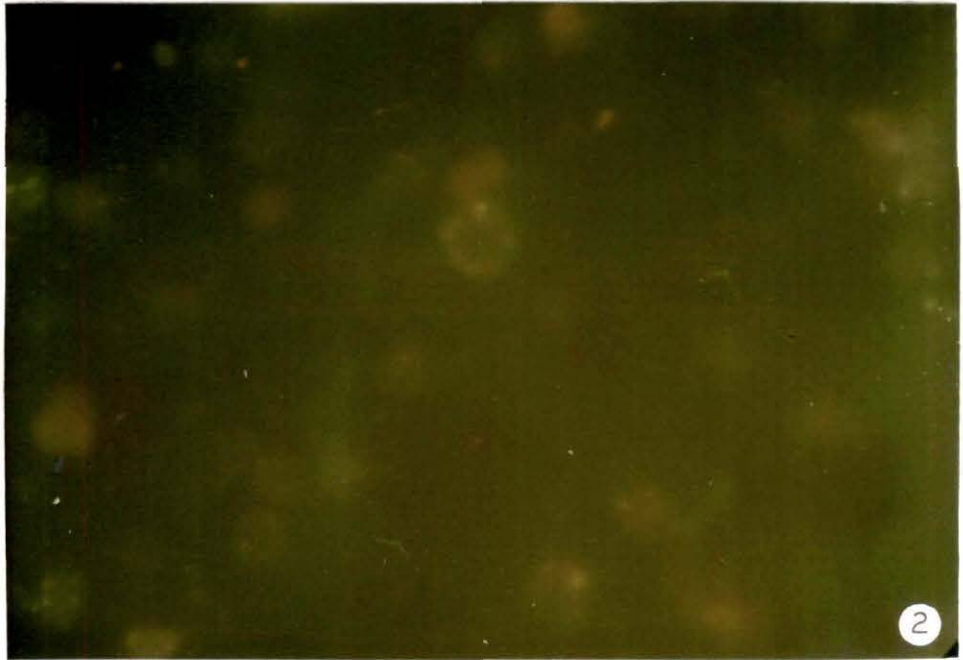
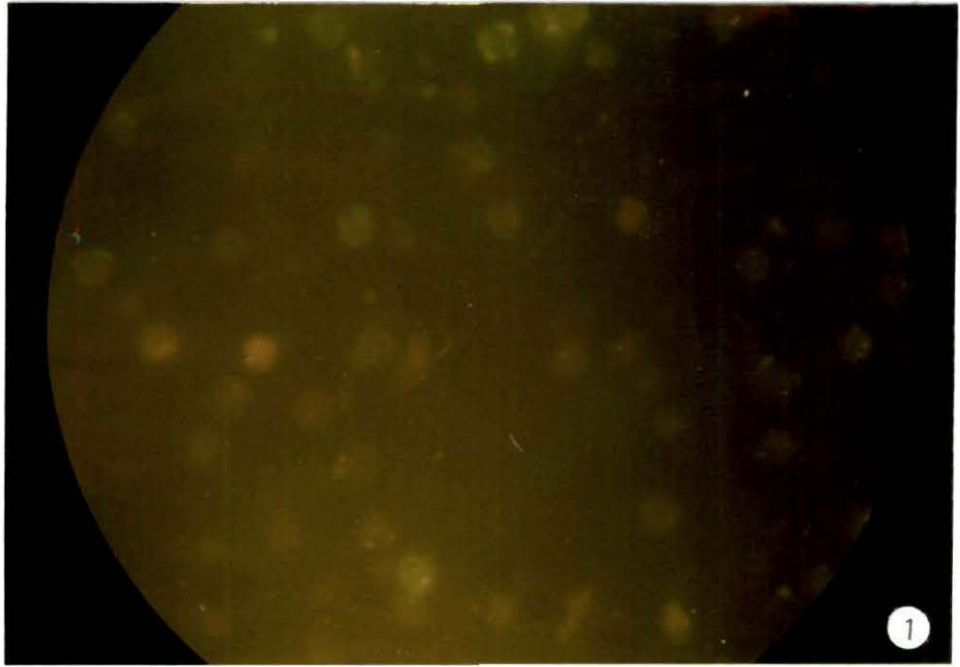


Plate - X

Fig. 1                   Photomicrograph of peripheral blood lymphocytes of bat, treated with anti-B Ig M and then Fl-anti-R Ig showing incomplete ring and patches of fluorescence . X 700.

Fig. 2                   Photomicrograph of peripheral blood lymphocytes treated with anti B Ig G and then Fl-anti-R Ig showing ring like fluorescence. X 700.

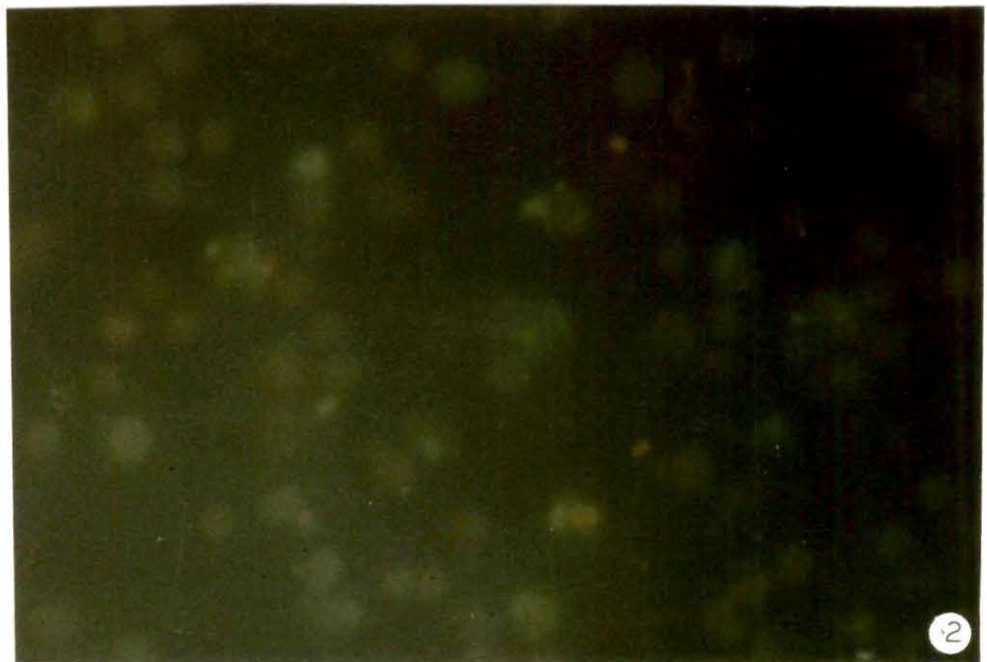
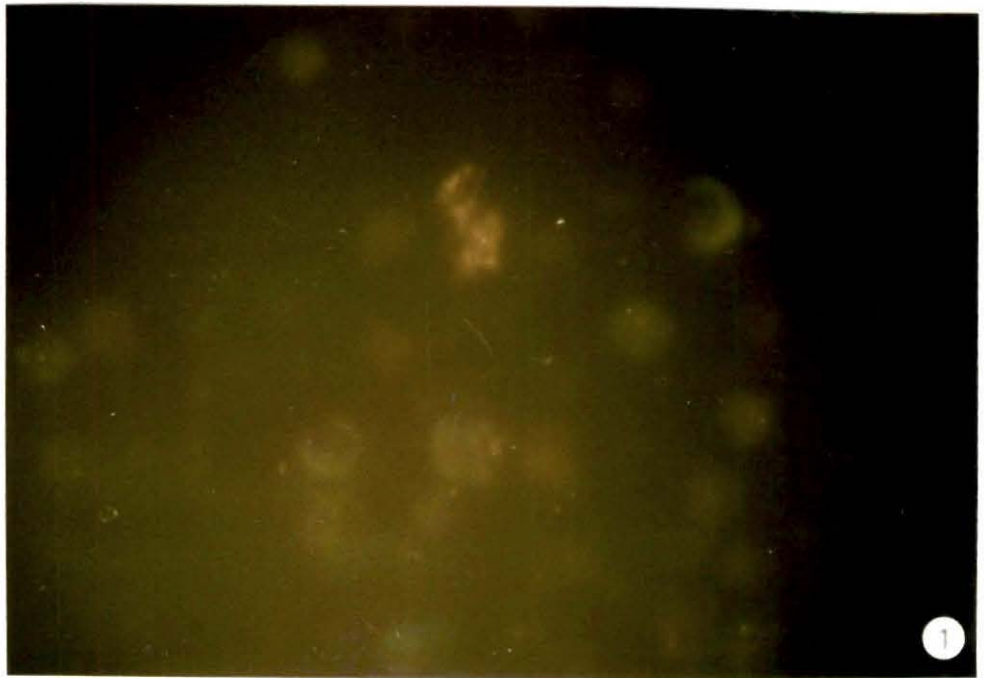


Plate - XI

- Fig. 1                      Photomicrograph of nylon wool adherent cells of bat treated with anti-B Ig M and then Fl-anti-R Ig showing indirect immunofluorescent staining of membrane Ig in the form of ring and patches. X 700.
- Fig. 2 & 3                  Photomicrographs of nylon wool adherent cells of bat treated with anti-B Ig G and Fl-anti-R Ig showing typical fluorescent ring for membrane Ig G on a cell, magnified view of which is shown in Fig. 3. X 700, X 900.

