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ULTRASTRUCTURE OF CUTANEOUS GRANULOMAS AND MONONUCLEAR CELLS FROM PATIENTS WITH SARCOIDOSIS

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Abstract. Routine electron microscopy was carried out on biopsy material taken in an active stage from cutaneous sarcoid granulomas of 5 patients. In addition, mononuclear cells isolated from the peripheral blood of 2 patients and 2 controls were studied prior to and after culture with phytohemagglutinin. The infiltrating cells of the sarcoid granulomas varied only slightly from patient to patient. The dominant cell types were macrophages and epithelioid cells rich in endoplasmic reticulum. Epithelioid cells with numerous secondary lysosomes were frequently seen. Typical as well as atypical lymphocytes, giant cells and epithelioid cells with many primary lysosomes were less frequent. No atypical lymphocytes were seen among the mononuclear cells from the peripheral blood, either before or after culture with phytohemagglutinin.

Key words: Cutaneous sarcoidosis; Ultrastructure; Mononuclear cells; Phytohemagglutinin

Previous ultrastructural studies on sarcoid granulomas from lymph nodes and the spleen have shown that the granulomas have a fairly consistent cellular structure. Epithelioid cells are generally described as belonging to one of two types characterized either by abundant, dense endoplasmic reticulum and vesicles containing fine granular material, or by lightly stained cytoplasm, abundant Golgi complex and numerous small mitochondria (4). Some authors have considered the vesicles to be lysosomes (10). A phagocytic function of these cells has been demonstrated on the basis of the uptake of carbon particles injected in a mixture with Kveim material (8). The cell membrane of either type of epithelioid cell interdigitates with neighbouring cells (8, 13). Lymphocytes are commonly seen in the granulomas. Jones-Williams (9) distinguished between resting and activated lymphocytes, the latter having more cytoplasm and a greater number of organelles. He suggested a transition between lymphocytes and epithelioid cells; this theory was subsequently refuted (12). Lymphocytes with strongly convoluted nuclei have been found in the peripheral blood of sarcoid patients (5).

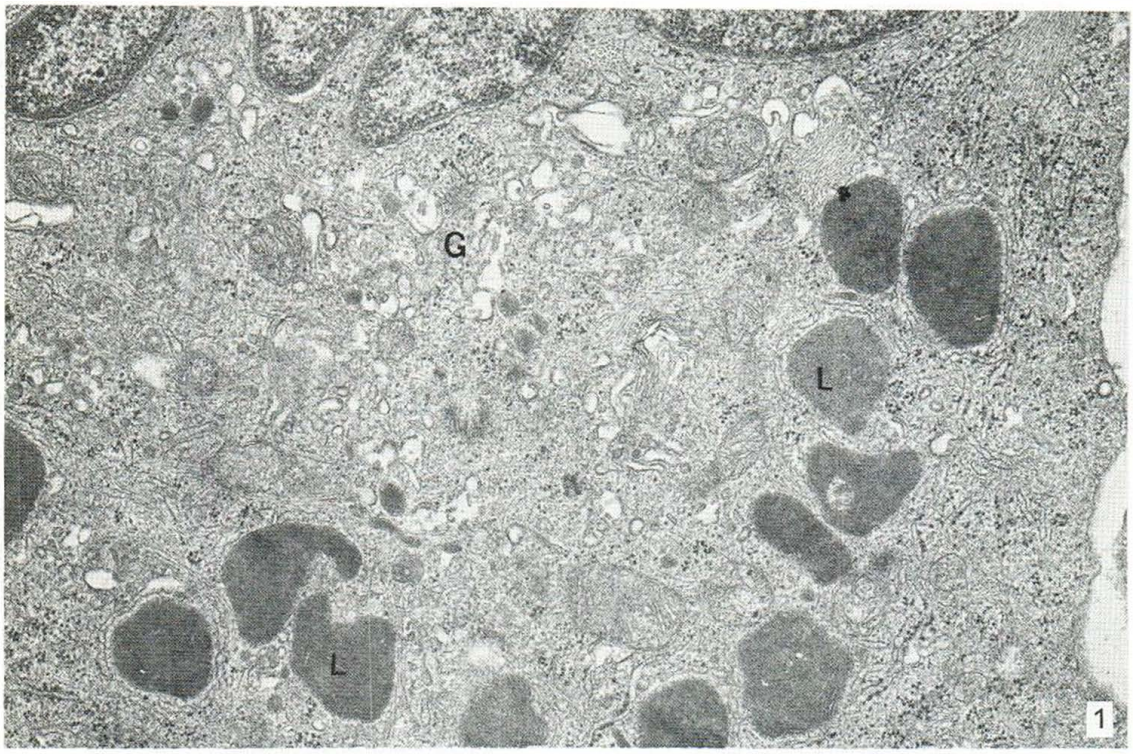
Sarcoidosis appears to be associated with certain immunological features, such as the formation of immune complexes (7). Immunoglobulin deposits have also been seen in the vessel walls of cutaneous sarcoid granulomas (11).

