



THE UNIVERSITY OF
SYDNEY

COPYRIGHT AND USE OF THIS THESIS

This thesis must be used in accordance with the provisions of the Copyright Act 1968.

Reproduction of material protected by copyright may be an infringement of copyright and copyright owners may be entitled to take legal action against persons who infringe their copyright.

Section 51 (2) of the Copyright Act permits an authorized officer of a university library or archives to provide a copy (by communication or otherwise) of an unpublished thesis kept in the library or archives, to a person who satisfies the authorized officer that he or she requires the reproduction for the purposes of research or study.

The Copyright Act grants the creator of a work a number of moral rights, specifically the right of attribution, the right against false attribution and the right of integrity.

You may infringe the author's moral rights if you:

- fail to acknowledge the author of this thesis if you quote sections from the work
- attribute this thesis to another author
- subject this thesis to derogatory treatment which may prejudice the author's reputation

For further information contact the University's Copyright Service.

sydney.edu.au/copyright

**AN IMMUNOHISTOCHEMICAL AND
ULTRASTRUCTURAL STUDY OF THE
GIANT CELL GRANULOMA OF THE JAWS**

LYDIA LIM, BDS (Hons) FRACDS

**A thesis submitted in partial fulfilment
of the requirements for the degree of**

MASTER OF DENTAL SCIENCE

Department of Oral and Maxillofacial Surgery

University of Sydney

1993

UNIVERSITY OF SYDNEY DENTAL LIBRARY

ABSTRACT

A panel of 5 immunohistochemical markers including MB1, Leukocyte Common Antigen, S-100 Protein, Smooth Muscle Specific Actin and Factor VIII Related Antigen were used to study 20 giant cell lesions. These included 8 central giant cell granulomas, 9 peripheral giant cell granulomas and 3 giant cell tumours of bone.

The multinucleated giant cells stained positively with MB1, the mono-nuclear round cells were positive to Leukocyte Common Antigen and the spindle-cells were unreactive to all the markers chosen in all the lesions.

The most interesting finding was the staining pattern of the blood vessels to Factor VIII Related Antigen in the giant cell granuloma. The blood vessels on the periphery of the lesions were strongly positive for this antibody. However, reaction product was not evident deeper in the lesion within the aggregations of giant cells. Two other endothelial cell markers, Ulex Europae 1 Lectin and QBend 10 were used to study 10 giant cell lesions and a similar pattern of staining was observed.

Transmission electron-microscopy was subsequently used to study the ultrastructure of the microvasculature of 3 peripheral giant cell granulomas. The findings indicated that the reasons for the differential staining may be in the differences in the structure of the micro-circulation.

STATEMENT OF AUTHORSHIP

The experimental work described in this thesis was undertaken at the Institute of Clinical Pathology and Medical Research (I.C.P.M.R), Westmead Hospital. The technical and laboratory procedures in this study were performed by the author unless otherwise stated. The sectioning and processing of tissue sections for immunohistochemistry and the immunoperoxidase staining procedures were performed with the assistance of Ms Jane Milliken. The processing of tissue sections for transmission electron-microscopy was undertaken by the staff of the Department of Electron-Microscopy. The ultrastructural analysis was performed under the guidance of Dr John Gibbins and with the assistance of Mr Ross Boadle.

To the best of my knowledge this work has not been submitted in part or whole for any other degree, and the immunohistochemical and ultrastructural findings described with respect to the micro-vasculature has not been previously published.

Lydia Lim

ACKNOWLEDGEMENTS

This study was financially supported by a grant from the Research Committee of the Faculty of Dentistry, University of Sydney.

I wish to thank my supervisor, Dr John Gibbins (Reader, Department of Pathology, University of Sydney) for his invaluable guidance and support, and especially for the education that I have received through working with him on this project.

I would like to thank Drs G. McKellar, M. Hanan, A. Collins (Oral and Maxillofacial Surgeons, Department of Oral and Maxillofacial Surgery, Westmead Hospital) for the permission to use their clinical material and surgical specimens in this study. Many thanks to Prof. J. E. deB Norman (Oral and Maxillofacial Surgeon, St George Hospital), Dr B. Austin (Oral and Maxillofacial Surgeon, Sydney) and the Department of Orthopaedics, Westmead Hospital for their donation and contribution of surgical specimens.

I am grateful to Dr Michael Bilous (Head, Department of Anatomical Pathology, Westmead Hospital) for his generosity with respect to the use of laboratory facilities. Special thanks to Ms Jane Milliken (Department of Histochemistry, Westmead Hospital) for her guidance and hospitality throughout the technical phase of the project. I am appreciative of the assistance I received from Mr Ross Boadle (Department of Electron-Microscopy, Westmead Hospital) and Ms Mara Cvejic (Research Support Unit, Westmead Dental Clinical School). I am grateful to Ms Jacqueline Siu for undertaking the tedious task of proof-reading this thesis.

TABLE OF CONTENTS

CHAPTER 1

GIANT CELL LESIONS

1.1	The Central Giant Cell Granuloma	1
1.1.1	Clinical Features	1
1.1.2	Radiographic Features.....	1
1.1.3	Pathology	2
1.1.4	Differential Diagnosis	3
1.1.5	Management.....	3
1.2	The Peripheral Giant Cell Granuloma	4
1.2.1	Clinical Features	4
1.2.2	Pathology	5
1.2.3	Management.....	5
1.3	The Giant Cell Tumour of Bone	6
1.3.1	Clinical Features	6
1.3.2	Radiographic Features.....	6
1.3.3	Pathology	7
1.3.4	Management.....	7

CHAPTER 2
CONTROVERSIES AND CURRENT RESEARCH

2.1 The Relationship of the Giant Cell Granuloma of the Jaws with the Giant Cell Tumour of Bone.....12

2.2 The Search for Histological Parameters to Predict Biological Behaviour 15

2.3 The Origin and Nature of the Giant Cells 18

 2.3.1 Macrophages 18

 2.3.2 Fibroblasts, Myofibroblasts 20

 2.3.3 Endothelial Cells, Pericytes 21

 2.3.4 Osteoclasts 23

2.4 The Identification of the Principal "Tumour" Cell 25

CHAPTER 3
FUNDAMENTALS OF IMMUNOHISTOCHEMISTRY

3.1 Antigens and Antibodies.....27

 3.1.1 Polyclonal Antibodies 28

 3.1.2 Monoclonal Antibodies 28

3.2 Immunoperoxidase Methods.....29

 3.2.1 Conjugate Methods 30

 3.2.2 Non-Conjugate Methods 31

 3.2.4 Inhibition of Endogenous Peroxidase Activity 31

3.2.5	Proteolytic Enzyme Pretreatment.....	32
3.2.6	Detection Agents	32
3.2.7	Controls	33

CHAPTER 4

THE ANTIBODIES

4.1	Leukocyte Common Antigen.....	35
4.2	MB1	37
4.3	S-100 Protein	38
4.4	Smooth Muscle Specific Actin.....	39
4.5	Factor VIII Related Antigen	41

CHAPTER 5

THE STUDY (PART 1)

5.1	AIMS	44
5.2	Materials and Methods	45
5.2.1	Tissue specimens.....	45
5.2.2	Clinical Data	46
5.2.3	Immunoperoxidase Procedure.....	48
5.2.4	Tissue Fixation and Preparation.....	48
5.2.5	De-waxing.....	48

5.2.6	Buffer	48
5.2.7	Primary Antibody.....	48
5.2.8	Enzymatic Pre-treatment.....	49
5.2.9	Rinsing	49
5.2.10	Inhibition of Endogenous Peroxidase	49
5.2.11	Secondary Antibody.....	50
5.2.12	Tertiary Antibody.....	50
5.2.13	Chromogen-Substrate Solution	50
5.2.14	Counterstaining and Mounting.....	51
5.2.15	Controls	51
5.2.16	Analysis.....	51

CHAPTER 6

RESULTS (PART 1)

6.1	Leukocyte Common Antigen.....	53
6.2	MB1	53
6.3	Smooth Muscle Specific Actin.....	54
6.4	S-100 Protein	54

CHAPTER 7

DISCUSSION (PART 1)

7.1 Comparison of the Staining Pattern between the Peripheral and Central Giant Cell Granuloma and the Giant Cell Tumour of Bone.....64

7.2 Leukocyte Common Antigen.....64

7.3 MB1 67

7.4 S-100 Protein 68

7.6 Smooth Muscle Specific Actin.....69

7.7 Factor VIII Related Antigen 71

CHAPTER 8

THE STUDY (PART 2)

8.1 Introduction 74

8.2 Aims.....75

8.3 Materials and Methods 76

8.3.1 Immunohistochemistry 76

8.3.1.2 Tissue specimens 76

8.3.1.3 Endothelial Cell Markers.....76

8.3.1.4 Immunoperoxidase Procedure 76

8.3.1.5 Controls.....77

8.3.2 Transmission Electron-Microscopy 78

8.3.2.1 Tissue Specimens.....78

8.3.2.2 Fixation and Embedding.....78

8.3.2.3 Preparation of Semi-Thin Sections 79

8.3.2.4 Sectioning for Transmission Electron-Microscopy 79

8.3.2.5 Counter-Staining 79

8.3.2.6 Analysis 80

CHAPTER 9

RESULTS (PART 2)

9.1 Immunohistochemistry 81

9.2 Transmission Electron-Microscopy 81

CHAPTER 10

DISCUSSION (PART 2)

10.1 Immunohistochemistry: Ulex Europaes 1 Lectin and QBend10 93

10.2 Transmission Electron-Microscopy 94

SUMMARY AND CONCLUSIONS 97

REFERENCES.....98

LIST OF FIGURES

Figure 1.1 Orthopantomogram illustrating the radiographic features of a central giant cell granuloma of the mandible	9
Figure 1.2 Colour photograph illustrating the clinical appearance of the peripheral giant cell granuloma	9
Figure 1.3 Histomicrograph illustrating the characteristic histological appearance of the giant cell granuloma of the jaws including the presence of multinucleated giant cells, mononuclear round cells, spindle-shaped cells and areas of haemorrhage	10
Figure 1.4 Histomicrograph illustrating the histological appearance of the giant cell tumour of bone.....	10
Figure 6.1 Histomicrograph illustrating the positive immunoperoxidase staining of the round mononuclear cells to Leukocyte Common Antigen	56
Figure 6.2 Histomicrograph illustrating the positive immunoperoxidase staining of the round mononuclear cells (some in the process of margination) to Leukocyte Common Antigen	56
Figure 6.3 Histomicrograph illustrating the positive immunoperoxidase staining of multinucleated giant cells to MB1	57
Figure 6.4 Histomicrograph illustrating the positive immunoperoxidase staining of multinucleated giant cells to MB1	57
Figure 6.5 Histomicrograph showing the negative immunoperoxidase staining of the stromal spindle-shaped cells to Smooth Muscle Specific Actin	58
Figure 6.6 Histomicrograph illustrating the positive immunoperoxidase staining of smooth muscle cells in the walls of blood vessels to Smooth Muscle Specific Actin which served as a positive control	58
Figure 6.7 Histomicrograph illustrating the negative immunoperoxidase staining of the giant cells and the stromal cells to S-100 Protein	59

Figure 6.8 Histomicrograph of the positive control for S-100 Protein illustrating the immunoperoxidase staining of the myoepithelial cells of salivary gland tissue. The ductal epithelial cells also show positive staining for S-100 Protein 59

Figure 6.9 Histomicrograph illustrating the differential immunoperoxidase staining of blood vessels in the giant cell granuloma. Intense staining of the blood vessels on the periphery of the lesion is demonstrated with little evidence of reaction product deeper within the lesion adjacent to the aggregations of the giant cells 60

Figure 6.10 Histomicrograph illustrating heavy uptake of Factor VIII Related Antigen in the blood vessels on the periphery of the lesion with progressively less evidence of staining deeper within the lesion towards the giant cell aggregations 60

Figure 6.11 Histomicrograph illustrating the intense immunoperoxidase staining of the blood vessels on the periphery of the lesion to Factor VIII Related Antigen 61

Figure 6.12 Histomicrograph illustrating the differential immunoperoxidase staining of the blood vessels to Factor VIII Related Antigen 61

Figure 6.13 Histomicrograph illustrating the differential immunoperoxidase staining of the blood vessels to Factor VIII Related Antigen 62

Figure 6.14 Histomicrograph illustrating the differential immunoperoxidase staining of the blood vessels to Factor VIII Related Antigen 62

Figure 6.15 Histomicrograph illustrating the differential immunoperoxidase staining of the blood vessels to Factor VIII Related Antigen 63

Figure 6.16 Histomicrograph illustrating the differential immunoperoxidase staining of the blood vessels to Factor VIII Related Antigen 63

Figure 9.1.1 Histomicrograph illustrating differential reactivity of the blood vessels to Ulex Europae 1 Lectin showing the positive staining of

the blood vessels on the periphery of the lesion while in the deeper areas of the lesion closer to the giant cell aggregations minimal reaction product was evident. The positive staining of the epithelial cells is also illustrated 83

Figure 9.1.2 Histomicrograph showing the gradual transition of reactivity of the blood vessels to Ulex Europaes 1 Lectin 83

Figure 9.1.3 Histomicrograph illustrating the intense staining of blood vessels (and epithelial cells) on the periphery of the lesion to Ulex Europaes 1 Lectin 84

Figure 9.1.4 Histomicrograph of the deeper areas of the lesion illustrating the negative staining of the blood vessels to Ulex Europaes 1 Lectin 84

Figure 9.1.5 Histomicrograph illustrating the intense staining of blood vessels on the periphery of the lesion to QBend 10 85

Figure 9.1.6 Histomicrograph illustrating the intense staining of blood vessels on the periphery of the lesion to QBend 10 85

Figure 9.1.7 Histomicrograph of the deeper areas of the lesion illustrating the negative staining of the blood vessels adjacent to the giant cell aggregations to QBend 10 86

Figure 9.1.8 Histomicrograph of the deeper areas of the lesion illustrating the negative staining of the blood vessels adjacent to the giant cell aggregations to QBend 10 86

Figure 9.2.1 Transmission electron-micrograph of blood vessels on the periphery of the lesion exhibiting high endothelial cells with well developed intercellular junctions 87

Figure 9.2.2 Transmission electron-micrograph of the peripheral areas of the lesion illustrating endothelial cells associated with inflammatory cells 87

Figure 9.2.3 Transmission electron-micrograph of endothelial cells on the periphery of the lesion illustrating the abundance of inflammatory cells within the lumen of the vessel 88

Figure 9.2.4 Transmission electron-micrograph illustrating the blood vessels on the periphery of the lesion with an abundance of inflammatory cells and a marginating macrophage 88

Figure 9.2.5 Transmission electron-micrograph of endothelial cells on the periphery of the lesion with an inflammatory cell in the process of diapedesis 89

Figure 9.2.6 Transmission electron-micrograph of an endothelial cell on the periphery of the lesion illustrating "splitting" or reduplication of the basal lamina 89

Figure 9.2.7 Transmission electron-micrograph illustrating the deeper areas of the lesion where structurally complete blood vessels could not be identified 90

Figure 9.2.8 Transmission electron-micrograph illustrating the deeper areas of the lesion where structurally complete blood vessels could not be identified 90

Figure 9.2.9 Transmission electron-micrograph illustrating the characteristic finding in the deeper areas of the lesion where areas with abundant erythrocytes were observed but normal endothelial structure could not be identified 91

Figure 9.2.10 Transmission electron-micrograph illustrating the characteristic finding in the deeper areas of the lesion where areas with abundant erythrocytes were observed but normal endothelial structure could not be identified 91

Figure 9.2.11 Transmission electron-micrograph illustrating erythrocytes in rouleaux formation but with no evidence of adjacent endothelial cell lining 92

Figure 9.2.12 Transmission electron-micrograph illustrating erythrocytes in rouleaux formation but with no evidence of adjacent endothelial cell lining 92

LIST OF TABLES

Table 5.1	Clinical Data	47
Table 6.1	Summary of Results	55

CHAPTER 1

GIANT CELL LESIONS

This chapter provides an overview of the clinical and histological features of the giant cell granuloma of the jaws and the giant cell tumour of bone.