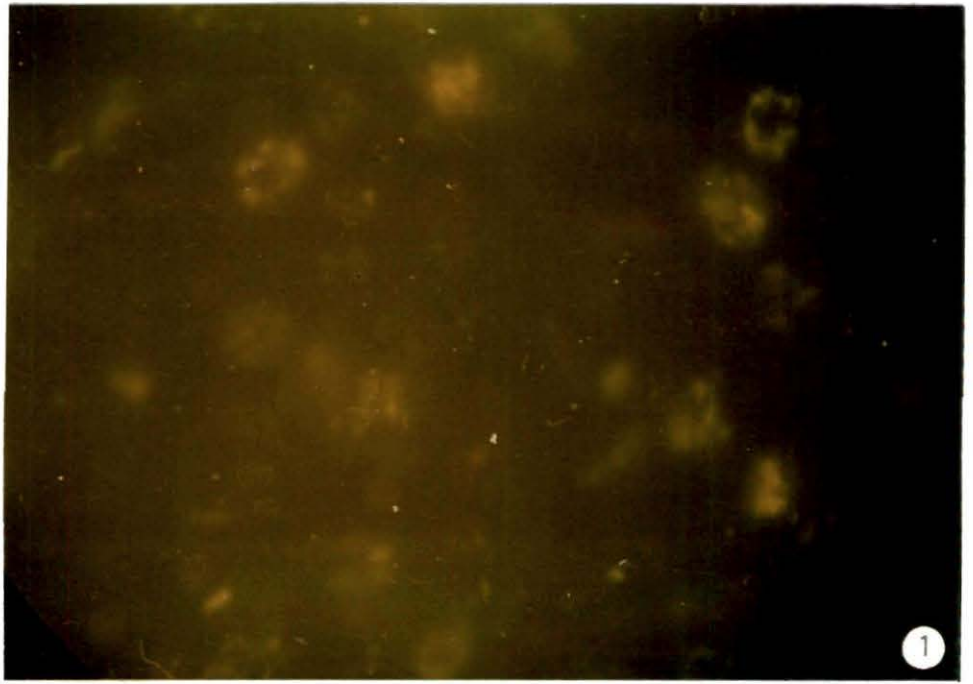


Plate - XII

Fig. 1 Photomicrograph of a histological section of spleen from normal bat showing a white pulp follicle. The periarteriolar lymphocytic sheath (PS) contains a good number of cells. CA-central arteriole. X 600

Fig. 2 Photomicrograph of a section of bat spleen removed 24 hours after in vivo anti-brain serum treatment. Density of lymphocytes in the PS region is slightly less than in the normal spleen. X 600.

Fig. 3 Photomicrograph of a section of bat spleen removed 72 hours after anti-brain serum treatment. PS region is loosely organized, with less number of lymphocytes than in normal, indicating depletion of cells in this region. X 600.

[All sections stained with Haematoxylin and Eosin ]

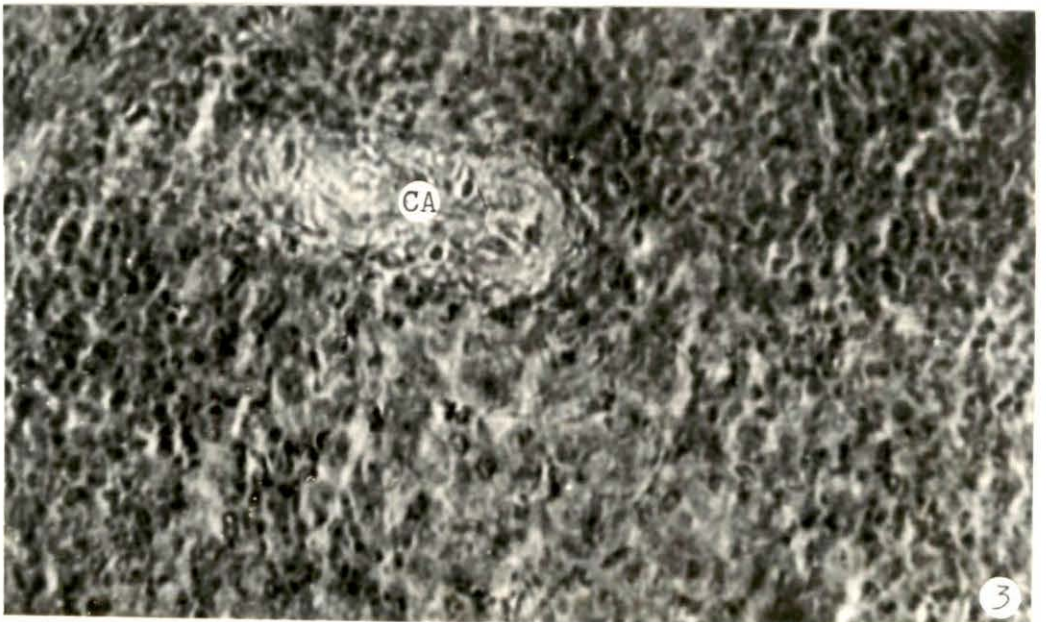
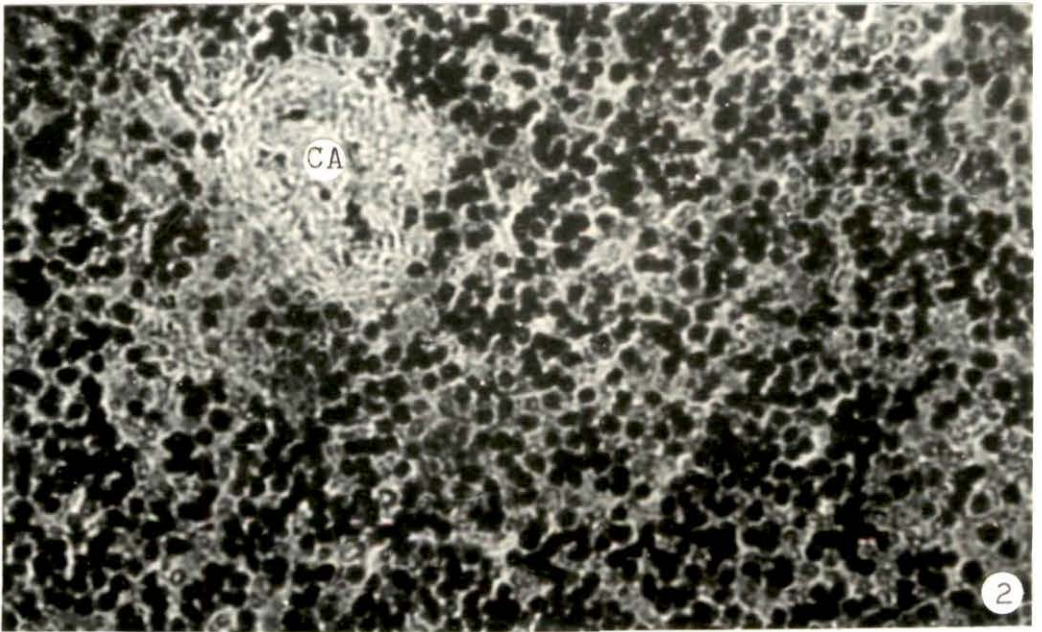
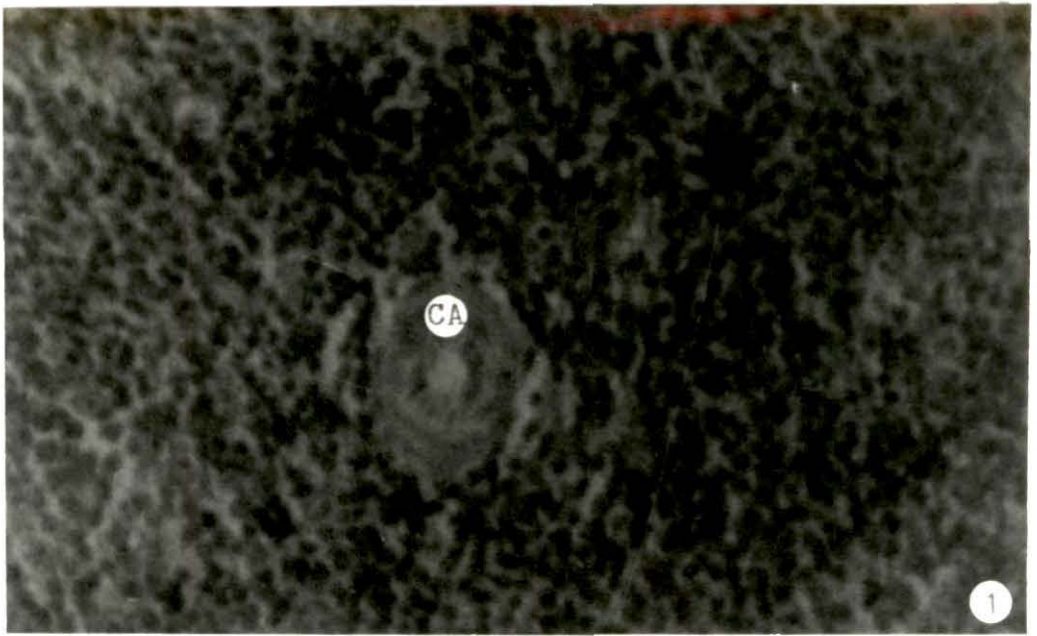


Plate - XIII

Photomicrograph of a section of mesenteric lymph node from normal bat, showing distribution of lymphocytes in lymphoid follicle (F) and paracortex region (PC). Haematoxylin-Eosin stain. X 450.