The aims of the present investigation were 1) to describe the fine structure of cutaneous sarcoid granulomas in a clinically active stage, 2) to look for atypical lymphocytes with convoluted nuclei in the granulomas, 3) to look for variations in the blood vessels of the granulomas, and 4) to determine whether atypical lymphocytes (Lutzner-type lymphocytes) could be identified in the peripheral blood and whether this type of lymphocyte can develop from the lymphocytes of the patient's peripheral blood after non-specific mitogenic stimulation.

PATIENTS AND METHODS

Five patients with multisystem sarcoidosis defined by the presence of epithelioid cell granulomas in more than one organ system and a clinical picture consistent with sarcoidosis participated in this study. All the patients had histologically verified sarcoid infiltrates in the skin with a duration from 2 months to 2 years. Four mm punch biopsies were taken from active cutaneous lesions of each patient.

Fifty ml samples of peripheral blood taken from 2 of the patients and 2 healthy controls were mixed with equal volumes of RPMI-1640 medium with 480 000 units penicillin, 0.5 g streptomycin, 50 mmol hepes, 1.2 mmol glutamine and 20 000 units heparin per litre and layered on Lymphoprep (Nyco). The mononuclear cells were isolated in a centrifuge at 2 000 rpm for 30 min. For each patient and each control, a culture of 10^5 cells in 0.5 ml of the above medium, to which 50 μl of phytohemagglutinin (Difco) 400 μg/ml had been added, was incubated for 3 days at 37°C in humidified air with 5% CO_2. Triplicate