1.1 The Central Giant Cell Granuloma

1.1.1 Clinical Features

The central giant cell granuloma occurs mainly in adolescents and young adults with a higher incidence in females. It is known to occur more commonly in the mandible than in the maxilla (Austin *et al* , 1959; Waldron and Shafer, 1966)

Clinically, it presents as a swelling and is only occasionally accompanied by pain. The clinical behaviour varies and is known to range from rapidly growing and aggressive lesions associated with pain, root resorption, bone destruction and high recurrence rates, to slow growing asymptomatic radiolucencies (Ficarra *et al*, 1987). Recurrence rates of the lesion are reported to be in the vicinity of 13% (Andersen *et al*, 1973)

1.1.2 Radiographic Features

Radiographically, the lesion appears as either a unilocular or multilocular radiolucent lesion (Figure 1.1). It may be well or ill-defined, and variable degrees of trabeculation may be observed. The cortex is often expanded and thinned but is

rarely perforated. Cohen *et al* (Cohen and Hertzanu, 1988) studied 16 cases of central giant cell granuloma radiographically and found that in a significant number of cases there was splaying of the roots of teeth adjacent to the lesion.

1.1.3 Pathology

Macroscopically, the lesion forms a vascular reddish-brown fleshy mass. The cortex is often thinned and occasionally can be expanded and perforated.

Histologically, the lesions are characterised by the presence of numerous multinucleated giant cells. The giant cells vary in their distribution and are surrounded by a vascular stroma consisting of plump spindle-shaped cells and round mononuclear cells. Areas of haemorrhage are frequently present with the occasional presence of newly formed bone (Adkins *et al*, 1969b; Andersen *et al*, 1973; Katsikeris *et al*, 1988; Waldron and Shafer, 1966). The giant cells are the most conspicuous component of the lesion and are known to be irregularly distributed with a tendency to form clusters. The number of nuclei in the giant cells varies from three to a hundred and mitoses are rare. The giant cells have been observed to be lying in close proximity to the blood vessels in the lesion (Chuong and Kaban, 1986).

The spindle-shaped cells are numerous and have pale to eosinophilic cytoplasm with oval shaped or round nuclei. They have been noted to resemble fibroblasts and ultrastructural studies have confirmed the presence of intracytoplasmic myofilaments with electron dense bodies similar to those of smooth muscle cells. Consequently, it has been proposed that they may be myofibroblasts (El-Labban and Lee, 1983).

The round mononuclear cells have intensely staining basophilic nuclei and resemble macrophages (El-Mofty and Osdoby, 1985; Regezi *et al*, 1987). Figure 1.3 illustrates the histological appearance of the lesion.

1.1.4 Differential Diagnosis

In the diagnosis of the central giant cell granuloma, the possibility of hyperparathyroidism should always be excluded as the histological features and clinical presentation of these lesions are difficult, if not impossible, to distinguish. Serology reveals an elevated serum calcium level in hyperparathyroidism. Parathyroid hormone assay is also a useful adjunctive test to confirm the presence of hyperparathyroidism.

The pathogenesis of the "brown tumour" of hyperparathyroidism is suggested to be a result of active bone resorption and replacement with fibrous tissue. Haemorrhage into the scar tissue and its discolouration by blood and haemosiderin results in the characteristic brown colour, and the appearance of multinucleate giant cells completes the resemblance to the giant cell granuloma (Jaffe, 1953a).

Other lesions where multinucleated giant cells may be present include fibrous dysplasia, cherubism and the aneurysmal bone cyst. Fibrous dysplasia and cherubism have distinctive clinical, radiographic and histological features that assist in distinguishing these conditions from the giant cell granuloma. The pathogenesis of the aneurysmal bone cyst is uncertain and there is some doubt as to whether it should be considered as a separate pathological entity or rather a variant of the giant cell granuloma (Bhaskar *et al*, 1958). The distinctive histological features observed in this lesion are the presence of numerous capillaries and large inter-communicating blood-filled spaces.

1.1.5 Management

The treatment of the central giant cell granuloma is generally by curettage and occasionally by excision. Most lesions are asymptomatic and slow-growing and these have a low rate of recurrence with curettage. The management of the more

aggressively behaving lesions has been a subject of some debate. Some have advocated surgical excision in clinically aggressive lesions due to the high incidence of recurrence (Chuong *et al*, 1986; Cohen, 1988; Thompson *et al*, 1983) while others believe that these lesions should always be managed conservatively since they are essentially benign and reparative in nature. Radiotherapy is contraindicated due to the possibility of post-radiation sarcoma.

1.2 The Peripheral Giant Cell Granuloma

The peripheral giant cell granuloma, also known as the "giant cell epulis", was described by Bernier and Cahn in 1954. It is not an uncommon lesion of the oral tissues and is regarded as the soft tissue variant of the central giant cell granuloma.

1.2.1 Clinical Features

They usually appear as sessile or pedunculated bluish-red swellings arising from the gingiva (Figure 1.2). They occur more frequently in young to middle aged adults and have a predilection for females in the ratio of 2:1 (Giansanti and Waldron, 1969). The preferred site of occurrence would appear to be the premolar and molar regions in the mandible (Katsikeris *et al*, 1988).

The recurrence rate has been reported at approximately 10%. Katsikeras *et al* (1988) found a 9.8% recurrence rate, Bhaskar *et al* (1971) 12% and Giansanti and Waldron (1969) reported a rate of 4.9%.

The aetiology of the lesion is unknown although some have suggested that trauma may be a predisposing factor. However, in the series of 224 cases by Katsikeras *et al* (1988) a history of previous extraction was found in only 8% of cases. Cooke (1952) suggested that these lesions were the result of the proliferation of cells able to form

multinucleated cells with osteoclastic function. He proposed that the area anterior to the molars were mainly prone to be affected since the transition of deciduous to permanent dentition occurred in this area. However, osteoclasts have now been shown to originate from haemopoietic stem cells rather than from local progenitor cells (Gothlin and Ericsson, 1972) and the high incidence of lesions in the area of permanent molars (Katsikeris et al, 1988) renders this hypothesis questionable.

1.2.2 Pathology

Macroscopically, the lesion is deep red in appearance and soft in consistency. The cut surface is reddish-brown and may be homogenous in appearance or may exhibit a peripheral brownish zone broken by paler radiating streaks (Lucas, 1976).

Histologically, the lesion is similar in appearance to the central giant cell granuloma described previously (Figure 1.3). However, being peripheral in nature, the lesion is covered by parakeratinised epithelium.

1.2.3 Management

The lesions are treated by excision.

1.3 The Giant Cell Tumour of Bone

The giant cell tumour of bone was defined as a distinct pathological entity by Jaffe *et al* (1940), Jaffe (Jaffe, 1953b), and Lichtenstein (1950, 1951, 1953).

1.3.1 Clinical Features

Giant cell tumours generally occur in an older age group in comparison with the giant cell granuloma of the jaws. They occur principally between the ages of twenty and forty and are extremely rare in children (Jaffe, 1953b; Schajowicz, 1961). There appears to be a predilection for females. In their series of 195 cases, Dahlin *et al* (1970) reported a 59% occurrence in females.

The most common sites for giant cell tumours are the lower end of the femur, the upper end of the tibia and the lower end of the radius. Dahlin *et al* (1970) found that more than 77% of tumours were located at or near the end of a major tubular bone of the extremities.

The usual symptoms are swelling, often accompanied by pain. Egg-shell crackling may be evident in large tumours. Neurologic signs and symptoms may accompany tumours of the sacrum, vertebrae and sphenoid.

1.3.2 Radiographic Features

Radiographically, the tumours produce an expanding zone of radiolucency and thinning of the cortex with very little peri-lesional new bone formation. There may be a soap-bubble or honeycomb appearance. These appearances are not pathognomic for the giant cell tumour.

1.3.3 Pathology

Macroscopically, the tumour is a deep red mass that is soft and may be friable. Greyish-white necrotic zones may be present. Degeneration may lead to cyst formation and lipid deposits may appear as prominent yellow zones with considerable fibrosis. Blood-filled spaces may be prominent and may simulate the aneurysmal bone cyst.

Microscopically (Figure 1.4), the lesion appears similar to the giant cell granuloma described previously. Although Jaffe *et al* (1940) and Hutter *et al* (1962) have attempted to grade the tumours histologically on the basis of pleiomorphism and mitotic activity, it is now widely accepted that there is little correlation between the histologic picture and the likelihood of the lesion to recur or undergo malignant transformation (Dahlin *et al*, 1970; Schajowicz, 1961).

1.3.4 Management

In general, the tumour is locally invasive with a high recurrence rate. Dahlin *et al* (1970) found a recurrence rate of 44.6% in tumours treated surgically without complete resection or amputation and Hutter *et al* (1962) reported that only 38% of giant cell tumours in his series were cured the first time treated.

A small percentage of cases has been shown to metastasise. There are two types of the so-called malignant giant cell tumour, the benign giant cell tumour that has "converted", usually following radiotherapy; and the primary malignant giant cell tumour. The latter is said to represent a form of fibrosarcoma and has been reported to have an incidence of as high as 30% of all giant cell tumours (Hutter *et al*, 1962).

In general, the recommended treatment of the giant cell tumour is surgical excision. However, there are varying approaches to treatment and it would appear that in the

management of these lesions, each case needs to be assessed individually. Thorough curettage may be preferred if the lesions are small and confined within bone without extending into the soft tissues. Radiation therapy may be employed either as primary or adjunctive measure however, there is a high incidence of sarcomatous transformation associated with radiotherapy. Hutter *et al* (1962) reported that 70% of malignant giant cell tumours were treated by radiotherapy at some stage prior to the final diagnosis.