Fig. 2

Photomicrograph of a section of mesenteric lymph node of bat removed 24 hours after anti-brain serum treatment. Density of lymphocytes in follicular and paracortical regions is slightly less than in normal lymph node. Masson's Trichrome stain. X 300.

Photomicrograph of a section of mesenteric lymph node of bat removed 120 hours after anti-brain serum treatment. The paracortical region (PC) shows empty spaces indicating **significant** depletion of cells in this region. Masson's Trichrome stain. X 300.

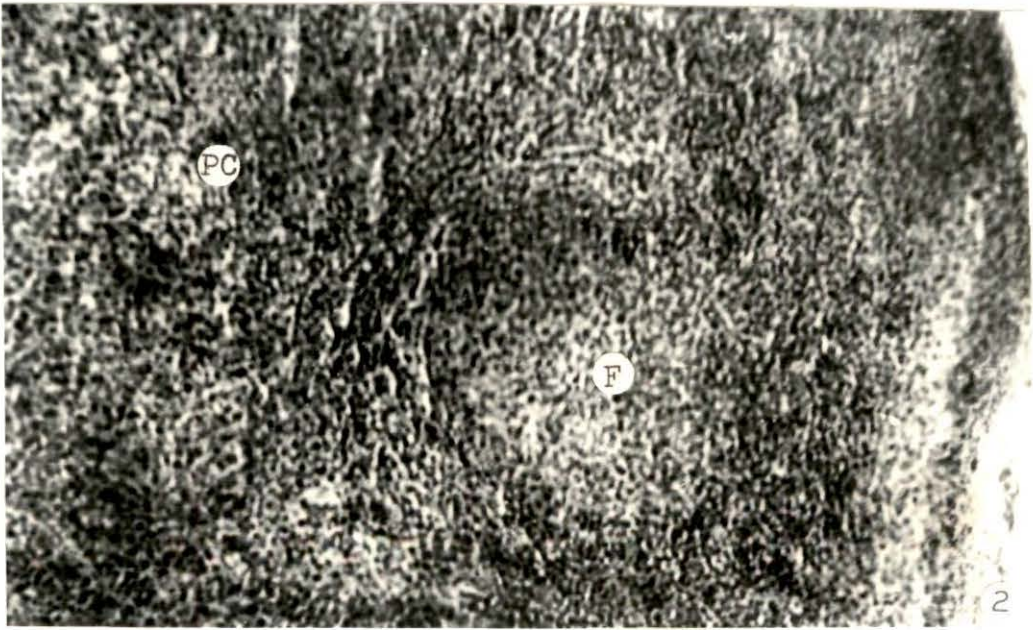
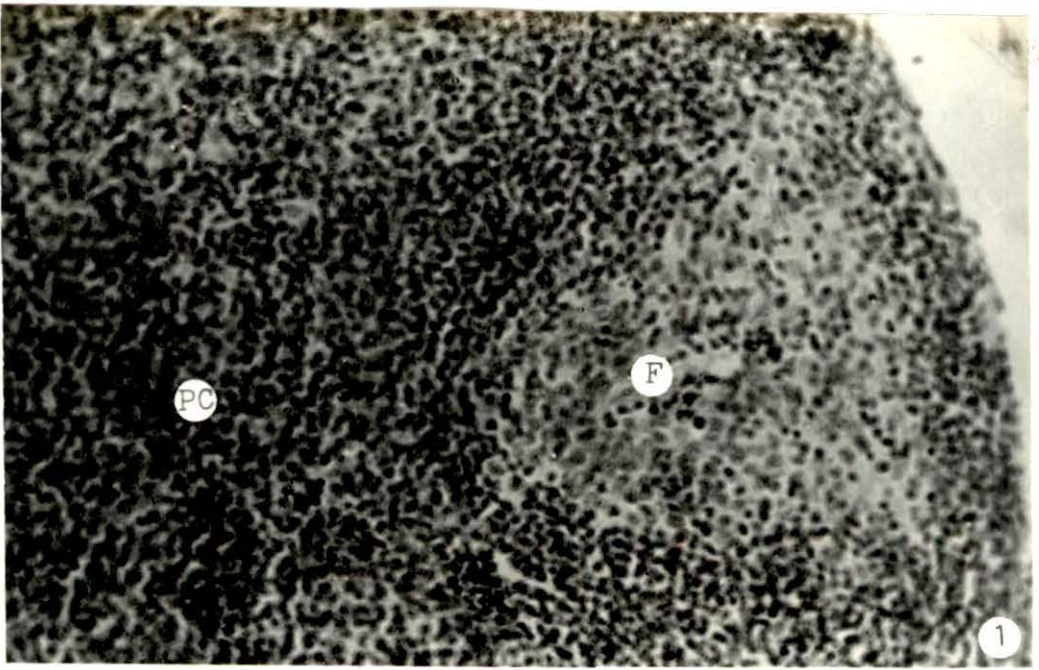


Plate - XIV

Fig. 1

Transmission electron micrograph of a section of spleen from normal bat. Lymphoid cells differ in size and cytoplasmic content. The smaller cells (SL) show a thin rim of cytoplasm with very few organelles. Nucleus shows thick patches of darkly stained heterochromatin mainly along nuclear margin, and nuclear pores. The larger lymphocytes (LL) show more amount of cytoplasm containing mitochondria (M), vesicles (V), some ribosomes and few lamellae of endoplasmic reticulum. Cytoplasmic extensions from other cells can be seen (arrow). X 12,300.

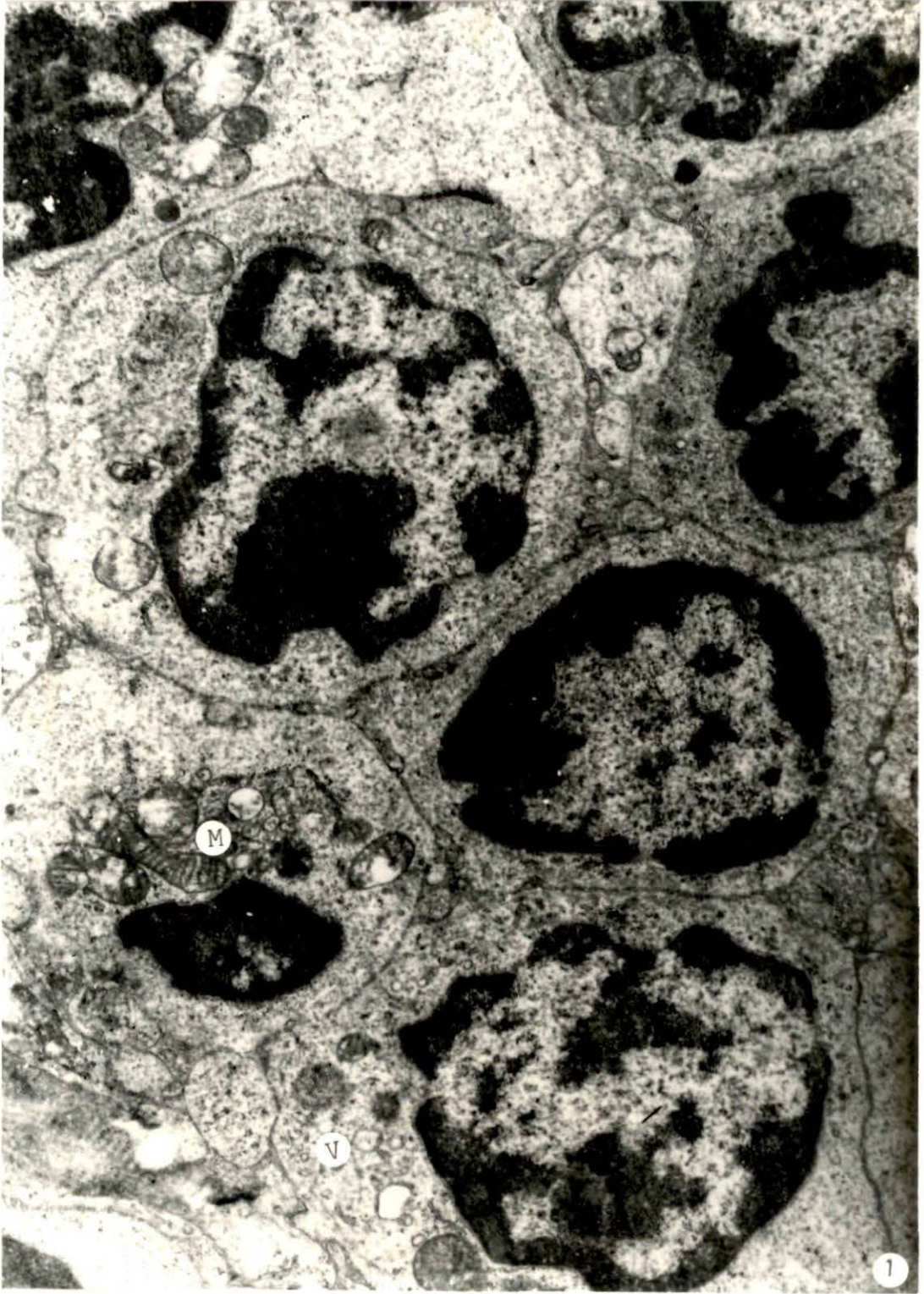


Plate - XV

Fig. 1

TEM photograph of lymphoid cells in normal bat spleen. Two large sized lymphocytes are seen with noticeable amounts of cytoplasm containing mitochondria (M) and vesicles (V). Occasional short profiles of endoplasmic reticulum (ER) can be seen. The cell nucleus is polygonal in shape, having fair amount of heterochromatin and a nucleolus (Nu). At the lower side, part of a capillary lined by endothelial cell (E) with typically elongated nucleus and microfilaments can be seen.