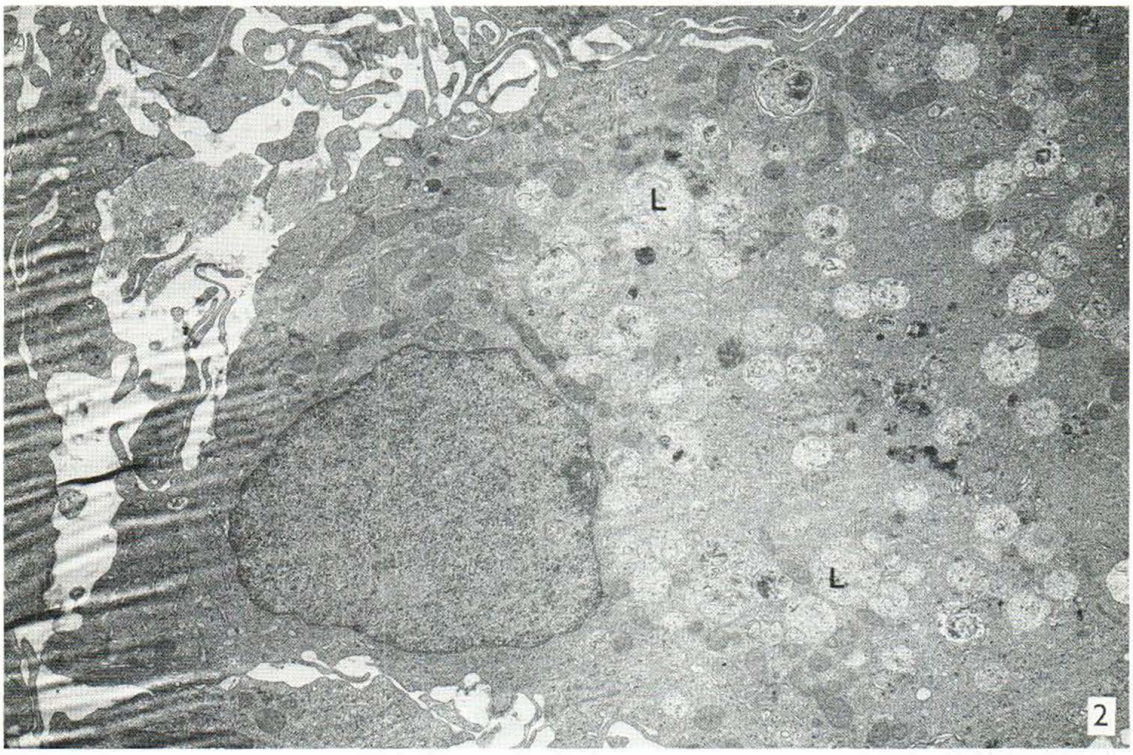


Fig. 1. Epithelioid cells with dense primary lysosomes (*L*) and widened Golgi area (*G*). ×22 500.

Fig. 2. Epithelioid cells with secondary lysosomes (*L*). ×45 000.