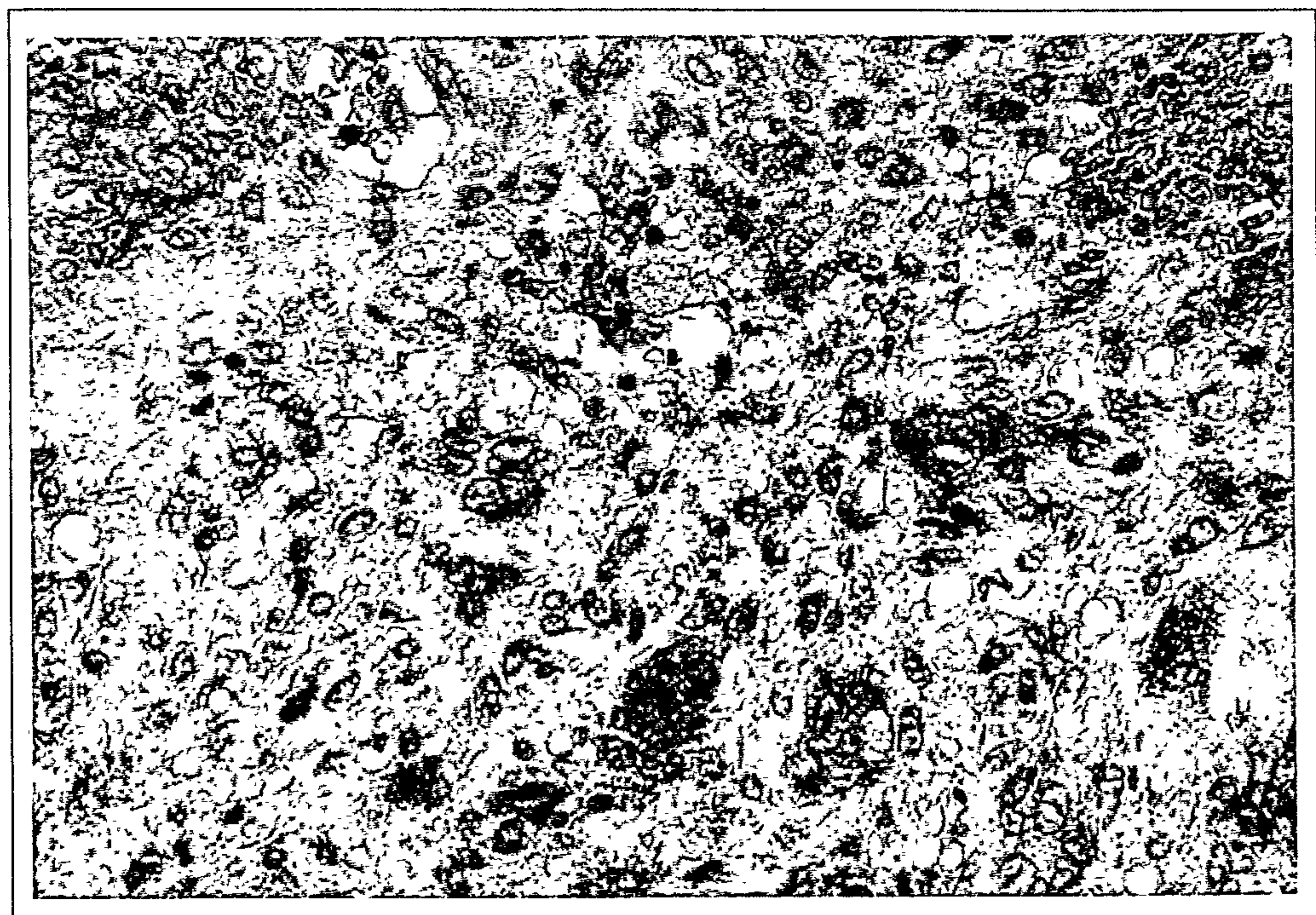
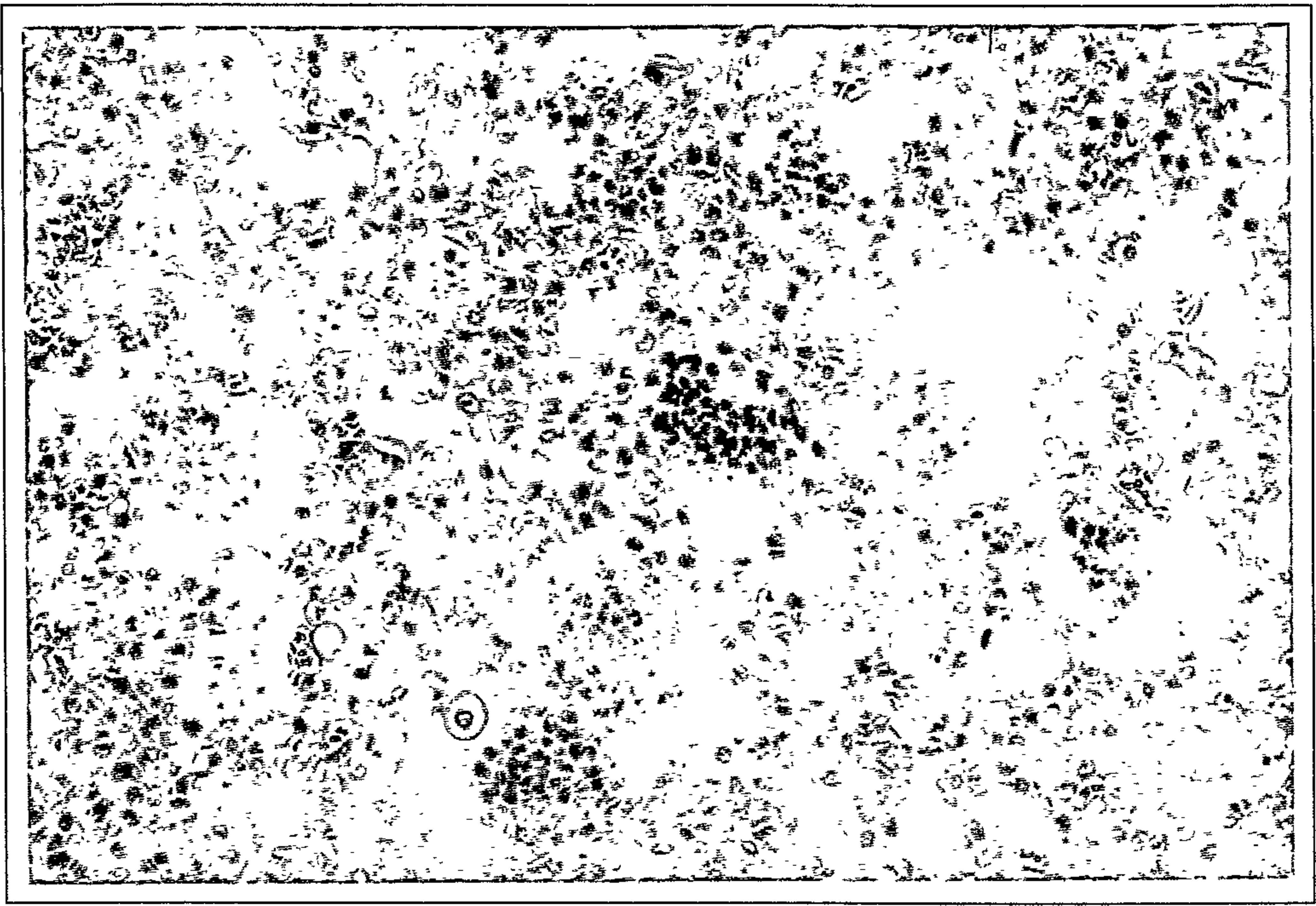
Figure 1.1 Orthopantomogram illustrating the radiographic features of a central giant cell granuloma of the mandible

Figure 1.2 Colour photograph illustrating the clinical appearance of the peripheral giant cell granuloma. Two lesions are demonstrated arising from the gingival tissues of the left mandible



Figure 1.3 Histomicrograph illustrating the characteristic histological appearance of the giant cell granuloma of the jaws including the presence of multinucleated giant cells, mononuclear round cells, spindle-shaped cells and areas of haemorrhage (X200)

Figure 1.4 Histomicrograph illustrating the histological appearance of the giant cell tumour of bone (X400)



CHAPTER 2

CONTROVERSIES AND CURRENT RESEARCH

The giant cell granuloma remains as one of the more controversial and lesser understood lesions in oral pathology.

In 1953, Jaffe (1953a) separated the giant cell granuloma of the jaws from the giant cell tumour of bone on the basis of the differences in their histological and clinical behaviour. He suggested that the giant cell granuloma was not a neoplasm but rather a reactive response to injury; hence, the term giant cell reparative granuloma that came to be widely adopted. This reparative concept has in recent times come into question as some of the giant cell lesions occurring in the jaws have been shown to behave extremely aggressively.

In fact, these lesions have been surrounded by much controversy. Does the giant cell tumour ever occur in the jaws? Why do some lesions behave more aggressively than others? Are there any histological parameters that can be used to predict biological behaviour? What is the nature and origin of the giant cells in the lesion?

A review of the current literature reveals four main areas of interest, controversy and on-going research.

1. The relationship of the giant cell granuloma of the jaws with the giant cell tumour of long bones.
2. The search for histological parameters to predicting biological behaviour.

3. The origin and nature of the giant cells.
4. The identification of the principal "tumour" cell.

The literature review will be divided into these four areas.

2.1 The Relationship of the Giant Cell Granuloma of the Jaws with the Giant Cell Tumour of Bone

Jaffe (1953b) separated the giant cell granuloma from the giant cell tumour on the basis of the perceived differences in their histological appearance and clinical behaviour. He believed that the giant cells in the giant cell granuloma represented a phagocytic response to haemorrhage and trauma, and that the giant cell tumour was extremely rare in the jaws.

Austin *et al* (1959) confirmed Jaffe's belief that the lesions were separate and re-emphasised the histological differences between the lesions. Shakler and Meyer (1961) however, believed that the giant cell tumour did occur in the jaws and was more common than had been previously thought.

Waldron and Shafer (Waldron and Shafer, 1966) reviewed 38 cases of giant cell granuloma of the jaws and agreed that these lesions were not neoplasms. They believed that they were analogous to the "benign giant cell tumour of bone". They questioned the distinction between the jaw lesions and the giant cell tumour of bone believing that "the differences in age, distribution and clinical behaviour, often cited as evidence that these processes are dissimilar, are more likely related to the anatomy of the jaws, which permits earlier diagnosis and easier surgical access than to any essential differences in biologic behaviour."

Many of the previous histological observations have been based on subjective criteria and more recently, efforts have been made to more objectively compare the giant cell granuloma with the giant cell tumour. Abrams and Shear (1974) studied 10 cases of each lesion and compared the giant cell component of the lesions. They found that the giant cells in the giant cell tumour were larger and contained more nuclei than the giant cells in the giant cell granuloma and supported the concept that the two lesions were distinct entities.

Following the work of Abrams and Shear (1974), Franklin *et al* (1979) applied stereological techniques to compare the histological parameters of the giant cell component of the giant cell tumour and giant cell granuloma. They also studied 10 cases of each lesion but also included 10 cases of peripheral giant cell granuloma. Unlike Abrams and Shear (1974), they found the giant cells in the giant cell tumour to be smaller than the giant cells in the giant cell granuloma. They found that the significant difference between the two lesions was that the nuclei of the giant cell tumour were more densely packed within cells of smaller volume.

The most recent and comprehensive comparative study was that of Auclair *et al* (1988). They compared the clinical, histologic and histomorphometric features of 42 giant cell tumours with 49 giant cell granulomas. The parameters studied were mean giant cell area, mean giant cell volume, mean giant cell form and mean giant cell nuclei. The clinical and radiographic data, including follow-up information was correlated with the quantitative assessment.

They found that although there were significant histologic differences between the lesions, there was also a significant percentage of the two lesions that were histologically similar. Their conclusions were that the two lesions were not separate entities but that they represent " a continuum of a single disease process modified by the age of the patient and the site of occurrence, and possibly by other factors."

It is interesting to note that while the clinical behaviour of the giant cell lesions in the jaws is generally more benign than those occurring in the long bones and that many believe that the giant cell tumour does not occur in the jaws, there have been several reports in the literature of "giant cell tumour" of the jaws.

Of particular significance is a case report by Mintz *et al* (1981) of a primary malignant giant cell tumour of the mandible which met the diagnostic criteria specified by Dahlin *et al* (1970) and was reported to have metastasised to the lungs and lymph nodes. The validity of the other reported cases of "malignant giant cell tumour of the jawbones" in the literature (Cones, 1953; Hamlin and Lund, 1967, Hayward, 1959) is questioned on the basis of the non-specific diagnostic criteria used.

It is evident that the controversy surrounding the relationship between the giant cell tumour and the giant cell granuloma of the jaws remains unresolved. However, the prevailing concept in the 1950's that the jaw lesions were separate and distinct from the giant cell lesions elsewhere has now shifted to a more moderate view which allows the possibility of overlap.

The findings of Auclair *et al* (1988) that there is a significant overlap of features between the two lesions appears to be consistent with the current experience of both clinicians and pathologists. The recent experience of the Department of Oral and Maxillofacial Surgery, Westmead Hospital, Sydney (1985-1992) in the management of several aggressive giant cell granulomas of the jaws has supported this belief (Table 5.4). Several lesions have been observed to behave in a manner markedly different from other histologically similar lesions and these were clinically inconsistent with the "reparative" concept proposed by Jaffe (1953).

It appears that these lesions may represent a spectrum of disease processes which histologically manifest in a similar way. Unfortunately, we have not as yet been able

to define or determine the clinical behaviour of these lesions through conventional means.

2.2 The Search for Histological Parameters to Predict Biological Behaviour

The biological behaviour of the giant cell granuloma of the jaws, in particular, the central giant cell granuloma, is known to range from quiescent and slow growing to aggressive and destructive.

There are several case reports in the literature of aggressive giant cell lesions of the jaws which have been shown to be rapidly growing and locally destructive with a high incidence of recurrence (Chuong and Kaban, 1985; Cohen, 1988; Eisenbud *et al*, 1988; Thompson *et al*, 1983). At the other end of the spectrum, there are the many slow growing giant cell granulomas presenting as asymptomatic radiolucencies on routine radiography, and which respond well to conservative curettage.

At present, there appears to be no histological correlation between the aggressive and non-aggressive lesions. Several recent studies have attempted to identify histological parameters by which clinical behaviour may be predicted and have met with little success.

Chuong *et al* (1986) retrospectively studied 17 patients with central giant cell granuloma and divided them into 2 groups, aggressive and non-aggressive, on the basis of their clinical and radiologic presentation. The parameters assessed were the fractional surface area occupied by giant cells, the size of the giant cells, the stromal characteristics, the mitotic index, the inflammatory cells present, and the haemosiderin content. Although they found the giant cells in aggressive lesions to

have a slightly higher relative surface area, they concluded that "the histologic differences between the two groups were not as clear as their biologic behaviour."

Following on from the work of Chuong *et al* (1986), Ficarra *et al* (1987) used computer-assisted image analysis to compare the same histological parameters on 22 patients divided into aggressive and non-aggressive groups. They claimed to have found "a statistically significant correlation between the number of giant cells, the fractional surface area (FSA) and the aggressiveness of the central giant cell granuloma." However, on close analysis of their results, the mean FSA of non-aggressive lesions was 0.041 (SD = 0.054) and for aggressive lesions was 0.087 (SD = 0.069), and the mean number of giant cells in non-aggressive lesions was 23.31 (SD = 19.19) and for aggressive lesions was 42.70 (SD=28.70). Unfortunately, these differences cannot be considered sufficiently significant to be routinely used as a parameter on which clinical and surgical decisions can be based.

Regezi *et al* (1987) attempted to answer the problem of differentiating between the range of giant cell lesions despite similar histopathological appearances by using immunoperoxidase techniques to evaluate HLA-DR expression. They believed that the detection of HLA-DR antigens may be a useful diagnostic tool and included in their series 21 peripheral giant cell granulomas, 21 central giant cell granulomas and 6 giant cell tumours of bone. They found that as a group, giant cell lesions exhibited HLA-DR antigens but unfortunately, the peripheral giant cell granuloma, the central giant cell granuloma and the giant cell tumour could not be differentiated on the basis of HLA-DR expression.

Nuclear DNA analysis using image cytometry was used by Eckardt *et al* (1989) to study the central giant cell granuloma and to predict clinical behaviour. They found the mean DNA-index of the aggressive lesions to be not significantly different from

non-aggressive lesions which indicated that the nuclear DNA content of giant cells is not useful as a predictor of the clinical behaviour of giant cell lesions of the jaws.

The search for histological parameters to predict clinical behaviour extends to studies involving the giant cell tumour of bone. Sara *et al* (Sara, 1990) used DNA flow cytometric analysis in an attempt to evaluate the biologic aggressiveness of giant cell tumours of bone. Their purpose was to investigate DNA ploidy patterns, a possible correlation between DNA ploidy and histologic type, and a correlation between DNA content and biologic behaviour. Sixty cases were studied and correlated with clinicopathological features. Unfortunately, they found that DNA ploidy had limited advantage in predicting biological behaviour of the giant cell tumour.

In keeping with the advances in the field of cellular genetics, the methods of cytogenetic analysis have been applied to correlate chromosomal abnormalities with biologic behaviour of the giant cell tumour of bone. The first cytogenetic study on giant cell tumour was undertaken by Schwartz *et al* (1989). The study included only 6 patients but they were able to show that all the tumours had a significantly higher incidence of non-clonal chromosome abnormalities than normal cells in culture. However, they found no correlation between any specific chromosomal change and the clinical behaviour or histology of giant cell tumours.

The most current serial cytogenetic study was performed by Bridge *et al* (1990) on 20 giant cell tumours over a three year period. Their results were interesting in that they found clonal and random chromosomal abnormalities in all but one of the aggressive tumours. Chromosomal abnormalities were detected in only one of the non-aggressive tumours. Although limited by a small sample size, this study suggests that cytogenetic analysis may become a useful tool in predicting biologic behaviour in the future.

However, although the results of Bridge *et al* (1990) may appear promising, the difficulty with this study as well as those outlined previously, was the underlying assumption that the giant cells are the most important component in the pathogenesis of the lesion.