X 12,300.

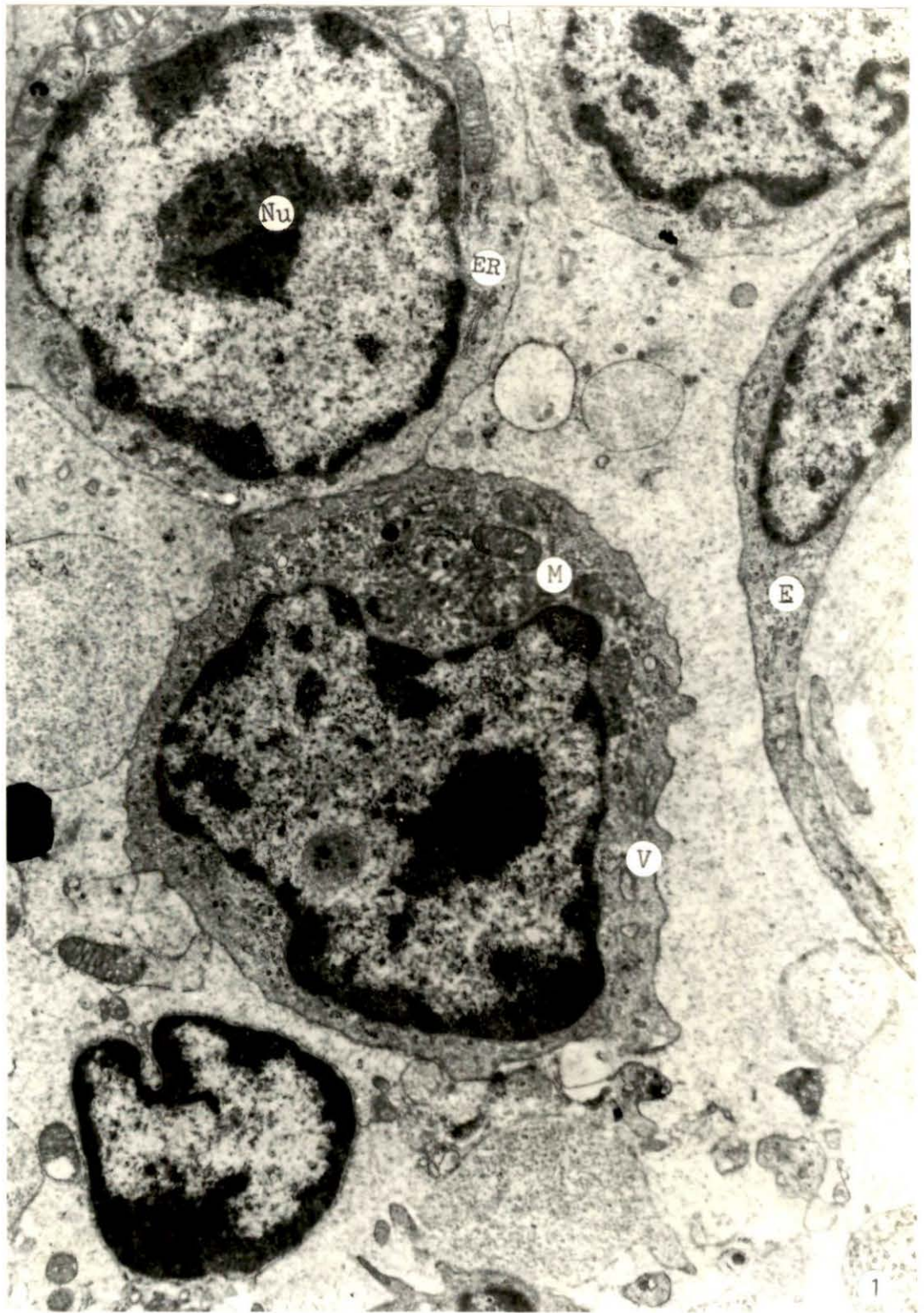
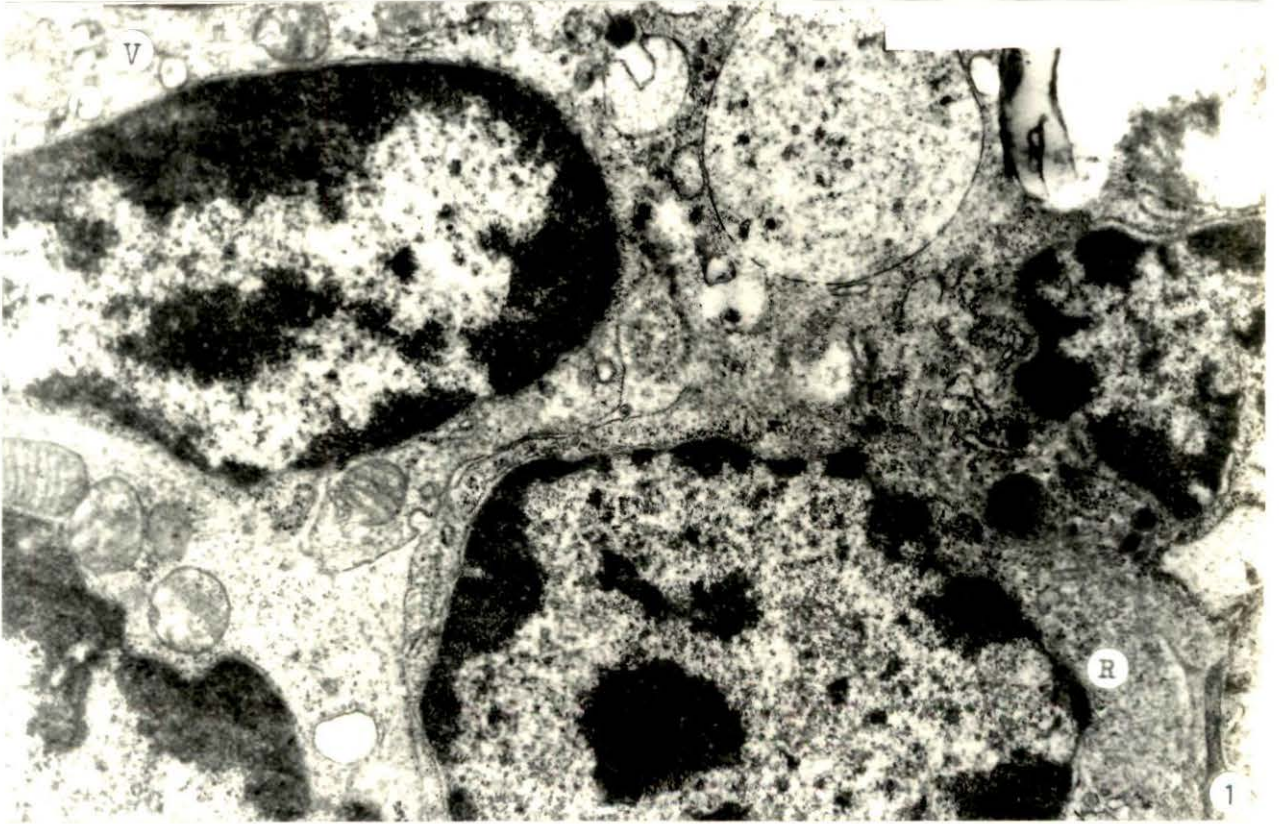


Plate - XVI

Fig. 1            TEM photograph of normal bat spleen showing large sized lymphocytes. Many free ribosomes (R) and mitochondria indicate that the cells are metabolically active. The cell at the upper side of the photograph has some vesicular structures (V) which may be part of a Golgi apparatus. Some short profiles of ER are also seen. X 22,500.

Fig. 2            Magnified view of a part of Figure 1 showing the details of a mitochondria and endo- or exocytotic vesicles. The vesicles with openings to the exterior, are surrounded by electron dense material (arrow). X 60,000.