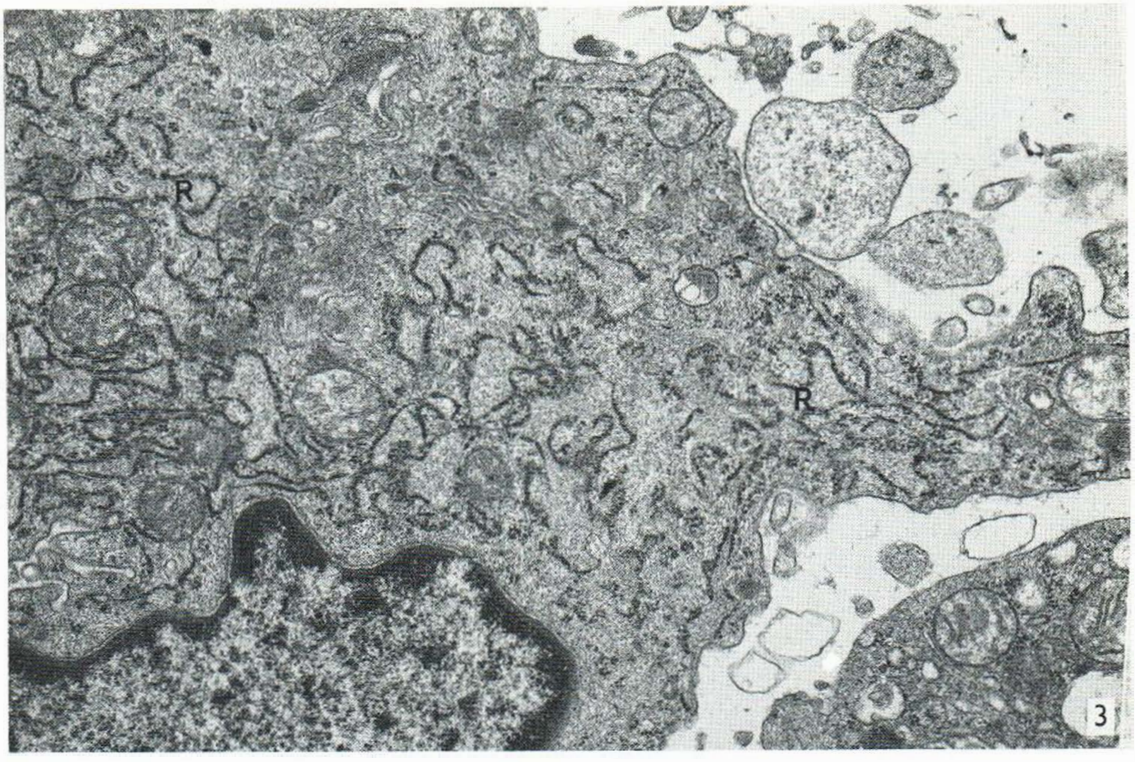


Fig. 3. Epithelioid cells with abundant granular endoplasmic reticulum (*R*). ×22 500.

unstimulated cultures were used for control. After the culture period the triplicates were pooled.

The biopsy specimens and the incubated lymphocytes were fixed in 6% and 3% glutaraldehyde solution in cacodylate buffer, pH 7.2, with 7.5% sucrose. After post-osmification and dehydration, the materials were embedded in Epon 812, and ultrathin sections were prepared. The ultrathin sections were stained with uranyl acetate and lead citrate. A Siemens electron microscope (Elmiskop IA) was operated at 80 kV.

RESULTS

The majority of the infiltrating cells were epithelioid cells. Three types could be distinguished. The first type, which occurred infrequently, was characterized by dense primary lysosomes in the cytoplasm (Fig. 1). The second type, seen more frequently than the first, had numerous lightly stained vesicles thought to be secondary lysosomes

Table I. *Distribution of cells (% of approximately 200 cells) in granulomas from each of 5 patients with sarcoidosis*

Types of cells	1	2	3	4	5	Mean
Lymphocytes	9	9	13	3.3	2.2	8
Macrophages	22	31	27.4	30	25.3	27
Atypical lymphocytes	6	7.6	7	3.3	1	5
Epithelioid cells						
(*a*) with primary lysosomes	6	1.5	1	1.7	2.2	3
(*b*) with secondary lysosomes	16	12	11.7	7.5	8.8	11
(*c*) with endoplasmic reticulum	36	34	37.5	51.5	58	43
Giant cells	1	0.6	1	1.7	2.2	1.2
Unclassified macrophage-like cells	4	4	1	0	0	1.8