To date, most studies have been directed at the giant cells in the lesion and understandably so, as they are the most prominent and conspicuous component of the lesion. However, evidence from recent studies have cast some doubt on the assumed primary role of the giant cells in the propagation of the lesion and this will be discussed in detail in section 2.4.

2.3 The Origin and Nature of the Giant Cells

The origin and nature of the giant cells have been the subject of much speculation. They have been thought to originate from the fusion of macrophages, endothelial cells, pericytes, myofibroblasts, osteoblasts and some have even labelled them as foreign body giant cells.

2.3.1 Macrophages

As early as 1954, Bernier and Cahn on the basis of light microscopical studies, believed that the giant cells in giant cell granulomas developed by the fusion of the mononuclear cells in the lesion. This view was possibly influenced by the prevailing opinion that giant cells originated from macrophages (Mallory, 1911).

In cell culture, macrophages have been shown to transform into epitheloid cells and then into giant cells by fusion (Sutton *et al*, 1966). Davis (1963) confirmed that lung macrophages transformed into giant cells in experimental asbestosis. This view of a macrophage origin of the giant cells is shared by Eisenstein (Eisenstein, 1969)

Ultrastructural studies on giant cell tumour by Hanaoka *et al* (1970) observed the merging of plasma membranes of mononuclear cells which led them to believe that cell fusion was occurring. Drepper *et al* (1961) noted the presence of irregular nuclei with deep indentations in the giant cells which was interpreted as resulting from amitotic nuclear division of mononuclear cells.

El-Mofty and Osdoby (El-Mofty and Osdoby, 1985) used cell culture to study the growth behaviour and lineage of cells from giant cell granulomas. Sections from the original lesion and cultured cells were analysed histochemically and they found that the giant cells and macrophage-like cells were identical in their staining characteristics for the histiocytic markers used, including Acid Phosphatase, Non-Specific Esterase, Lysozyme and Alpha-1-Antitrypsin. Therefore, they believed that giant cells could have been derived from the macrophage-like cells.

Regezi *et al* (1987) showed that both the giant cells and the round mononuclear cells in giant cell granulomas were positive for the histiocytic markers Alpha-1-Antitrypsin and Alpha-1-Antichymotrypsin supporting the view of a common haemopoietic cell lineage.

A review of the studies involving the giant cell tumour of bone also reflect the uncertainties with respect to the origin of the multinucleated giant cells. Wood *et al* (1977) analysed giant cell tumours to determine if giant cells expressed the characteristics commonly associated with macrophages, in particular, IgGFc and C3 receptors, phagocytosis and non-specific esterase activity. Their findings were that the giant cells failed to exhibit IgGFc and C3 receptors as well as being non-phagocytic for opsonised sheep red blood cells and heat killed yeast and were negative for non-specific esterase. They therefore concluded that the giant cells were not derived from the macrophage.

Horton *et al* (Horton, Lewis, 1985) used giant cells in giant cell tumour of bone for immunization producing mouse hybridomas secreting monoclonal antibodies reacting with the giant cells. They found no cross-reactivity of the antibodies with macrophages and concluded that the giant cells are phenotypically distinct from macrophages.

The phenotypic differences have been particularly emphasised by the failure of the giant cells to express Leukocyte Common Antigen (Aqel *et al*, 1988; Athanasou *et al*, 1986; Horton *et al*, 1984). However, in 1987, Athanasou *et al* were able to show that Leukocyte Common Antigen was present on giant cells in giant cell tumour of bone, thus supporting the more popular belief that the giant cell originates from a common haemopoietic cell line to the monocyte-macrophage.

Although there appears to be valid evidence that could argue against the common cell lineage of the giant cells with the monocyte (Wood *et al*, 1977), the possibility of a monocyte precursor to the giant cells cannot be excluded. In the process of differentiation, aggregation and formation, the giant cells may have lost some of the phenotypic characteristics common to the monocyte-macrophage and may have acquired other more specific cell markers. Ridley *et al* (1979) showed that the process of fusion of mononuclear cells in vitro was associated with modification of cell membrane receptors.

2.3.2 Fibroblasts, Myofibroblasts

From their studies of the oral giant cell fibroma using electron-microscopy, Weathers and Campbell (1974) believed that the giant cells formed by the fusion of fibroblasts and were probably induced by a virus. Their view was poorly substantiated and speculative and was primarily influenced by the findings of

Valazquez *et al* (1971) who produced giant cells in tissue culture from fusion of fibroblasts induced by Sendai virus.

Bartel and Piatowska (Bartel and Piatowska, 1977) favoured the concept of a fibroblastic origin as a result of their ultrastructural study of the peripheral giant cell granuloma. This was simply based on the observation that "fibroblasts with increased mitochondria often formed aggregates in which their membranes become indistinct".

El-Labban and Lee (1983) conducted an ultrastructural study of two cases of central giant cell granuloma in an attempt to elucidate the histogenesis of the giant cells. They found that the stromal fibroblast-like cells contained intracytoplasmic myofilaments with electron dense bodies similar to smooth muscle cells and proposed that they may be myofibroblasts, as introduced by Gabbiani *et al* (1971). They believed that the giant cells formed by fusion of the myofibroblasts on the observation that "many of the fibroblasts aggregated around giant cells with signs of fusion and close contact between their plasma membranes."

The concept of the myofibroblastic origin of the giant cells appears to be simply based on the close proximity of the cells either to each other or to the giant cells with "signs" of membrane fusion. Therefore, there is very little evidence to substantiate the myofibroblast hypothesis.

2.3.3 Endothelial Cells, Pericytes

A well recognised feature of the lesion is the close apposition of the giant cells to blood vessels. In some areas, it may almost appear as though the giant cells are either invading the vessel wall or detaching themselves from it. Not surprisingly, this has lead to speculation that the giant cells are of endothelial origin (David and Korth, 1959; Drepper *et al*, 1961).

The view that the giant cells originate from fusion of pericytes was proposed by Andersen *et al* (1973) as a result of a histochemical and ultrastructural study of central and peripheral giant cell granulomas. They found clusters of stromal cells which they considered to be pericytes due to the presence of lamina densa-like material surrounding the cells, with intimate intercellular relations. This morphologic relationship and the similar enzyme histochemical reactivity of the pericytes with the giant cells lead them to believe that the giant cells formed from fusion of pericytes.

2.3.4 Osteoclasts

Recent studies have provided strong evidence that the multinucleated giant cells in the giant cell granuloma and the giant cell tumour of bone may be osteoclasts.

Flanagan *et al* (1988) demonstrated that the giant cells in the giant cell granuloma possessed several osteoclast-specific characteristics. These included the ability to resorb bone *in vitro* and the responsiveness to the hormone calcitonin. The inhibition of bone resorption was demonstrated by a decrease in excavation patterns on bone using scanning electron-microscopy and motility inhibition to calcitonin was observed on time-lapse video recordings. Osteoclast specific monoclonal antibodies characterised by Horton *et al* (1985) was demonstrated to bind to the giant cells.

Chilosi *et al* (1988) showed that the recently introduced monoclonal antibody MB1 reacted with foetal bone osteoclasts. Subsequently, Bonetti *et al* (1990) demonstrated that the giant cells in peripheral giant cell granulomas also showed strong immunoreactivity with MB1.

Studies investigating the giant cell tumour of bone have provided similar evidence of the osteoclastic nature of the giant cells. Chambers *et al* (1982) showed that the addition of calcitonin to giant cell tumour cells in culture resulted in morphological changes in the giant cells and decreased their ability to resorb devitalised bone.

Goldring *et al* (1987) further developed these findings by using an autoradiographic technique in order to identify the individual hormone-responsive cell population. They found that over 90% of the multinucleated giant cells bound ^{125} I Calcitonin. The presence of calcitonin receptors is regarded as a distinctive marker of osteoclasts in bone tissue. Hattersley and Chambers (1989) demonstrated a strong and specific

correlation between the generation of bone resorptive function and calcitonin receptor positive cells *in vitro* which suggests that calcitonin receptor expression is a reliable marker for identification of osteoclastic differentiation.

In summary, recent studies have been able to show the presence of calcitonin receptors on the multinucleated giant cells along with bone resorptive capacity, and the responsiveness of the giant cells to calcitonin in both the giant cell granuloma of the jaws and the giant cell tumour of bone. In the light of the evidence presented, it would be reasonable to conclude that the giant cells in these lesions are likely to be osteoclasts or osteoclast-like in nature.

However, there still remain many unanswered questions. If the multinucleated giant cells are osteoclasts, then what are their precursors? The similarities between the macrophage and the osteoclast have prompted the proposal that the macrophages are appropriate osteoclast precursors. However, as previously discussed, osteoclasts and macrophages have been shown to differ in many significant respects, including the different phenotypic characteristics immunohistochemically, the lack of C3 and Fc receptors, and the non-responsiveness of monocytes to calcitonin.

The origin of the osteoclast has been debated at great length over the years (Marks, 1983). It is now acknowledged that they arise from haemopoietic stem cells and arrive via the circulation. The parabiotic study by Gothlin and Ericsson (1972) on irradiated rats with labelled ^3H thymidine showed that osteoclasts came from proliferating cells of the shielded rat via the bloodstream. However, it is still unclear if the osteoclasts originate from a monocyte precursor or from a separate haemopoietic stem cell line.

If the giant cells in giant cell granulomas are osteoclasts, what is the stimulus for their presence in the lesion? In addition, why are osteoclasts present in the soft tissue counterpart of the lesion, the peripheral giant cell granuloma?

Furthermore, what is the role of the osteoclasts in these giant cell lesions? It is evident that they are responsible for the osteolytic activity seen in the central giant cell granuloma, but are they the significant component in the development of the lesion or the so-called principal "tumour" cell of the lesion?

Evidence from more recent studies has suggested that this may not be the case and that the giant cells may simply be reactive in nature. This will be discussed in detail in the following section.

2.4 The Identification of the Principal "Tumour" Cell

It is evident that much of the past research activity with respect to these giant cell lesions has been directed primarily at the giant cell component of the lesion. The underlying assumption therefore has been that the giant cells represent the important component in its pathogenesis. However, recent studies using cell culture and transplantation have demonstrated the early disappearance of the giant cells, with continued proliferation of the stromal spindle-shaped cells, which suggests that the giant cells may simply be reactive in nature.

El-Mofty and Osdoby (1985) fractionated a central giant cell granuloma of the mandible into its cellular constituents which were subsequently grown in tissue culture. The giant cells and the macrophage-like cells were found to have a limited life span in tissue culture and survived two and five weeks respectively. However, the spindle-shaped cells continued to proliferate in culture, with exponential growth demonstrated by a doubling time of forty-eight hours. No mitotic activity was seen in the giant cells in the lesion or in culture, while the spindle-shaped cells displayed mitotic activity *in vivo* and were shown to replicate continuously in culture. These findings indicate that the giant cells may represent a fully differentiated end cell and may possibly be reactive in nature.

Cohen *et al* (Cohen, Grossman, 1988) transplanted 3 central giant cell granuloma lesions into nude mice. Xenografts were harvested at intervals of three weeks, five weeks, eight weeks and thirteen weeks, and examined histologically and ultrastructurally. The multinucleated giant cells were found to disappear at an early stage suggesting that they represent a stimulus dependent, differentiated end-stage cell population.

Studies involving the giant cell tumour of bone have demonstrated similar findings. Goldring *et al* (1987) noted the disappearance of the multinucleated giant cells of the giant cell tumour in cell culture after 2 to 3 weeks, with the predominant cell type being the spindle-shaped cells. Treatment with hormones 1,25-dihydroxyvitamin D3, parathyroid hormone, calcitonin, and prostaglandin E2 did not affect the survival of the giant cells.

In summary, these findings suggest that the giant cells in these lesions may simply be a reactive component and are possibly attracted to the lesion by an unknown stimulus from the stroma. Hence, they may only present by "accident".

This recent concept has several implications. In particular, there is the need to redirect the attention away from the giant cells in the study of these lesions. The stromal components which have been largely ignored in previous studies may therefore be the important component in the pathogenesis of these giant cell lesions.

Future studies should therefore be directed at understanding the nature of the stromal components of the lesion and hence, this concept will be incorporated as an integral part in the aims of this study.

CHAPTER 3

FUNDAMENTALS OF IMMUNOHISTOCHEMISTRY

3.1 Antigens and Antibodies

Immunohistochemical procedures are based on the antigen-antibody reaction of the immune system.

An antigen is any foreign substance which is able to stimulate the body's immune response to produce specific antibodies. Antibodies are proteins belonging to a large family of proteins called immunoglobulins and are produced by the B lymphocytes of the immune system.

There are five classes of immunoglobulins and each consist of two identical light (L) and two identical heavy (H) polypeptide chains. The H chains are antigenically distinct and determine the class and subclass of the molecule. The L chains are either kappa or lambda type.

Of the five classes of immunoglobulins, IgG and IgM are the most frequently utilised antibodies in immunohistochemistry. IgG has the basic four chain structure whilst IgM is pentameric consisting of five groups of four chains. The two L chains and two H chains are held together by disulphide bonds. IgG is a particularly stable molecule because of the large number of disulphide bonds between its chains.

Each polypeptide chain is divided into a variable (V) region and a constant (C) region. The amino acid sequence is variable in the V region and forms the basis of immunoglobulin diversity. The V region is also the basis of the specificity of the antibody as it represents the binding site for the antigen.

The immunoglobulin molecule consists of two portions, the antigen binding fragment (Fab) containing the binding sites of the antigen and the crystallisable fragment (Fc) which regulates biologic activity.

3.1.1 Polyclonal Antibodies

When an antigen is injected into an animal an immune response is mounted and specific antibodies are produced. As antigens can have several antigenic determinants more than one clone of the antibody producing cells will be activated resulting in a polyclonal population of antibodies.

The most frequently used animal for production of polyclonal antibodies is the rabbit although goat, pig, sheep and other animals may be used.

The disadvantage of polyclonal antibodies is the lack of specificity as cross-reactions with similar antigens are commonly encountered. Some of the problems of cross-reactivity with polyclonal antibodies can be overcome with affinity chromatography where specific immunoglobulins can be absorbed onto columns that contain ligands for specific antibody.

3.1.2 Monoclonal Antibodies

It would obviously be desirable to be able to obtain a specific antibody to a specific antigen. The work of Kohler and Milstein (Kohler and Milstein, 1975) was a major

advancement in the field of immunohistochemistry with the development of a technique for the fusion of myeloma cells with isolated specific B lymphocytes.

Mice are almost exclusively used for the production of monoclonal antibodies. After injection with the antigen and subsequent immune response, B lymphocytes are isolated from the spleen or lymph nodes and fused with non-secreting mouse myeloma cells. The B lymphocytes convey specific antibody and the myeloma cells provide the longevity to the hybrid cells so that theoretically, unlimited amounts of antibody can be produced. The hybridoma is tested for reactivity and propagation of the antibody is either through culture medium or by transplantation into peritoneal cavities of syngeneic mice.