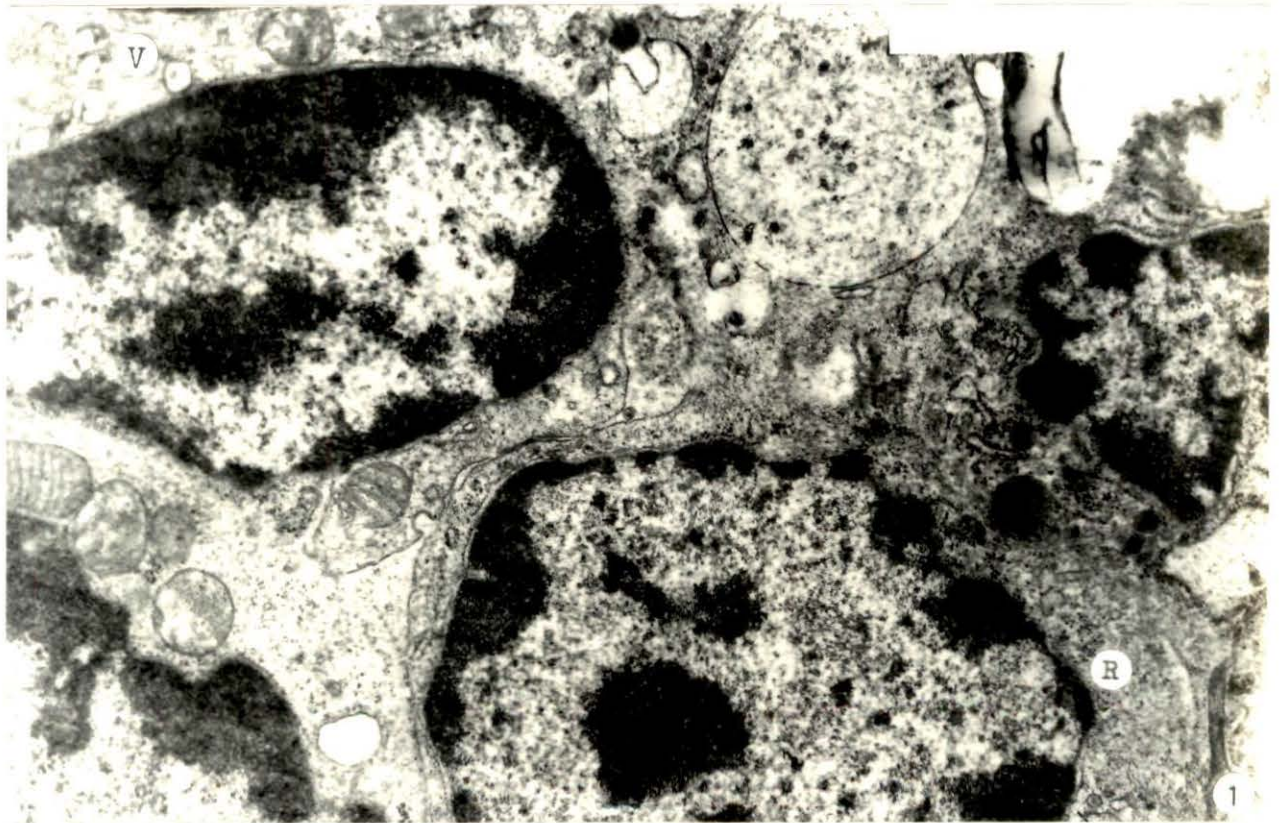


Plate - XVII

Fig. 1

TEM photograph of normal bat spleen. Two large cells with paucity of cytoplasmic organelles are seen. Differences in nuclear morphology and heterochromatin content among the cells is noticeable. Cytoplasmic extensions from adjacent cells can be seen. X 14,000.

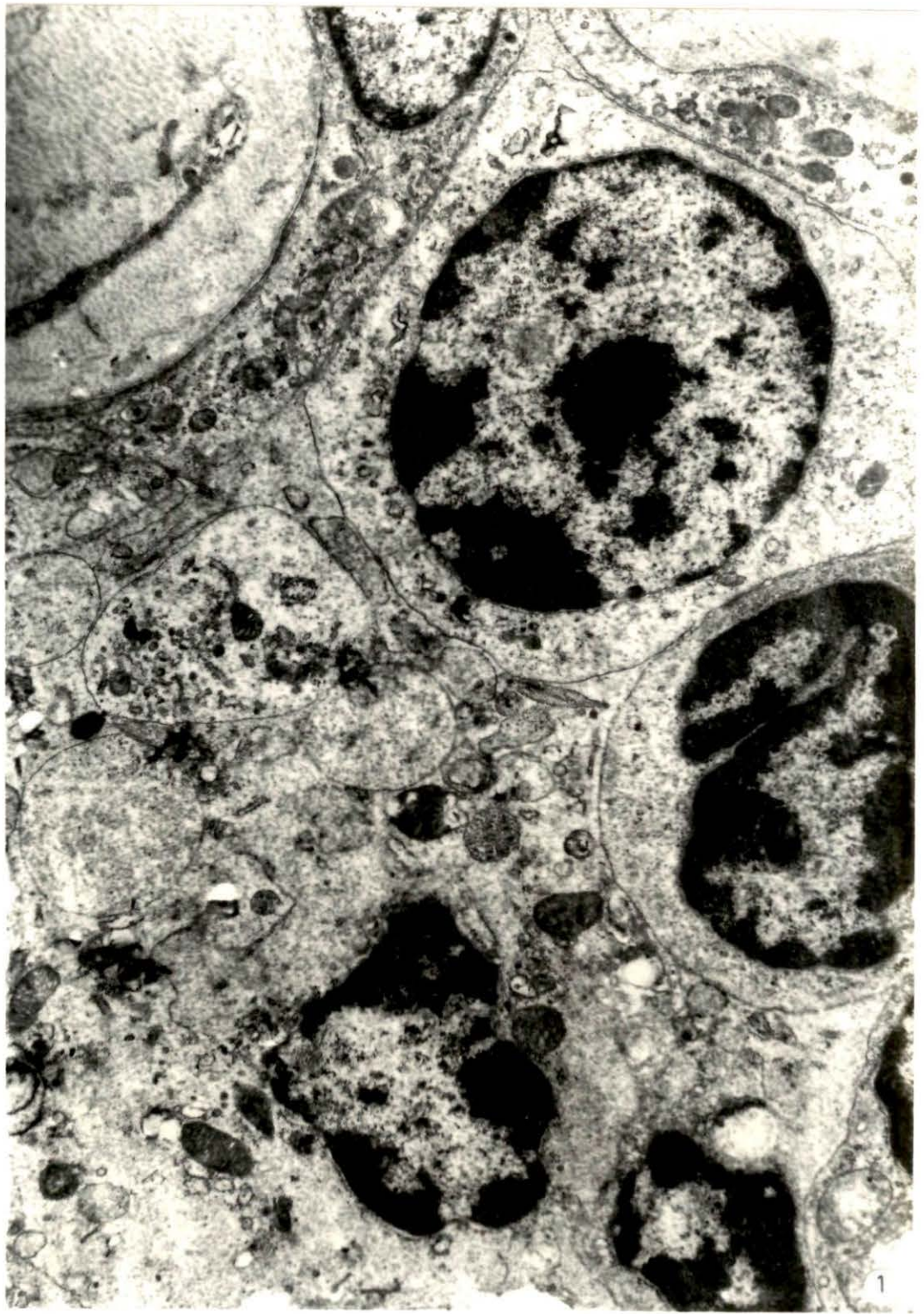


Plate - XVIII

- Fig. 1 High power view of a part of the figure in Plate-XVII. The large cell has a round nucleus, many ribosomes and a very few other organelles. Few small vesicles are present. The cell looks like a plasma cell. X 18,200.
- Fig. 2 Magnified view of a nucleolus in a lymphocyte of normal bat spleen. Dark granules (arrow) inside the nucleolus are visible, probably indicative of ribonucleoprotein particle synthesis. X 18,800.

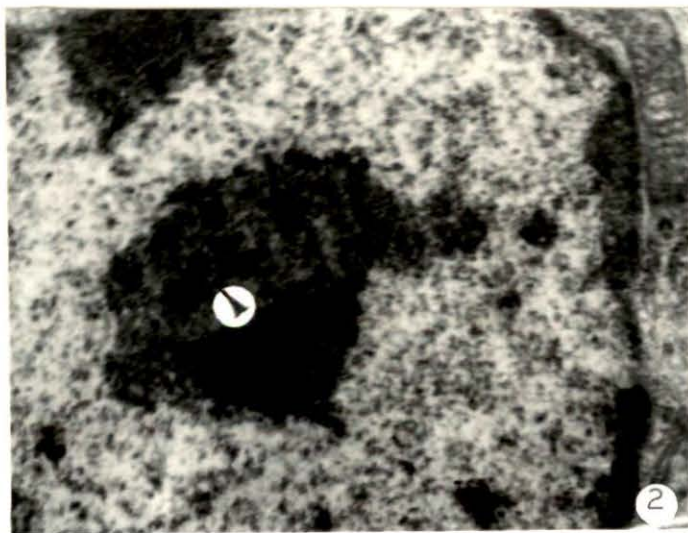
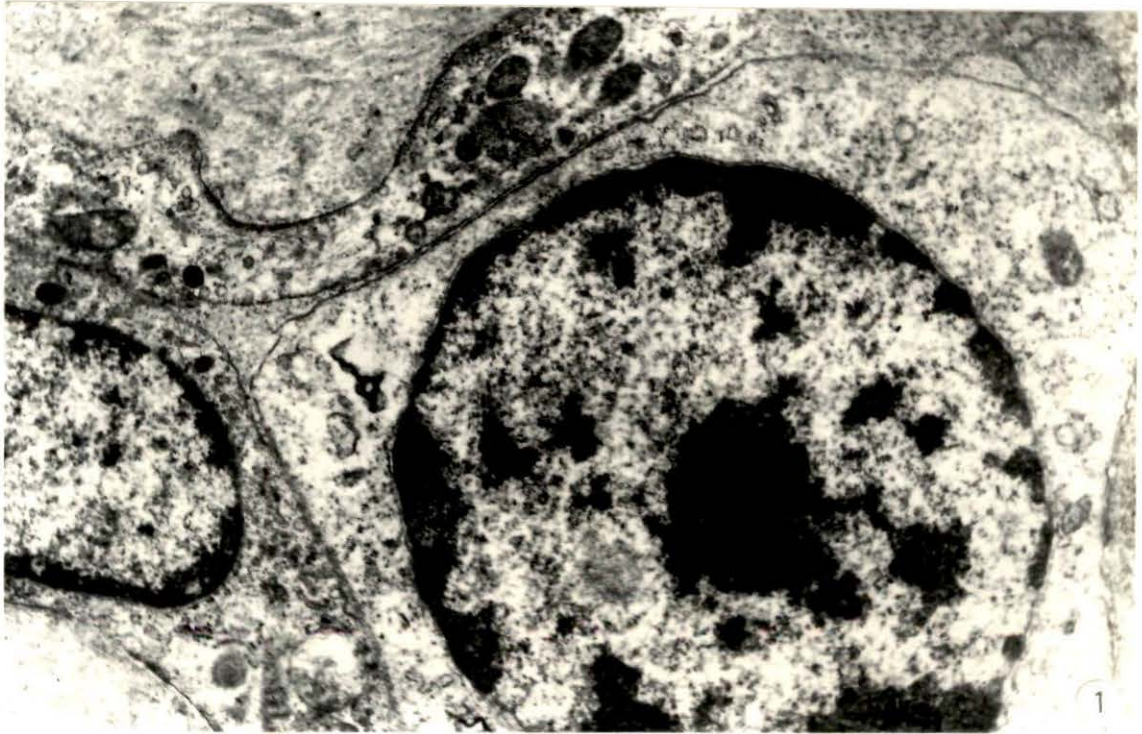