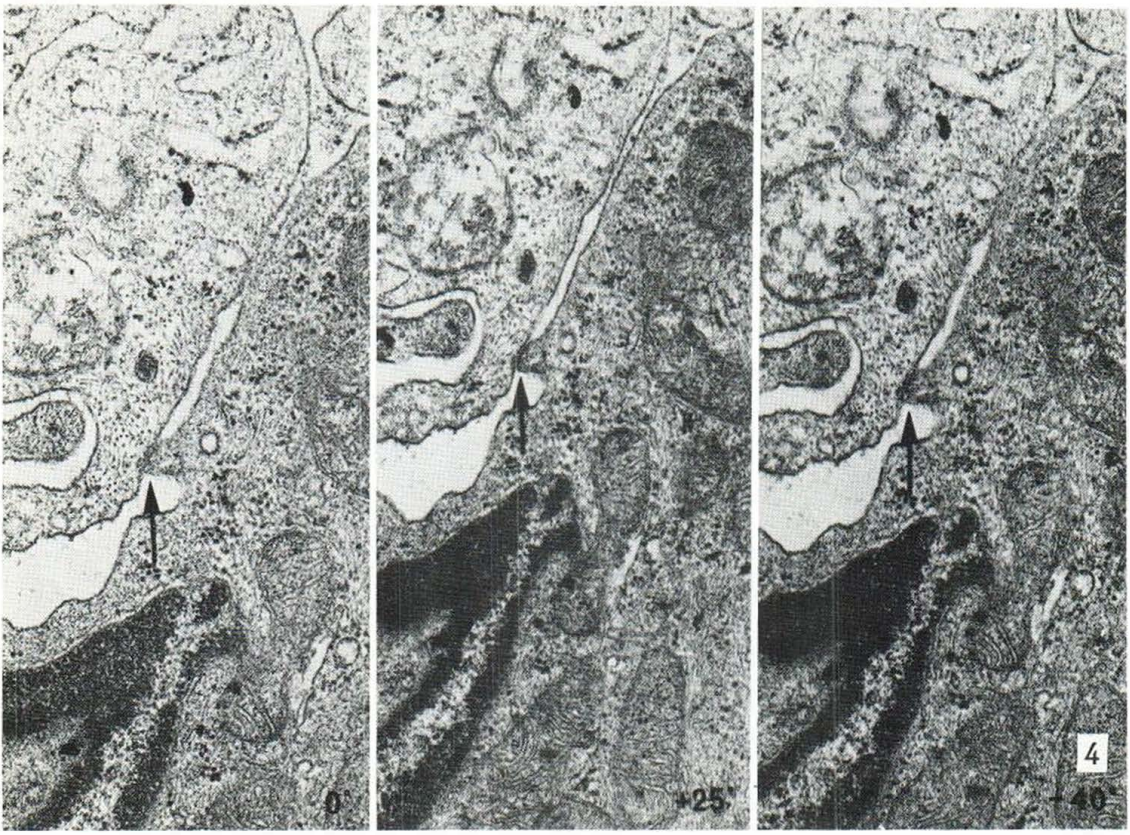


Fig. 4. The contact areas of an atypical lymphocyte and an epithelioid cell. Definite cell membrane was always found when the ultrathin sections were tilted. The arrows point toward an area of contact. The angles of tilting appear in the lower right corner. ×30000.

(Fig. 2), while the third was characterized by the presence of a well-developed granular endoplasmic reticulum with numerous ribosomes (Fig. 3).

A few very large multinucleated giant cells were seen. The nuclei had the appearance of epithelioid cells, and the cytoplasm had epithelioid cell features with many secondary lysosomes and endoplasmic reticulum.

Macrophages were frequently seen, often in close contact with epithelioid cells. Cytoplasmic protrusions were seen at the contact sites. There was similar contact between lymphocytes and epithelioid cells. A sharply outlined plasma membrane could be seen on neighbouring cells when the grid was tilted (Fig. 4). No fusion of cells was seen.

Not all of the cells with a macrophage/epithelioid cell appearance could be readily classified into one of the above-mentioned types (Table I).