There are many advantages of monoclonal over polyclonal antibodies including their high degree of specificity, the minimisation of cross-reactions and ease of characterisation. However, in order to benefit from the advantage of specificity, the targeted epitope must be unique to a given antigen. If the antibody is directed at an epitope shared by two or more antigens then specificity will be lost.

3.2 Immunoperoxidase Methods

The current immunoperoxidase methods developed from a technique introduced by Coons *et al* (1942) which demonstrated that fluorescein labelled antibodies could be used to locate antigens in tissues.

Graham and Karnovsky (1966) introduced a reaction whereby the enzyme peroxidase could be located by the formation of a coloured end product. Horseradish peroxidase first forms a complex with Hydrogen Peroxide causing it to release oxygen and water. The reaction consists of the liberated oxygen oxidising the

electron donor or chromogen 3,3'-Diaminobenzidine to a brown coloured end product.

Peroxidase can be attached to other proteins either covalently or non-covalently. There are two general classes of reactions available: the conjugate or enzyme labelled antibody procedures or the non-conjugate or unlabelled antibody procedures described by Sternberger *et al* (1970).

3.2.1 Conjugate Methods

In the conjugate procedures, the enzyme label is covalently linked to the appropriate antibody. There are two methods available, the direct and indirect conjugate methods. The direct method (Avrameas, 1970) involves the application of one antibody covalently linked to the peroxidase enzyme. This method tends to yield conjugates of unknown composition.

The indirect method (Nakane and Pierce, 1966) provides a more consistent labelling ratio and involves two antibodies, the second antibody conjugated with the enzyme and directed against the first antibody.

The disadvantages of using the conjugate methods are due to the procedures involved in attaining a chemical union, for example with glutaraldehyde, between the enzyme and the antibody which can cause the antibody to denature or lose activity. Sensitivity of the method further suffers from the presence of remaining unconjugated antibody which can compete with the staining of labelled antibodies. The conjugate methods are faster but are also known to produce a great deal of background staining.

3.2.2 Non-Conjugate Methods

In the non-conjugate methods, the chemical procedures involved in linking the antibody to the enzyme is avoided, the antibody remains unaltered and the enzyme activity is maintained.

The unlabelled antibody method (Sternberger *et al*, 1970) utilises a preformed anti-enzyme immune complex. The staining sequence involves an unconjugated primary antibody followed by the secondary antibody and finally the soluble enzyme anti-enzyme complex and substrate solution. Of the enzyme complexes available, the Peroxidase-Anti-Peroxidase (PAP) method is most commonly used and involves the use of a Peroxidase-Anti-Peroxidase complex.

3.2.3 Avidin-Biotin Methods

The avidin-biotin technique is an extension of the unlabelled antibody principles and utilises the high affinity of avidin or streptavidin for biotin. A biotinylated link antibody is required. Biotinylation is a mild process where the biotin is covalently linked to the antibody. The open sites on avidin from the avidin-biotin complex bind to the biotin on the link antibody. The strong affinity of avidin for biotin and the mild biotinylation make this technique extremely sensitive. It is for this reason that the labelled avidin-biotin method was the technique utilised in this study.

3.2.4 Inhibition of Endogenous Peroxidase Activity

Naturally occurring peroxidase molecules in the tissue section will react in the substrate-chromogen step in the procedure and lead to false-positive staining.

Of the methods available, the use of Hydrogen Peroxide (Streefkerk, 1972) was used in this study. This is possible through the mechanism of substrate inhibition where the presence of an excess of substrate will reversibly or irreversibly inhibit an enzyme. To block endogenous activity, a hundred-fold excess of Hydrogen Peroxide (3% H₂O₂) is sufficient to irreversibly inhibit the endogenous enzyme.

3.2.5 Proteolytic Enzyme Pretreatment

Proteolytic enzyme pretreatment introduced by Huang *et al* (Huang, Minassian, 1976) may be required in certain procedures to "unmask" antigenic sites. The mechanism of action of proteolytic enzymes to enhance binding of the antibody is unknown. Radioimmunologic assays have suggested that there are larger amounts of immunoactive antigens present in tissues than can be demonstrated on routine immunohistochemical techniques (Finley and Grossman, 1978). It has been suggested that proteolytic enzymes can uncover antigenic sites possibly obstructed by the cross-linking of various proteins from aldehyde fixatives (Finley and Petrusz, 1982). Proteolytic enzyme pretreatment is also known to reduce non-specific background staining and it is suggested that this may be due to the removal of ground substance protein molecules (Reading, 1977). Enzymes available for pretreatment include protease, pronase and trypsin.

3.2.6 Detection Agents

The detection of a specific enzyme within a tissue is accomplished by the localisation of a reaction product obtained in the presence of a specific substrate. The reaction product must be insoluble and as it accumulates at the site of the enzyme, visualisation can be made possible.

The substrate for peroxidase is Hydrogen Peroxide. The reaction yields a primary complex and with the presence of an electron donor, the complex dissociates,

regenerating the reduced state of the enzyme and releasing the oxidised electron donor. The electron donor is the histochemical marker and include compounds such as 3,3'-Diaminobenzidine Tetrahydrochloride (DAB), 3-Amino-9-Ethyl Carbazole (AEC), 4-Chloro-1-Naphthol (CN), p-Pheynylenediamine Dihydrochloride (PDC) and Pyrocatechol (PC) or Hanker-Yates reagent (HYR).

In this study, DAB was chosen as the electron donor. DAB was introduced by Graham and Karnovsky (1966) and is now probably the most widely used electron donor in immunohistochemical procedures. On oxidation, a polymer is formed which is brown in colour and highly insoluble. The major disadvantage of DAB is that it is a suspected carcinogen and should be used and disposed of with caution.

3.2.7 Controls

Positive and negative controls are required in order to validate immunohistochemical staining. Controls center around the primary antibody to determine if positive staining is the result of specific binding to the antigen.

Positive tissue controls are sections processed identically to the specimen and contain the target protein. Internal tissue controls are "built-in" controls and are ideal because the variables of tissue preparation, processing, and staining are eliminated. Internal controls contain the target marker in adjacent normal elements.

Negative controls involve either reagent control procedures or tissue controls. Reagent substitution involves the replacement of the primary anti-serum with a serum sample from a non-immunised animal of the same species as the primary serum. This serves as an indicator of non-specific binding which may give false positive results. Negative tissue controls involve the inclusion of a tissue sample that has been fixed and processed identically with the unknown and does not contain the

antigen in question. Omitting the primary anti-serum also serves as a negative control and demonstrates non-specific staining of the secondary anti-serum.

CHAPTER 4

THE ANTIBODIES

This chapter will provide a review of the characteristics of the antibodies used in the first part of the study and the reasons for their choice with respect to the aims of the study will be discussed.

4.1 Leukocyte Common Antigen

The human Leukocyte Common Antigen (LCA) is a cell surface glycoprotein complex of approximately 200 kDa that is present on all haemopoietic cells except mature erythrocytes.

LCA consists of a family of five glycoproteins. The heterogeneity of the different LCA proteins is caused by several mRNAs which code for specific glycoproteins and by the variable carbohydrate content.

Streuli *et al* (1988) showed that this antibody reacts with an epitope of the CD45 gene. Monoclonal antibodies directed at human LCA have shown differing specificities probably due to the recognition of individual antigenic components on the LCA molecule. There are two well characterised antigenic specificities which have been demonstrated by biochemical and tissue distribution studies. These are the conventional CD45 which is present on all leukocytes and the CD45R which is selectively expressed by B lymphocytes and a sub-population of T cells (Dalchau and Fabre, 1984).

It is suggested that LCA may be a cell surface receptor involved in signal transduction and in regulating cell activation and proliferation by dephosphorylating proteins which have been phosphorylated by kinase (Clark and Ledbetter, 1989).

In normal tissue, LCA labels lymphoid cells and is unreactive with non-haemopoietic tissues. Macrophages and histiocytes have been shown to be labelled by LCA while polymorphs are only weakly labelled. Plasma cells are generally unreactive.

LCA is a widely used marker for discriminating between tumours from lymphoid origin and those of other cell lineages. Neoplastic B and T cells in tumours such as non-Hodgkin's lymphoma and in B and T cell leukaemias are reactive to LCA.

This antibody was chosen to demonstrate the probable cell lineage of the cellular components in the giant cell lesions studied. In particular, it was directed at the giant cells and the round mononuclear cells in the lesion. The round mononuclear cells are macrophage-like and it has been suggested that the giant cells originate from the fusion of these cells.

Previous studies have demonstrated the presence of LCA on the round mononuclear cells. However, there is conflicting evidence on whether the giant cells possess the CD45 epitope (Aqel *et al*, 1988).

It is believed that the giant cells are osteoclasts (Flanagan *et al*, 1988). The presence of LCA on osteoclasts has not been demonstrated in studies by Horton *et al* (1985) and it has therefore been suggested that osteoclast precursors are not derived from the mononuclear phagocyte system. However, Athanasou *et al* (Athanasou, Quinn, 1987) using a panel of monoclonal antibodies to LCA showed that LCA is present on foetal and adult human osteoclasts.

The antibody chosen for this study was the conventional LCA which recognises epitopes shared by the 220, 205, 190, and 180 kDa glycoproteins known to be present on all leukocytes.

4.2 MB1

MB1 is one of five new antibodies recently introduced by Poppema *et al* (1987). It is known to be reactive with leukocyte subsets and can be used on formalin-fixed paraffin-embedded tissue sections.

MB1 reacts with mature B lymphocytes and a minority of T lymphocytes and monocytes. This monoclonal antibody recognises three bands of 200, 110, and 100 kDa when characterised by the immunoblot procedure. Since it is reactive with antigens with molecular weights near 200 kDa it is thought to be directed at members of the Leukocyte Common Antigen family.

MB1 is reactive with all B cells, excluding mature plasma cells and with approximately 50% of mature T lymphocytes. It is known to be reactive with all B cell lymphomas except for plasmacytomas. It is unreactive with lymphoblastic T cell lymphomas but reactive with some peripheral T cell lymphomas (Poppema, Hollema, 1987)

Chilosi *et al* (Chilosi, Gilioli, 1988) showed that MB1 is strongly reactive against osteoclasts in foetal bone. This study also demonstrated that MB1 was present in the giant cells in giant cell tumour of bone and was in giant cells of granulomatous lesions such as sarcoid lymphadenopathy and Histiocytosis X.

Recently, Bonetti *et al* (Bonetti, Pelosi, 1990) demonstrated that MB1 was reactive in giant cells in nine cases of peripheral giant cell granuloma.

MB1 was chosen in this study to investigate the presence of this antibody in the multinucleated giant cells of the central giant cell granuloma, the peripheral giant cell granuloma and the giant cell tumour. The demonstration of the presence of MB1 in the the giant cells in this study would be further evidence in support of the belief that they may be osteoclasts (Flanagan *et al*, 1988).

4.3 S-100 Protein

S-100 Protein was studied by Moore (1965) as a nervous tissue specific protein. It was so-named because of its solubility in 100% ammonium sulphate solution at neutral pH.

It is a relatively small thermolabile protein with a molecular weight of 20 - 25 kDa. S-100 Protein is a mixture of two similar proteins, S-100a and S-100b with molecular weights of 20 907 and 21 014 respectively. Structural analysis revealed the presence of E-F hand structure common to the group of calcium binding proteins including calmodulin, troponin C and the light chain of myosin (Isobe and Okuyama, 1978).

The function of S-100 Protein is unknown. It has the ability to bind calcium and is able to induce microtubule disassembly with greater efficiency than calmodulin (Baudier *et al*, 1982). Hence, it is suggested that S-100 Protein may interact with the microtubule system. S-100 Protein has also been shown to interact with artificial lipid membranes, inducing an increase in their permeability to cations under physiological conditions (Calissano and Bangham, 1971).

In the nervous system, this protein is present mainly in astrocytes, oligodendrocytes, schwann cells and in some neurons. Clinical applications have been mainly directed at central nervous tissue tumours. Malignant melanoma has been reported to contain

variable amounts of S-100 Protein (Nakajima, Watanabe, 1982) and S-100 detection has now become a frequently used diagnostic indicator of the disease.

Nakajima *et al* (1982) also demonstrated that S-100 Protein is not restricted specifically to nervous tissue and showed the presence of this protein in other non-neural tissue such as in Langerhan cells of the skin and in the interdigitating reticulum cells in the lymph nodes. S-100 Protein was also demonstrated in chondrosarcoma, chordomas, Langerhans cell granulomatosis, myo-epithelial cells of breast tumours and pleomorphic adenomas of the salivary gland.

In immunohistochemistry, S-100 Protein can be used on formalin-fixed paraffin-embedded tissue specimens. Cells containing S-100 Protein display both cytoplasmic and nuclear staining.

Why was S-100 Protein chosen as one of the antibodies for this study? Although the giant cell granuloma of the jaws and the giant cell tumour of long bones are similar histologically, they are known to behave differently. Unlike the mesodermal origin of the connective tissue in the rest of the body, the structures of the face originate embryologically from the neural crest. S-100 Protein was chosen in the hope that it may be a useful indicator for the observed behavioural differences between the giant cell lesions from the jaws and those of long bones.

4.4 Smooth Muscle Specific Actin

Actin is a major component of the cytoskeleton and is present in every cell type. It is a globular protein with a mass of approximately 47 kDa. The actins are divided into six iso-types based on differences in iso-electric points and amino acid sequence and can be resolved into alpha, beta and gamma components. Four muscle actins have

been identified: skeletal alpha, cardiac alpha, vascular smooth muscle alpha and enteric smooth muscle gamma actin.

The monoclonal antibody to smooth muscle specific actin named HHF35 was described by Tsukada *et al* (1987). Following immunoelectrophoresis and immunoblotting techniques, HHF35 was shown to recognise the alpha and gamma isotypes of skeletal, cardiac and smooth muscle cells. In gel electrophoresis immunoblot experiments, this antibody detects a 42 kDa polypeptide from tissue extracts of uterus, ileum, aorta, diaphragm, heart and extract from smooth muscle cells. HHF35 is non-reactive on immunoblots of extracts from all tested non-muscle cell extracts (Tsukada *et al*, 1987). This antibody is therefore a useful marker for muscle and muscle derived cells.

The distribution of Smooth Muscle Specific Actin in normal, reactive and pathologic tissues was also investigated by Tsukada *et al* (1987). It was found that in addition to muscle tissues, the antibody was present in myo-epithelium, myofibroblasts of healing wounds, tumours such as leiomyomas and rhabdomyosarcoma, myo-epithelial cells of pleomorphic adenoma, hyperplastic fibrous tissue responses and in lesions of atherosclerosis.