Plate - XIX

Fig. 1

TEM photograph of spleen from bats immunized with 25% SRBC for 10 days. Large cells, elongated in appearance are seen. The nuclei show less heterochromatin clumps. Cytoplasmic organelles are less prominent, except some ribosomes and mitochondria. X 19,900.

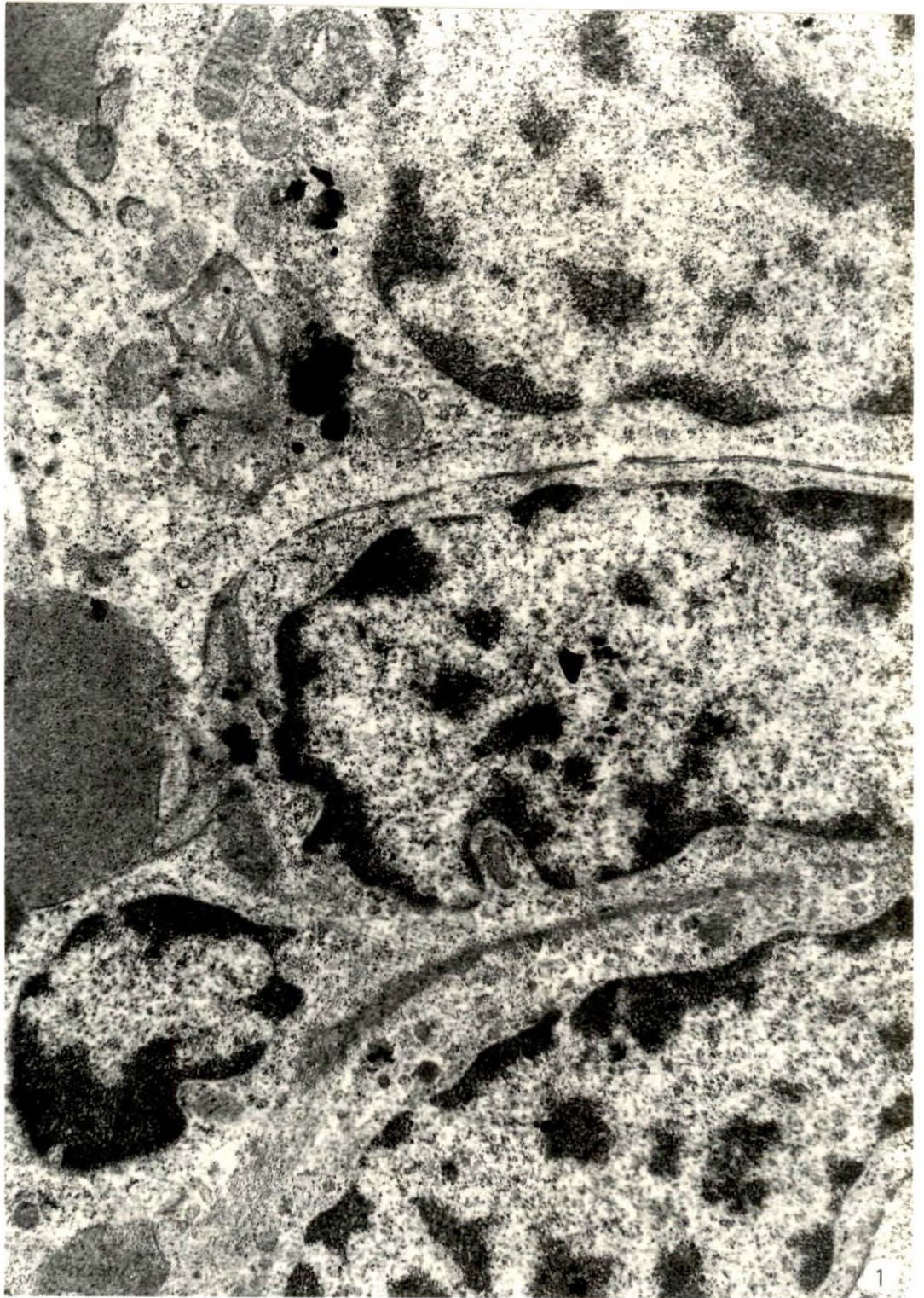


Plate - XX

Fig. 1

TEM photograph of spleen from immunized bat. A cell, resembling a macrophage is seen with irregular outline. Nucleus is with scanty heterochromatin. Membrane bound vesicles containing granular or homogeneously osmophillic material probably represent lysosomes (L). Faint cutlines of microfilaments may be seen (arrow). X 16,700.

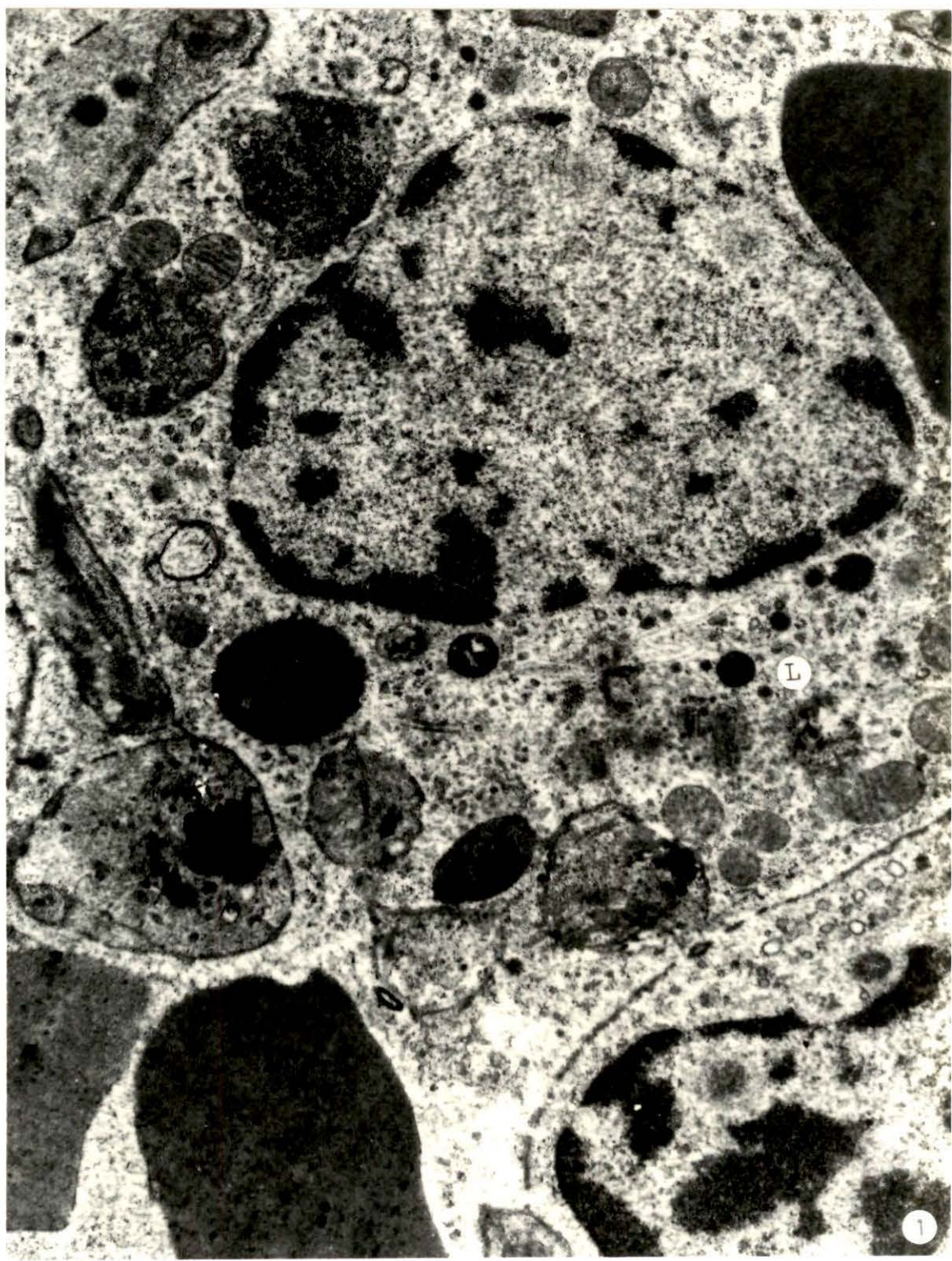


Plate - XXI

Fig. 1

TEM micrograph of spleen from immunized bat. A small lymphocyte is easily recognized from its thin cytoplasmic rim lacking organelles. In the adjacent cell, the nucleus is large and indented deeply (arrow). Euchromatic nucleus in the large lymphocyte is in contrast to the heterochromatic nucleus of the smaller cell. X 12,500.