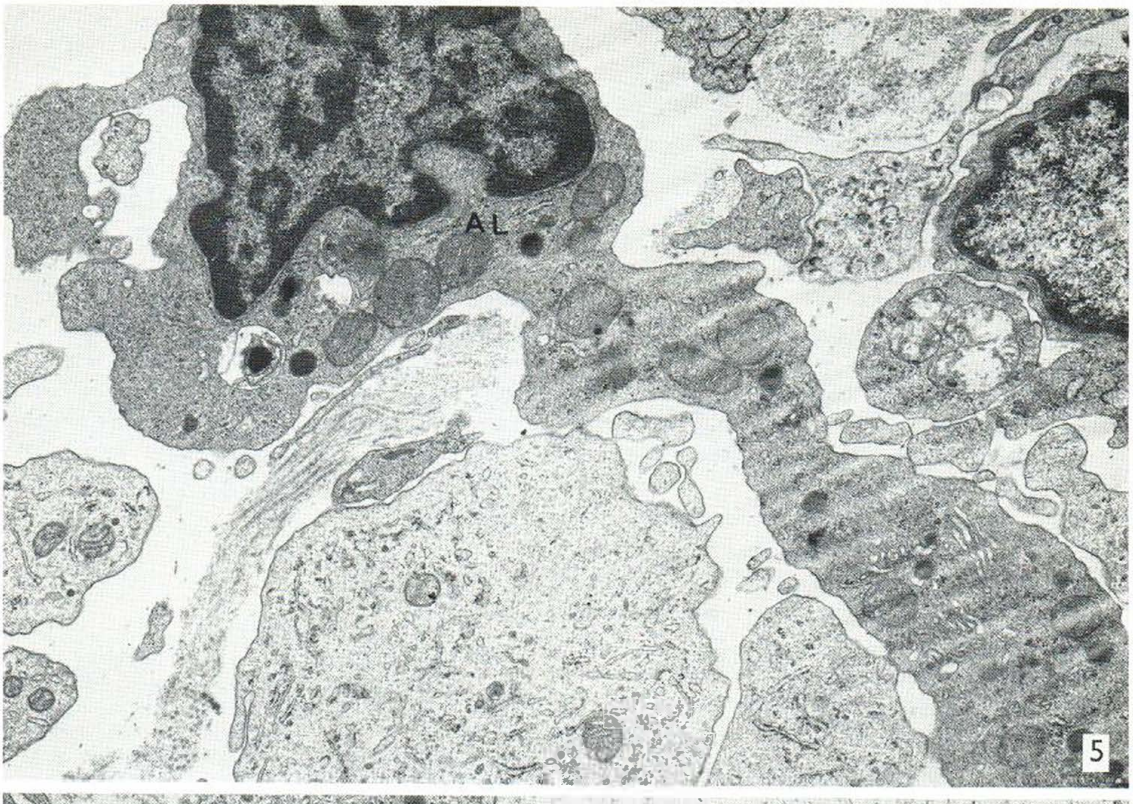
Atypical lymphocytes characterized by a nucleus having dense chromatin and numerous deep indentations, sparse granular endoplasmic reticulum, a few lysosomes and mitochondria were seen in several areas of the granulomas (Fig. 5).

Approximately 200 cells from various parts of the granulomas of each biopsy were classified and counted to determine the frequency of occurrence of the various cells (Table I).

The distribution of the cell types varied slightly from patient to patient. There was a predominance

Fig. 5. Atypical lymphocyte (*AL*). The lymphocyte shows a strongly convoluted nucleus, irregular shape of the cytoplasm with sparse granular endoplasmic reticulum, and a small number of mitochondria and lysosomes. The lymphocyte is in contact with the surrounding cells. ×6750.