In this study, Smooth Muscle Specific Actin was directed in particular at the spindle-shaped cells in the stroma of the giant cell lesions. These cells have been described as being myofibroblasts (Dayan *et al*, 1989; El-Labban and Lee, 1983) on the basis of their ultrastructural features which are similar to the myofibroblasts found in healing wounds as described by Gabbiani *et al* (1971) and said to be involved in wound contracture.

In an experimental model, Gabbiani *et al* (1971) found that fibroblasts in healing wounds were able to develop certain modifications including a fibrillar system and electron dense areas within the fibrillar bundles similar to smooth muscle cells. They

also possessed nuclear deformations which have been correlated with cellular contractions in smooth muscle, myocardial fibres and endothelial cells. Finally, the presence of surface differentiations including peripheral attachment sites related to an extra-cellular layer of basement membrane-like material parallel to the cell wall was observed which was also present in smooth muscle cells. The surface differentiations may be the means whereby the mechanical contractions could be transmitted to the surrounding tissues.

The ultrastructure of the spindle-shaped cells in giant cell lesions was investigated by El-Labban and Lee (1983) and Dayan *et al* (1989) who studied the central giant cell granuloma and the peripheral giant cell granuloma respectively. Both studies reported the presence of parallel intracytoplasmic myofilaments with electron dense bodies as well as prominent rough endoplasmic reticulum and Golgi apparatus. The nuclei of these cells were irregular in outline possessing many indentations and folds and some cells were surrounded by a basement membrane-like material. These features strongly suggest that the spindle-shaped cells may be myofibroblasts.

Tsukada *et al* (1987) demonstrated that the myofibroblasts in a tissue specimen of a healing leg ulcer were positive to Smooth Muscle Specific Actin. Therefore, this antibody was chosen in this study to investigate whether the "myofibroblasts" of the giant cell lesions are immunohistochemically similar to the myofibroblasts described by Gabbiani *et al* (1971) in healing wounds.

4.5 Factor VIII Related Antigen

Factor VIII or anti-haemophilic factor is a macromolecular complex involved in the intrinsic pathway of coagulation. It is a plasma glycoprotein present in normal human plasma in trace amounts, approximately 5-10 micrograms per millilitre. It

consists of three functionally distinct components : a clot promoting factor (FVIII C), a Factor VIII Related Antigen and Von Willebrand Factor.

Anti-haemophilic factor consists of a high molecular weight and a low molecular weight sub-unit (McKee *et al*, 1975). Factor VIII Related Antigen and Von Willebrand Factor are located in the high molecular weight sub-unit and Factor VIIIIC in the low molecular weight sub-unit. The antibody to Factor VIII Related Antigen is raised against the high molecular weight sub-unit.

Immunofluorescent and tissue culture studies have shown that the vascular endothelial cells synthesise Factor VIII Related Antigen and Von Willebrand Factor (Hoyer *et al*, 1973). As well as endothelial cells, it has also been demonstrated to be present in platelets and megakaryocytes (Piovella *et al*, 1978).

Mukai *et al* (1980) using an immunoperoxidase method, demonstrated that Factor VIII Related Antigen was localised in vascular endothelial cells in formalin-fixed paraffin-embedded tissues. In a subsequent study, Burgdorf *et al* (1981) investigated the presence of Factor VIII Related Antigen in endothelial cells of a range of lesions of alleged vascular nature. They found that the endothelial cells of small, normal cutaneous vessels gave the strongest reaction. The cells of haemangioma and angiokeratoma were also stained positive. However, the cells of poorly differentiated angiosarcoma and the spindle-shaped cells of Kaposi's sarcoma were generally negative.

Factor VIII Related Antigen is a widely used marker for endothelial cells in formalin-fixed paraffin-embedded tissues. In this study, Factor VIII Related Antigen was selected to investigate the blood vessels in the giant cell lesions for several reasons. Giant cell granulomas appear to be vascular in nature. The peripheral giant cell granuloma appears as a purplish-red haemorrhagic epulis and bleeds readily on biopsy or excision. The central giant cell granuloma macroscopically appears as a

deep red, vascular mass. In addition, the giant cells in the giant cell granuloma have been noted to be intimately associated with the blood vessels in the lesion and some have been described as appearing to either be forming part of, or emanating from the vessel wall (Andersen *et al*, 1973). Some have even speculated on the possibility of an endothelial origin of the giant cells (David and Korth, 1959; Drepper *et al*, 1961).

CHAPTER 5

THE STUDY (PART 1)

5.1 AIMS

The aim of the study was to investigate the phenotypic characteristics of the cellular components of giant cell granuloma of the jaws using a panel of immunohistochemical markers. The panel of antibodies were: Leukocyte Common Antigen, MB1, S-100 Protein, Smooth Muscle Specific Actin and Factor VIII Related Antigen.

The following summarises the specific objectives of the study:

1. To compare the phenotypic characteristics of the central giant cell granuloma, the peripheral giant cell granuloma and the giant cell tumour of bone following immunoperoxidase staining with the antibodies chosen.
2. To investigate the presence of Leukocyte Common Antigen in the giant cells and round mononuclear cells in the lesion to examine their histogenesis.
3. To investigate the reactivity of the giant cells to MB1, a monoclonal antibody shown to be present in osteoclasts of foetal bone.

4. To examine the reactivity of the cells of the jaw lesions to S-100 Protein and to compare it with the giant cell lesions of extragnathic sites. The possibility for this antibody to be an indicator of the behavioural differences observed between the lesions of the jaws and long bones was hypothesised.
5. To investigate the presence of Smooth Muscle Specific Actin in the spindle-shaped cells of the lesion in the light of its alleged myofibroblastic nature.
6. To study the blood vessels in the lesion with the widely used endothelial cell marker Factor VIII Related Antigen.

It was previously discussed that although most of the past research work has been directed at studying the giant cell component of these lesions, recent evidence has suggested that the giant cells may simply be reactive in nature.

Therefore, it was hoped that in this study, the attention would not simply be directed at the multinucleated giant cells that dominate these lesions but to also investigate the characteristics of the surrounding stromal components.

5.2 Materials and Methods

5.2.1 Tissue specimens

The tissue specimens were retrieved from the archives of the Department of Anatomical Pathology, Institute of Clinical Pathology and Medical Research, Westmead Hospital.

A total of 20 formalin-fixed paraffin-embedded tissue specimens were obtained.

These included:

8 central giant cell granulomas

9 peripheral giant cell granulomas

3 giant cell tumours of bone

The histological diagnosis was determined by Consultant Pathologists of the Department of Anatomical Pathology, Westmead Hospital.

5.2.2 Clinical Data

The tissue specimens were obtained from 18 patients. Two of the specimens studied were recurrent lesions.

All patients with giant cell granuloma of the jaws were treated at the Department of Oral and Maxillofacial Surgery at Westmead Hospital. The investigator was involved in the treatment and follow-up of the majority of these patients.

The patients with giant cell tumour of bone were treated at the Department of Orthopaedics, Westmead Hospital.

Table 5.1 summarises the clinical features of the patients with the giant cell lesions selected in the study.

Table 5.1 Clinical Data

NO.	PATIENT	AGE	SEX	TYPE	SITE	TREATMENT	RECURRENCE	FOLLOW-UP	COMMENTS
1	A.B.	16	M	CGCG	Maxilla	Enucleation	No	No record	
2	B.B.	9	M	CGCG	Mandible	Excision	Yes (18 mths)	3 years	Recurrence within rib graft
3	B.B.	9	M	CGCG	Mandible	Enucleation	No	3 years	
4	W.D.	50	M	CGCG	Mandible	Excision	No	5 years	
5	R.G.	35	M	CGCG	Mandible	Enucleation	No	18 months	In site of mandibular osteotomy and orthodontic uprighting of teeth
6	K.J.	30	F	CGCG	Maxilla	Excision	No	3 years	
7	T.L.	41	F	CGCG	Mandible	Enucleation	Yes (19 mths)	3 years	Recurrence at previous site of PGCG
8	F.B.	66	M	CGCG	Maxilla	Enucleation	No	2 years	CGCG with perforation of alveolus and peripheral presentation
9	J.K.	58	M	PGCG	Mandible	Excision	No	7 months	
10	D.O.	46	F	PGCG	Maxilla	Excision	No	6 months	
11	M.K.	7	M	PGCG	Mandible	Excision	No	3 months	
12	C.M.	4	M	PGCG	Mandible	Excision	Yes (twice)	2 years	Recurrence in periods less than 6 months of excision
13	T.L.	41	F	PGCG	Mandible	Excision	No	3 years	Recurrent lesion of previous PGCG
14	K.K.	65	F	PGCG	Mandible	Excision	No	2 months	
15	T.S.	20	F	PGCG	Mandible	Excision	No	1 month	
16	R.Q.	7	M	PGCG	Mandible	Excision	No	14 months	
17	H.T.	76	F	PGCG	Maxilla	Excision	No	No record	
18	E.W.	22	F	GCT	Tibia	Enucleation	No	No record	
19	D.H.	34	M	GCT	Heel	Enucleation	No	No record	
20	V.W.	16	F	GCT	Sacrum	Enucleation	No	No record	

5.2.3 Immunoperoxidase Procedure

The Avidin-Biotin complex method based on the indirect unlabelled antibody technique of Sternberger *et al*, (1970) was used.

5.2.4 Tissue Fixation and Preparation

The tissue specimens were fixed in 10% formalin and conventionally embedded in paraffin.

The specimen blocks were serially sectioned into 4 μ m sections using a Leitz rotary microtome. Each section was placed on slides pre-coated with poly-o-lysine.

The sections were heat-fixed at 60 degrees Celsius for a period of 15 minutes.

5.2.5 De-waxing

The sections were de-paraffinised in Xylene. They were then immersed in Absolute Alcohol, followed by 70% Alcohol and then into the buffer, Phosphate Buffered Saline, for several minutes.

5.2.6 Buffer

Phosphate buffered saline (PBS) of pH 7.2 was used as the buffer for rinsing and dilution. It is prepared by dissolving three tablets of commercially prepared PBS (Oxoid) in 500 mls of distilled water.

5.2.7 Primary Antibody

The tissue sections were incubated with the diluted primary antibody in a moist chamber at room temperature for a period of 30 minutes.

The following is a list of the primary antibodies and the dilutions used:

1. Leukocyte Common Antigen PD 7/26, 2B11 (DAKO, California, U.S.A.), 1:50
2. MB1 (Biogenex, California, U.S.A.), 1:50
3. S-100 Protein (Biogenex, California, U.S.A.), 1:60
4. Smooth Muscle Specific Actin HHF35 (Biogenex, California, U.S.A.), 1:40
5. Factor VIII Related Antigen (DAKO, California, U.S.A.), 1:50

5.2.8 Enzymatic Pre-treatment

Of the above, only Factor VIII Related Antigen required enzymatic pre-treatment. Prior to incubation with the primary antibody the sections were pre-treated with 0.05% Protease at 37 degrees Celsius for 5 minutes. The enzyme Protease was diluted in a Tri-Amino-Methane-Maleate buffer.

5.2.9 Rinsing

In between steps, the sections were routinely rinsed in a bath of running tap water for a few seconds and then immersed in PBS for one minute.

5.2.10 Inhibition of Endogenous Peroxidase

After incubation with the primary antibody, the sections were incubated in a solution of 3% Hydrogen Peroxide for a 5 minute period to inhibit the production of endogenous peroxidase.

5.2.11 Secondary Antibody

The sections were subsequently incubated with the secondary antibody for 30 minutes at room temperature.

The secondary antibody consisted of biotinylated goat anti-mouse serum (TAGO, California U.S.A.) if the primary antibody was monoclonal (LCA, MB1, Smooth Muscle Specific Actin, and Factor VIII Related Antigen) and biotinylated goat anti-rabbit serum (TAGO, California U.S.A.) if the primary antibody was polyclonal (S-100 Protein). A 1:500 dilution was used in either case.

5.2.12 Tertiary Antibody

Finally, the sections were incubated in Steptavidin Peroxidase (TAGO, California U.S.A.) in a 1:4000 dilution for a further 30 minutes at room temperature.

5.2.13 Chromogen-Substrate Solution

Following the third antibody, the sections were placed in PBS for a 5 minute period.

The chromogen 3,3'-Diaminobenzidine Tetrachloride (DAB) was used. The preparation of DAB consisted of diluting 1 gram of DAB (Sigma) with 165 mls of PBS buffer. 5 mls of this solution was further diluted with 45 mls of PBS with the addition of 5 drops of 3% Hydrogen Peroxide.

The sections were incubated in this solution for a period of 5 minutes then washed in a bath of running tap water for 5 minutes.

5.2.14 Counterstaining and Mounting

The sections were counter-stained in Haematoxylin for several seconds and rinsed in running tap water.

They were then dehydrated in graded alcohols starting with 75% Alcohol and ending in Absolute Alcohol.

Finally, the sections were cleared in Xylol and mounted manually with cover-slips using DPX glue.

5.2.15 Controls

Positive controls involved using tissue of known antigenic positivity including a section of salivary gland tissue for the reactivity of the myo-epithelial cells to S-100 Protein, and the use of internal tissue controls which involved the presence of small diameter blood vessels for Factor VIII Related Antigen and larger diameter blood vessels for Smooth Muscle Specific Actin. Negative controls consisted of omitting the primary antibody.

5.2.16 Analysis

Random numbers were allocated to each slide to avoid bias in analysis.

The sections were analysed under a conventional light microscope. Since DAB was the chromogen used, positive uptake of the antibody was indicated by a brown coloured staining of the cells in the tissue.

Each antibody was assessed for staining of the cellular constituents in the lesions. The cells were grouped into the following categories: the multinucleated giant cells, the round mononuclear cells, the spindle-shaped cells and the endothelial cells.

CHAPTER 6

RESULTS (PART 1)

Analysis of the stained tissue sections revealed no differences in staining pattern of the the giant cells, mononuclear round cells and spindle-shaped cells between the central and peripheral giant cell granuloma, and the giant cell tumour of bone. Table 6.1 summarises the results obtained and the figures illustrate the immunohistochemical staining of the giant cell granuloma.

6.1 Leukocyte Common Antigen

The round mononuclear cells stained strongly for Leukocyte Common Antigen while the multinucleated giant cells and the spindle-shaped cells did not stain with this antibody (Figure 6.1 and 6.2).

6.2 MB1

The giant cells reacted positively for MB1 whilst the other cellular components of the lesion were unreactive (Figure 6.3 and 6.4).