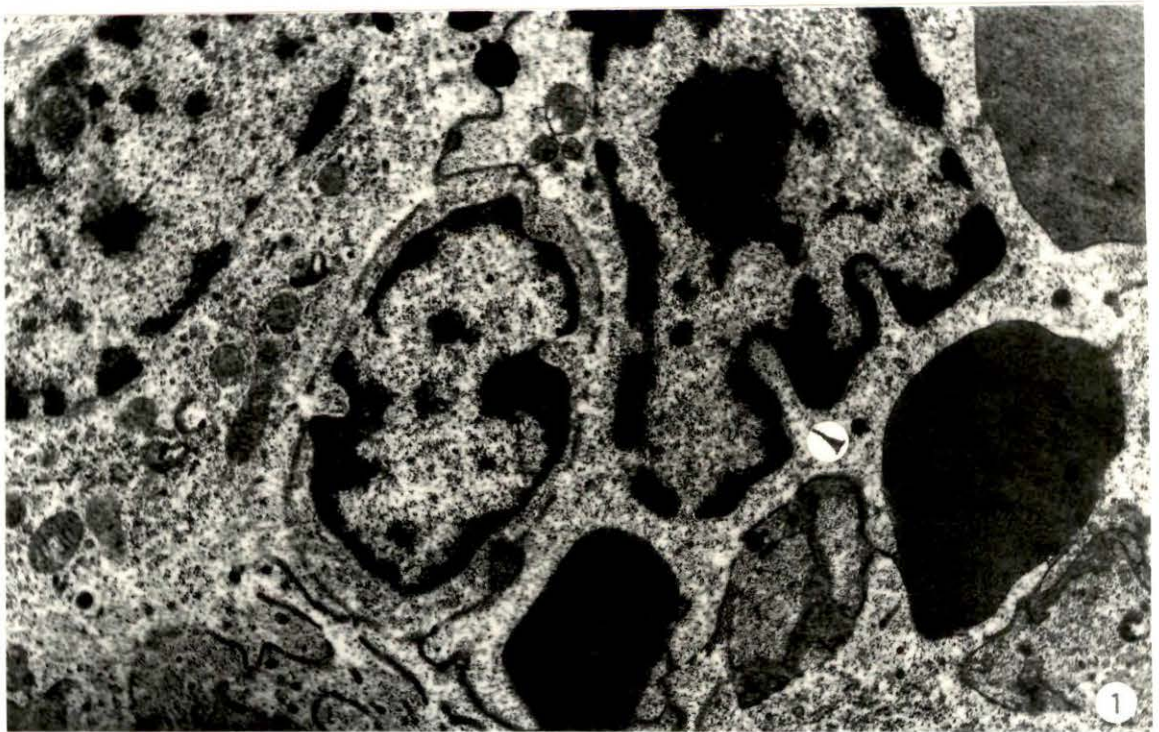


Plate - XXII

- Fig. 1            TEM photograph of isolated nylon wool adherent lymphocytes from immunized bat. Two large lymphocytes (LL) with abundant cytoplasm are seen along with some small lymphocytes having less cytoplasm. Surface projections as observed in SEM photographs are not prominent, certain vesicles (V) adjacent to the plasma membrane are visible. X 9000.
- Fig. 2            TEM photograph of spleen from immunized bat. The cells are loosely organized, show large nuclei with scattered smaller clumps of heterochromatin. Cytoplasmic organelles are not very much prominent. X 14,000.

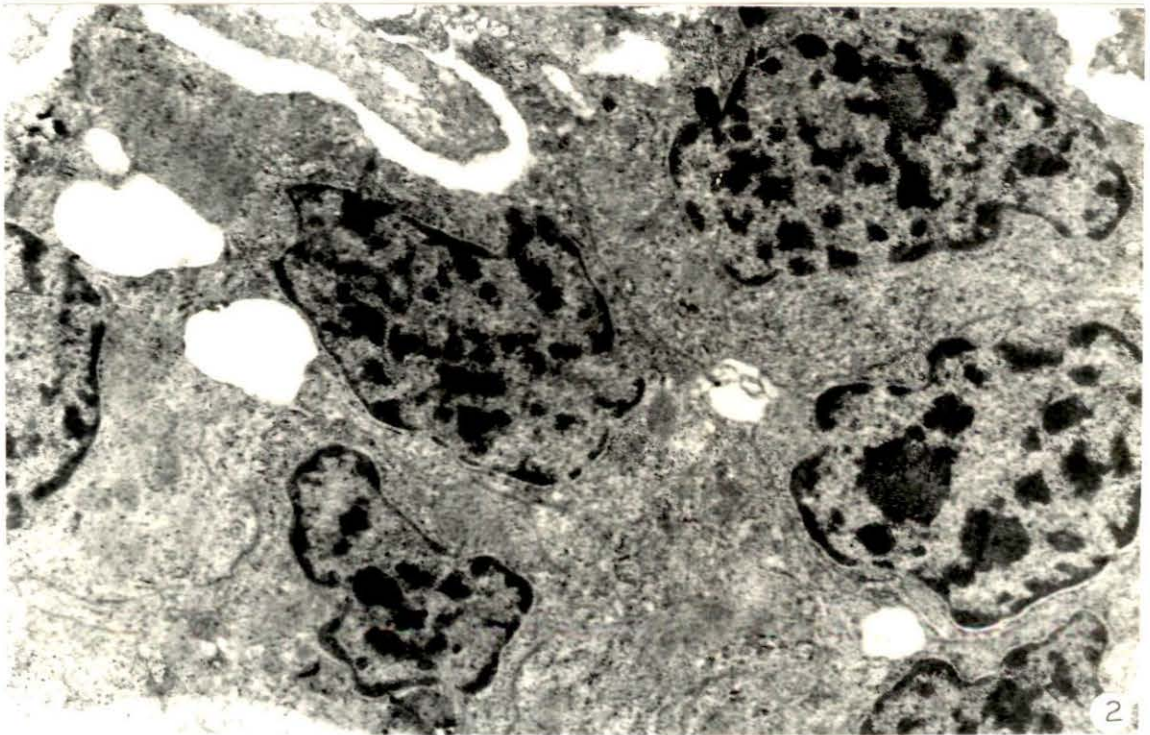
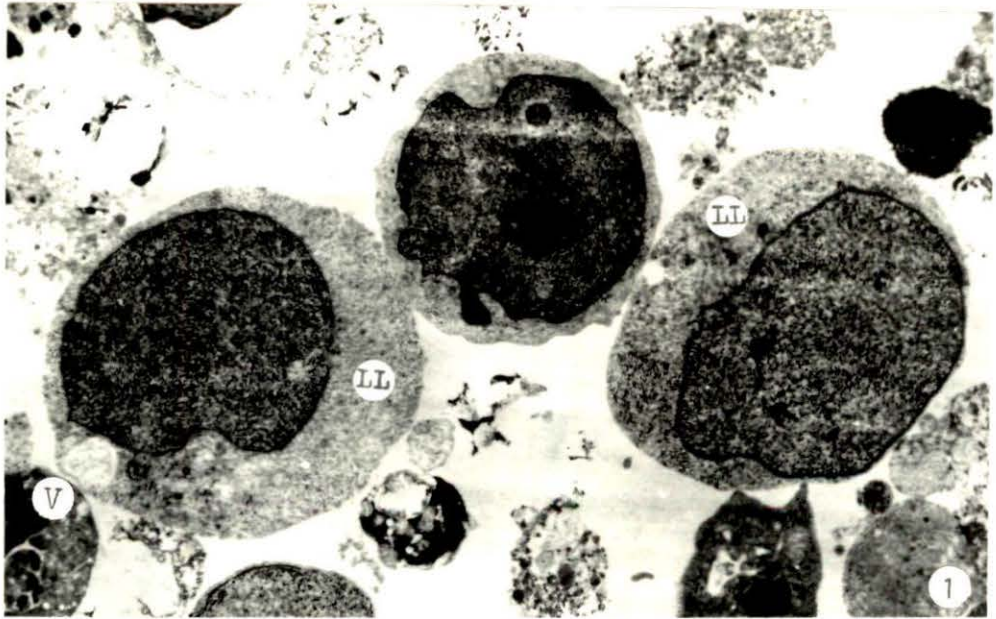


Plate - XXIII

Figs. 1 & 2      TEM micrographs of mesenteric lymph node from immunized bat. The small lymphocytes (SL) show less cytoplasm and notched nuclei (arrow) containing thick heterochromatin patches, while the larger lymphocytes (LL) have more cytoplasm containing several mitochondria (M) and vesicles (V). Nuclei in these cells have less heterochromatin, and are less indented. X 13,500, X 9,250.