Fig. 6. Vascular endothelial cells displaying a tortuous surface. ×22 500.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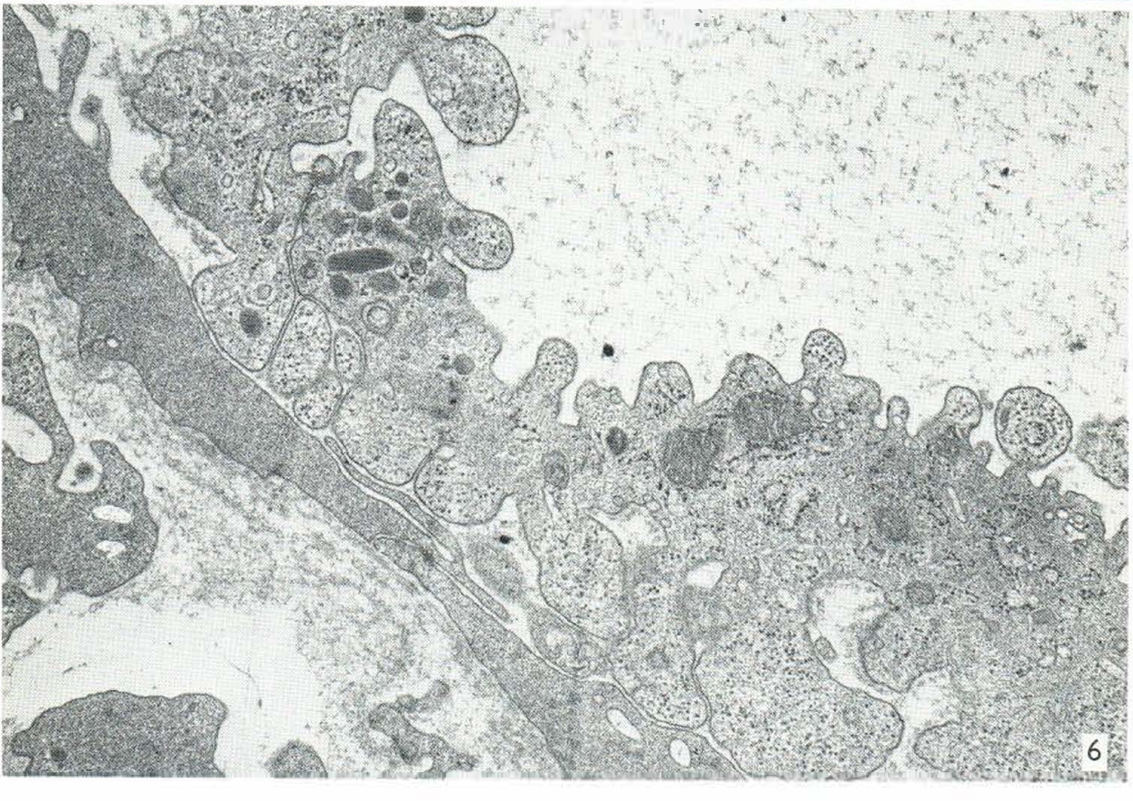
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of epithelioid cells with well-developed endoplasmic reticulum; macrophages were the second commonest cell type. Epithelioid cells with many secondary lysosomes were also frequently seen, while typical as well as atypical lymphocytes and epithelioid cells with primary lysosomes were less frequent; there were few giant cells.

Examination of a number of blood vessels in the granulomas showed most of these to have normal endothelial cells and a normal basal lamina. In a few vessels, however, the endothelial cells had a convoluted luminal surface, and gaps were seen between neighbouring endothelial cells. All the endothelial cells appeared to be viable. In some areas the basal lamina was thickened but showed no other ultrastructural alteration (Fig. 6).

The lymphocytes from the peripheral blood of patients as well as controls appeared to be normal and mature prior to culture with phytohemagglutinin. No atypical lymphocytes were seen among a count of approximately 200 cells from patients and controls before or after culture with phytohemagglutinin. After culture with phytohemagglutinin, the cells from patients as well as controls increased in size. They had an abundant pale cytoplasm containing few organelles, and nuclei with scarce chromatin.

DISCUSSION

The results presented here suggest that dermal sarcoid granulomas have the same ultrastructural appearance as the granulomas in lymph nodes and spleen.

The major cell component is an epithelioid cell rich in granular endoplasmic reticulum, which finding supports the theory that synthetic rather than phagocytic properties are characteristic of sarcoid granulomas. Atypical lymphocytes in the granulomas have not been described previously. Hedfors (6) found similar cells in the peripheral blood of sarcoidosis patients. She suggested that they represented specifically sensitized cells, while morphologically identical cells in patients with mycosis fungoides are considered to be malignant cells probably derived from T-lymphocytes (14). The location of these cells in the granulomas did not give any clue as to their function. This morphology of cells did not appear following non-specific mitogenic stimulation of mononuclear cells from the peripheral blood. Jones-Williams (9) did not mention it in his study of peripheral blood lymphocytes from tuberculin-positive sarcoid patients. Nor were the cells described by Douglas (3) who cultured lymphocytes from healthy individuals with pokeweed mitogen.

The study of blood vessels did not elicit any definite evidence of immunological damage to the vessel walls, as would be expected if immune complex formation were to play an essential part in the pathogenesis of sarcoidosis (7, 11). Some endothelial cells had a convoluted luminal surface, and intercellular cohesion was interrupted in a few instances, though the cells appeared to be viable and the overall structure of the vessels was normal. Cellular abnormalities such as those seen in the vascular walls by Basset et al. (1, 2) were not found in this study.

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