6.3 Smooth Muscle Specific Actin

The stromal spindle-shaped cells and the giant cells were unreactive to Smooth Muscle Specific Actin (Figure 6.5). Smooth muscle cells in the walls of blood vessels in the connective tissues to some of the lesions stained positively with this marker (Figure 6.6).

6.4 S-100 Protein

S-100 Protein failed to stain the stromal and cellular components of the lesions (Figure 6.7). The myoepithelial cells (and ductal epithelial cells) within the section of salivary gland tissue used as the positive control stained positively with this antibody (Figure 6.8).

6.5 Factor VIII Related Antigen

This was the most interesting and unexpected finding in the study. In the giant cell granulomas, the blood vessels on the periphery of the lesion stained intensely with this antibody however, deeper within the lesion and closer to the aggregation of the multinucleated giant cells reaction product was not evident (Figures 6.9 to 6.16).

Table 6.1 Summary of Results

ANTIBODIES	GIANT CELL GRANULOMA				GIANT CELL TUMOUR			
	Multi- Nucleated Giant Cells	Round Mono- nuclear Cells	Spindle Shaped Cells	Blood Vessels	Multi- Nucleated Giant Cells	Round Mono- nuclear Cells	Spindle Shaped Cells	Blood Vessels
Leukocyte Common Antigen	-	+	-	-	-	+	-	-
MB1	+	-	-	-	+	-	-	-
S-100 Protein	-	-	-	-	-	-	-	-
Smooth Muscle Specific Actin	-	-	-	-	-	-	-	-
Factor VIII Related Antigen	-	-	-	+/-	-	-	-	-

LEGEND : + Positive Staining
 - Negative Staining
 +/- Differential Staining

Figure 6.1 Histomicrograph illustrating the positive immunoperoxidase staining of the round mononuclear cells to Leukocyte Common Antigen (X400)

Figure 6.2 Histomicrograph illustrating the positive immunoperoxidase staining of the round mononuclear cells (some in the process of margination) to Leukocyte Common Antigen (X400)

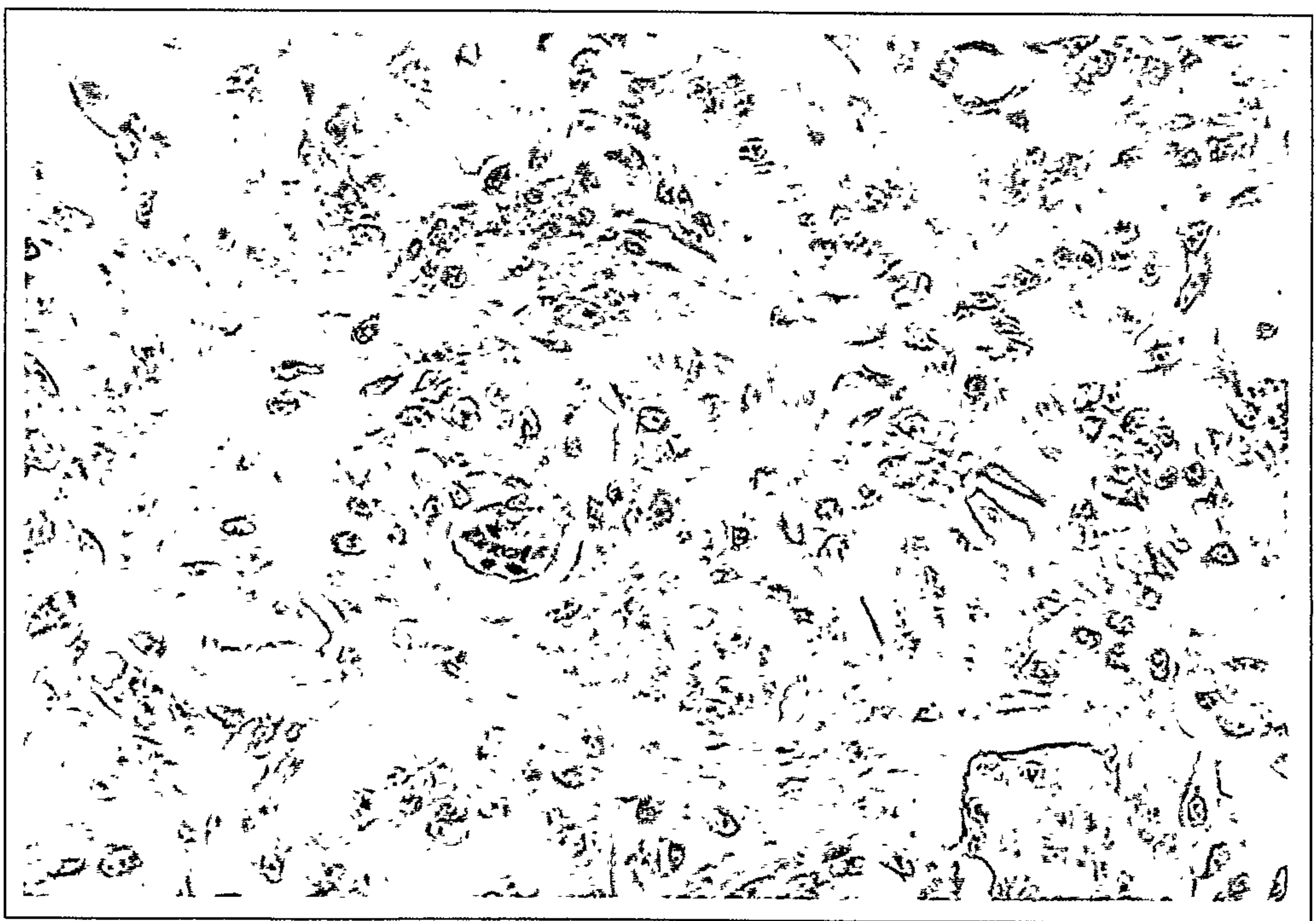
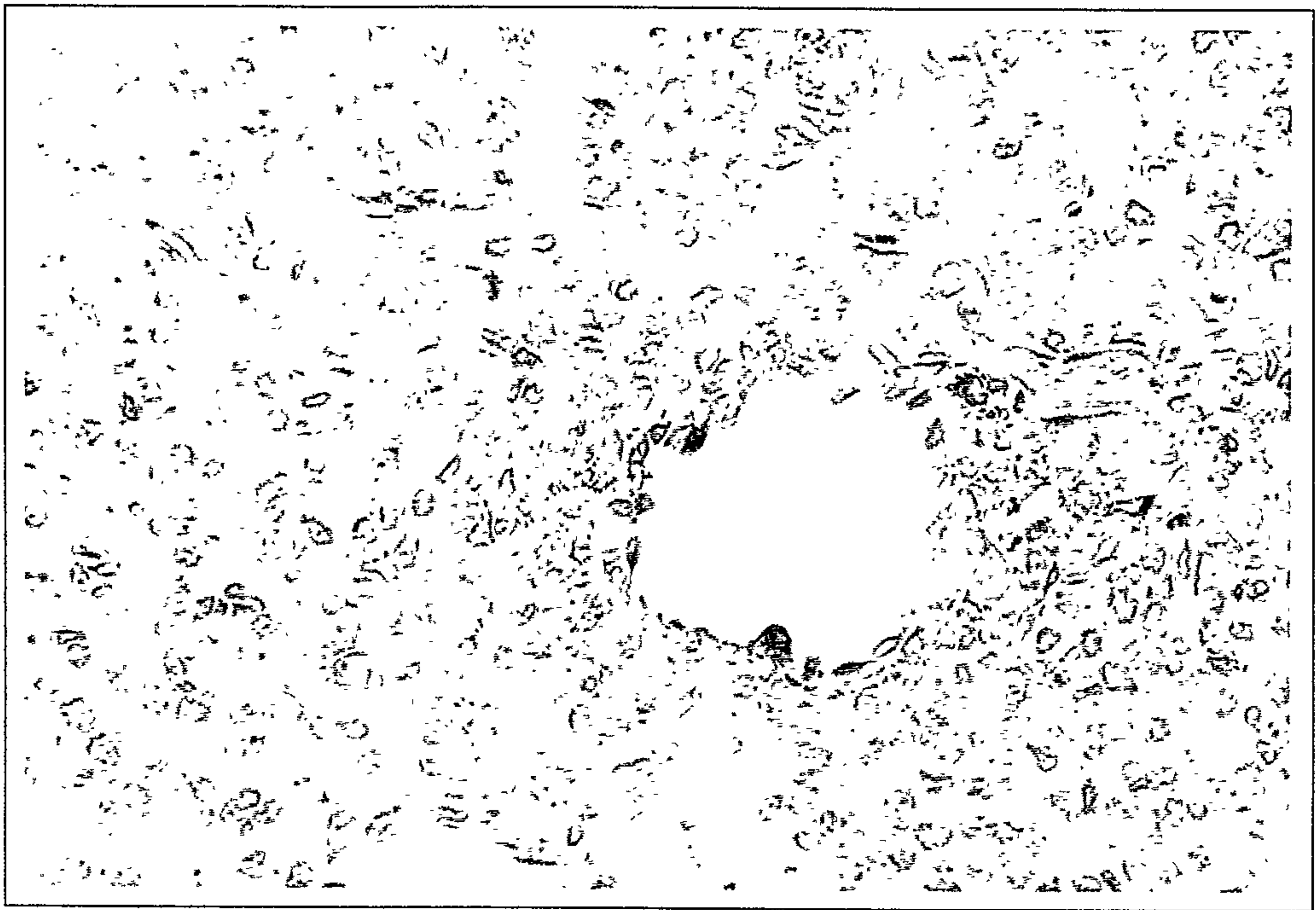


Figure 6.3 Histomicrograph illustrating the positive immunoperoxidase staining of multinucleated giant cells to MB1 (X400)

Figure 6.4 Histomicrograph illustrating the positive immunoperoxidase staining of multinucleated giant cells to MB1 (X400)

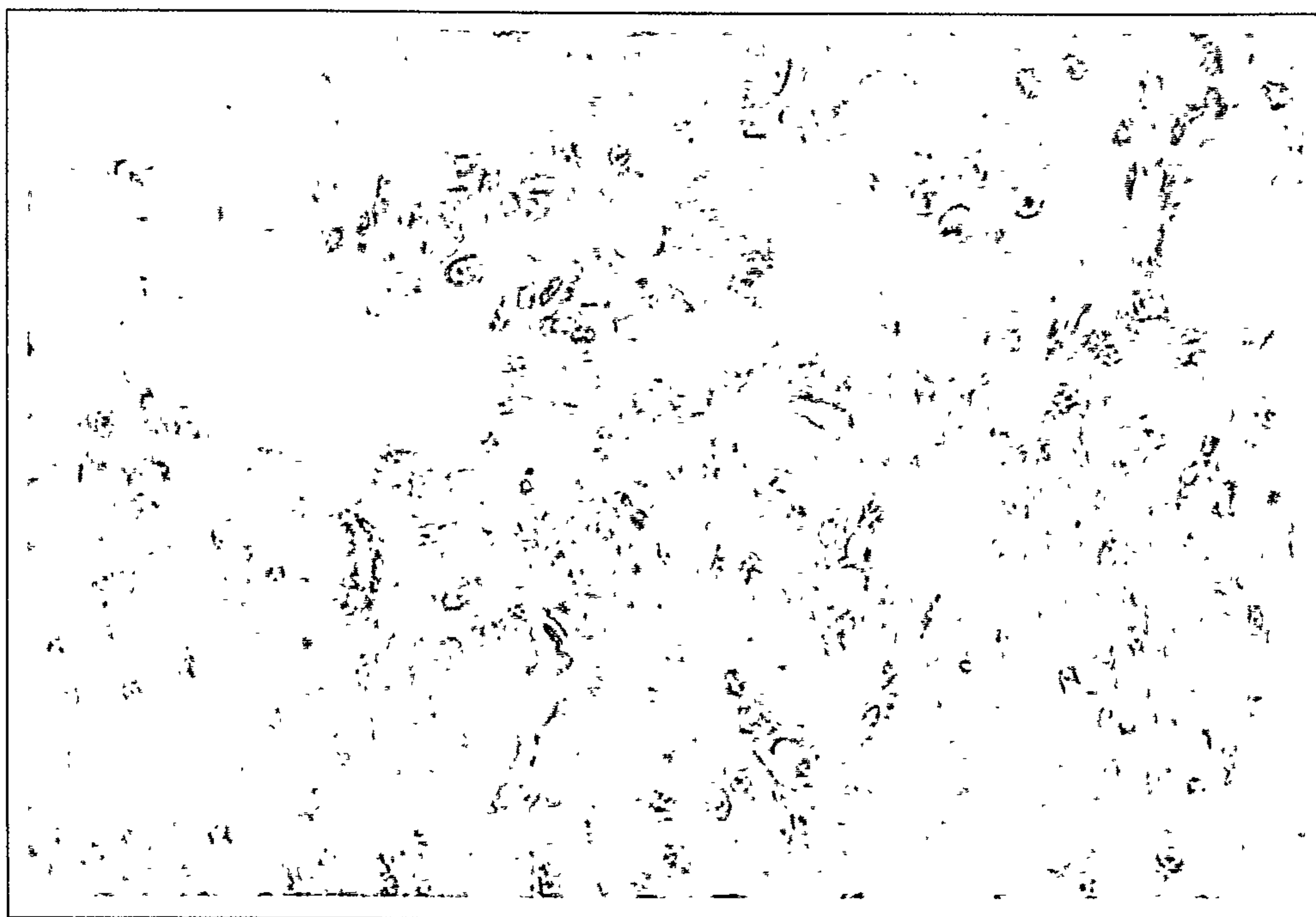
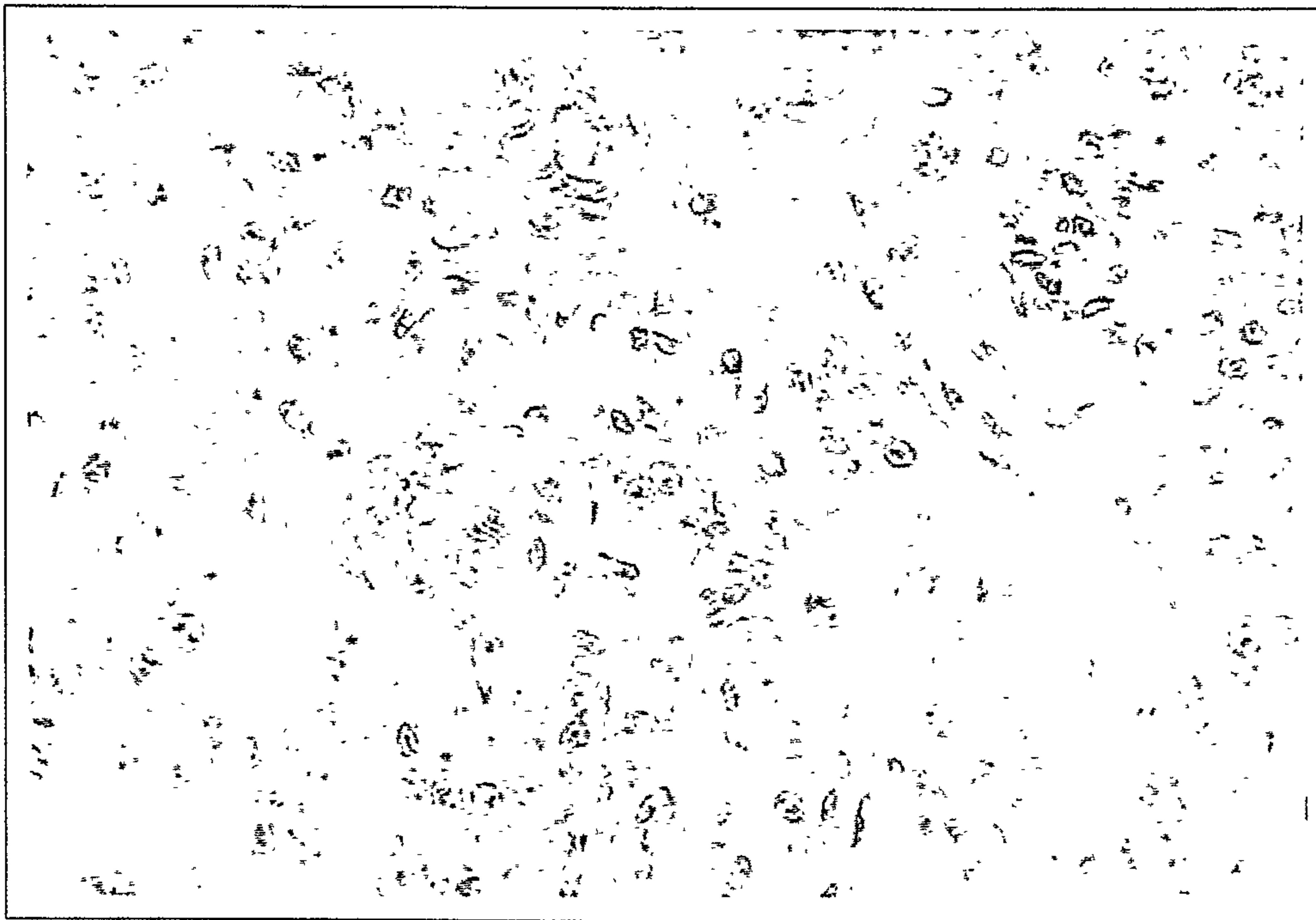