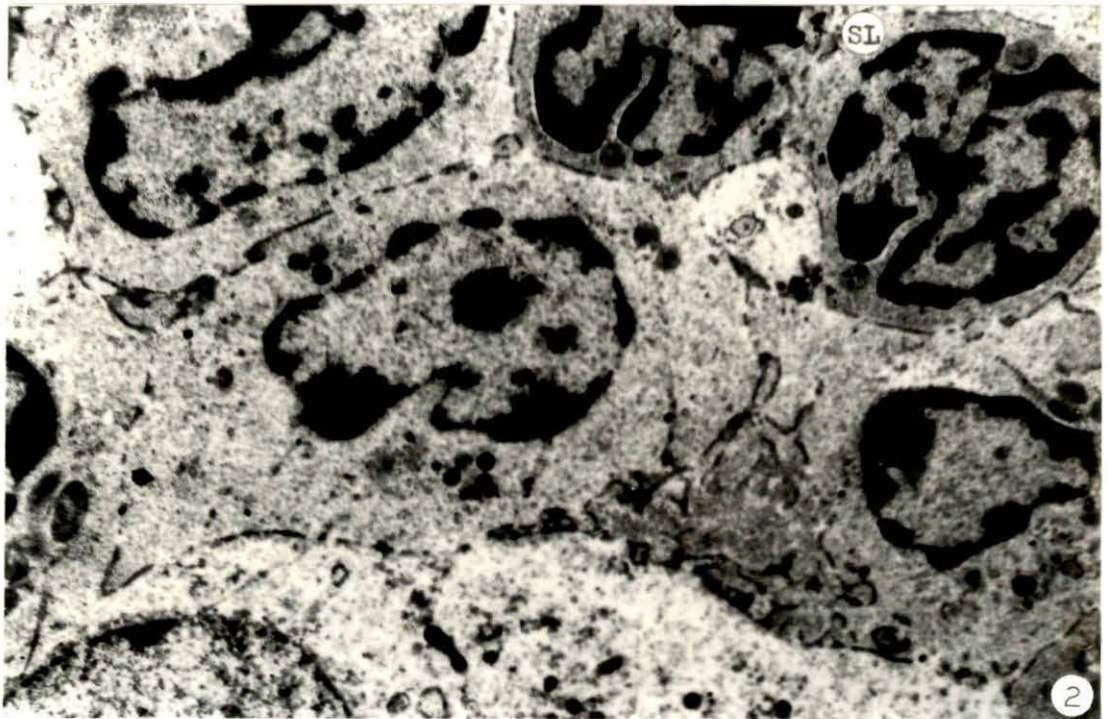
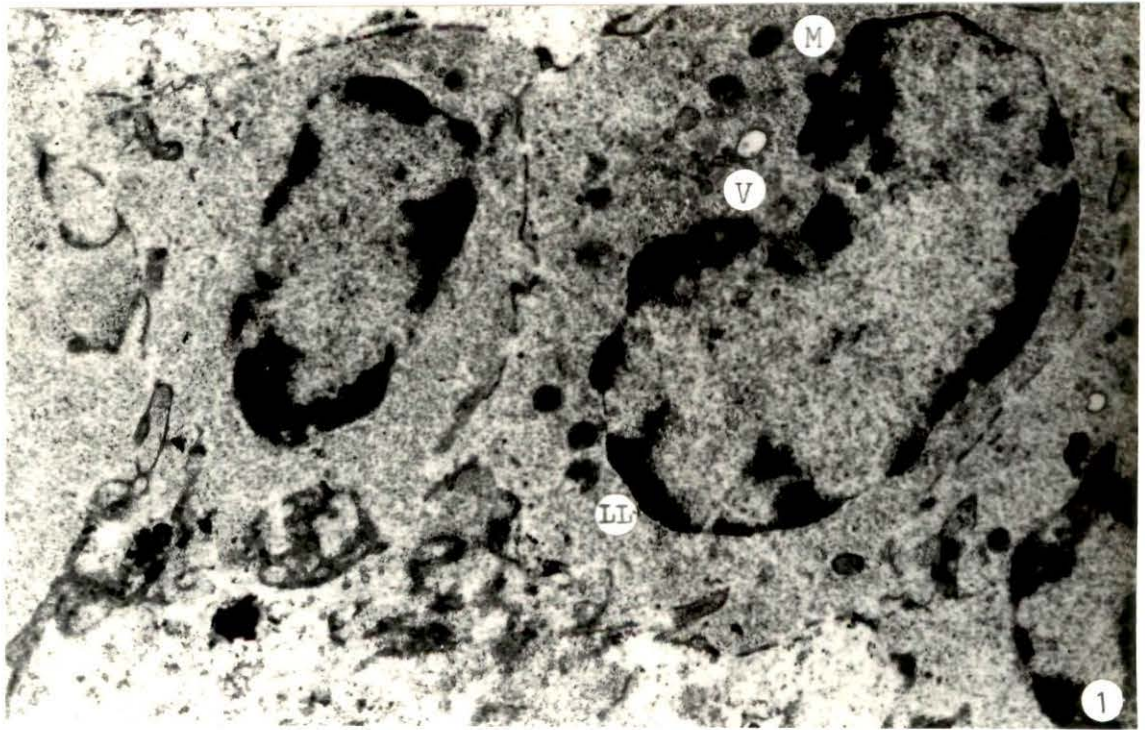


Plate - XXIV

Fig. 1

TEM photograph of mesenteric lymph node from immunized bat showing cells differing in size, cytoplasmic content, nuclear morphology and heterochromatinization. The small lymphocytes (SL) typically has less cytoplasm while large lymphocytes (LL) have more cytoplasm and deeply indented nucleus. Several mitochondria and vesicles are seen in these cells. In one cell, faint outlines of ER can be seen. X 13,600.

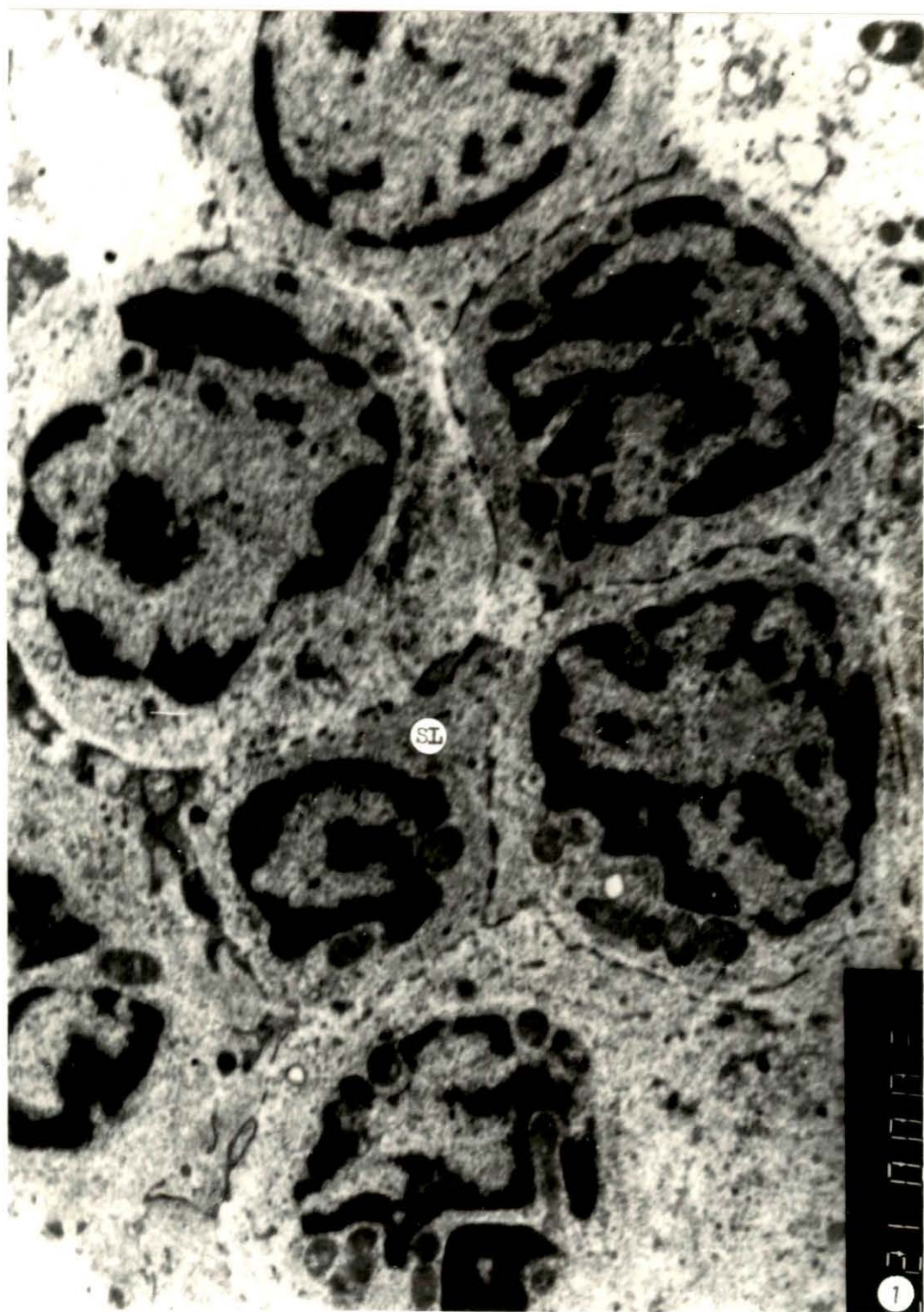


Plate - XXV

Fig. 1

TEM micrograph of mesenteric lymph node from an immunized bat, showing part of a large lymphocyte. Some mitochondria, vesicles and scattered ribosomal particles are seen. Nucleus shows moderate amounts of heterochromatin. X 23,000.