Figure 6.5 Histomicrograph showing the negative immunoperoxidase staining of the stromal spindle-shaped cells to Smooth Muscle Specific Actin (X400)

Figure 6.6 Histomicrograph illustrating the positive immunoperoxidase staining of smooth muscle cells in the walls of blood vessels to Smooth Muscle Specific Actin which served as a positive control (X400)

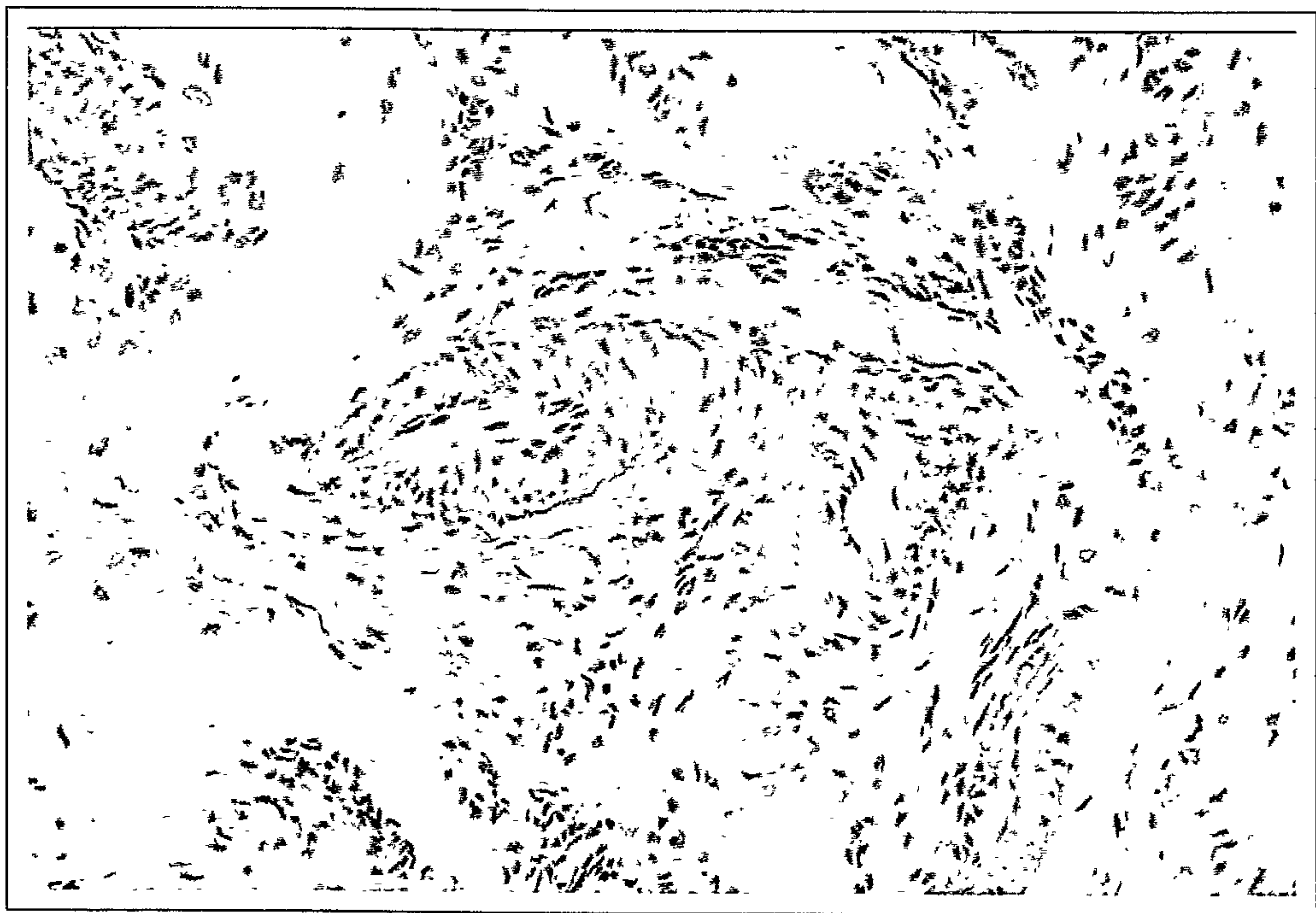
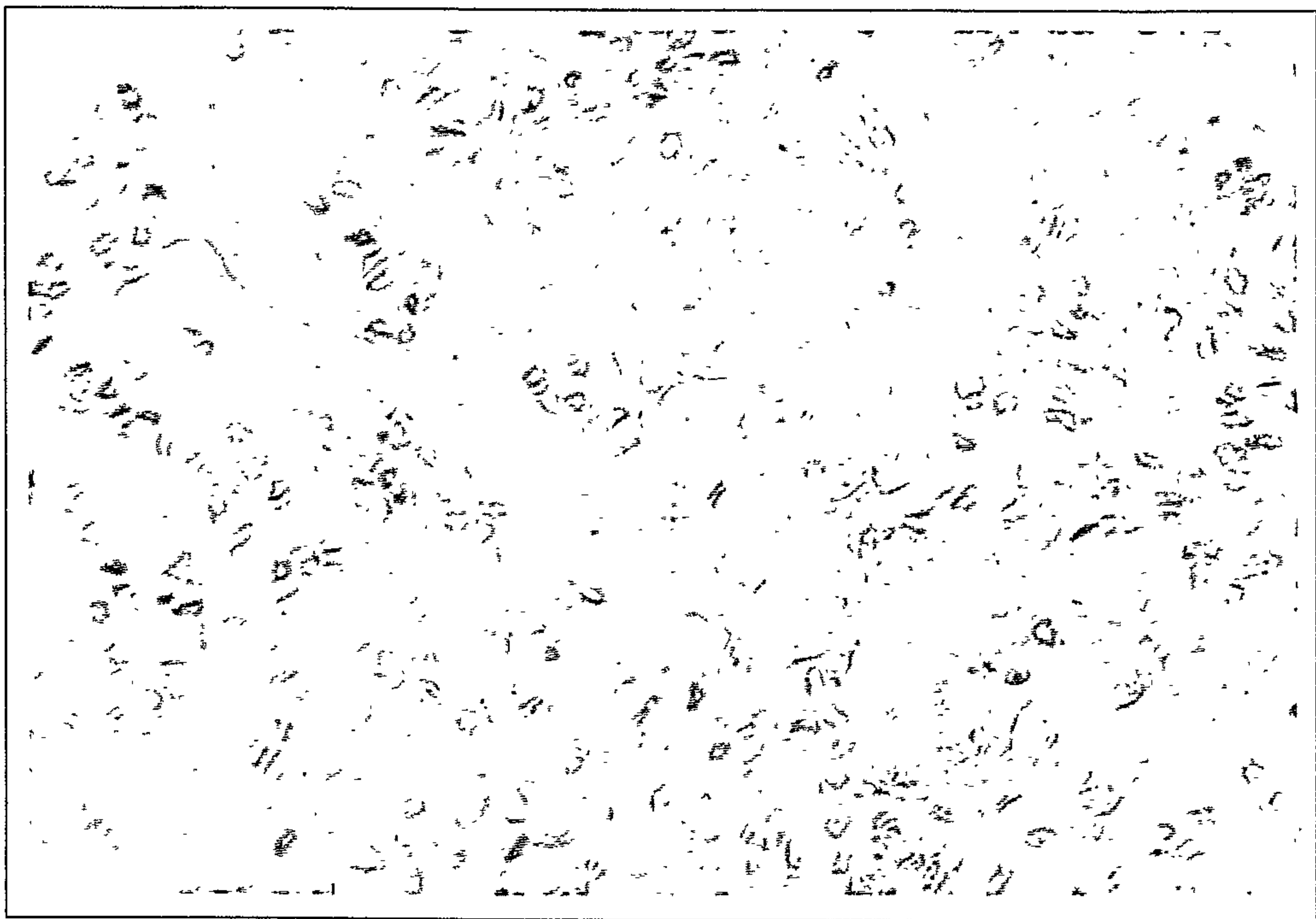


Figure 6.7 Histomicrograph illustrating the negative immunoperoxidase staining of the giant cells and the stromal cells to S-100 Protein (X400)

Figure 6.8 Histomicrograph of the positive control for S-100 Protein illustrating the immunoperoxidase staining of the myoepithelial cells of salivary gland tissue. The ductal epithelial cells also show positive staining for S-100 Protein (X400)

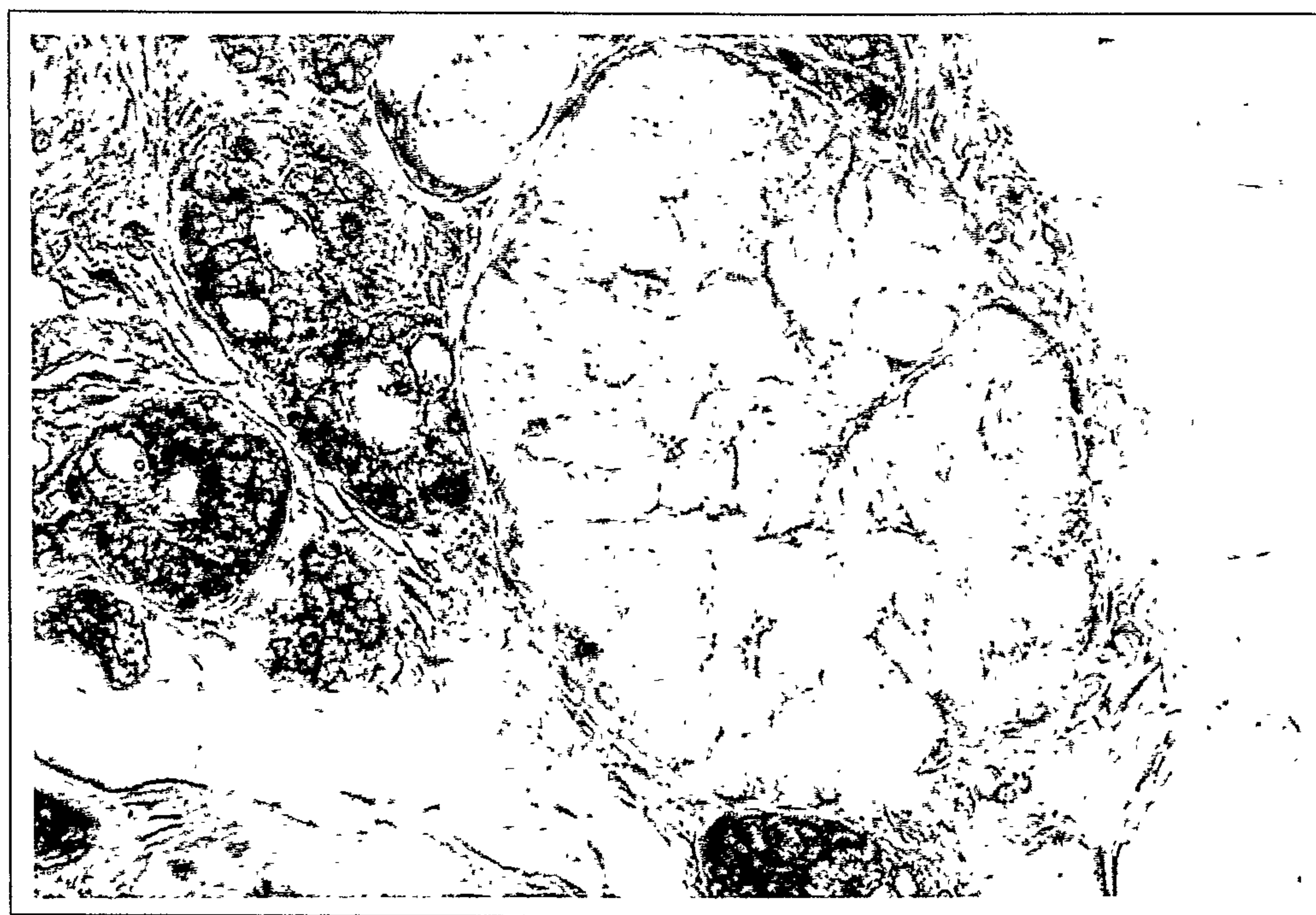
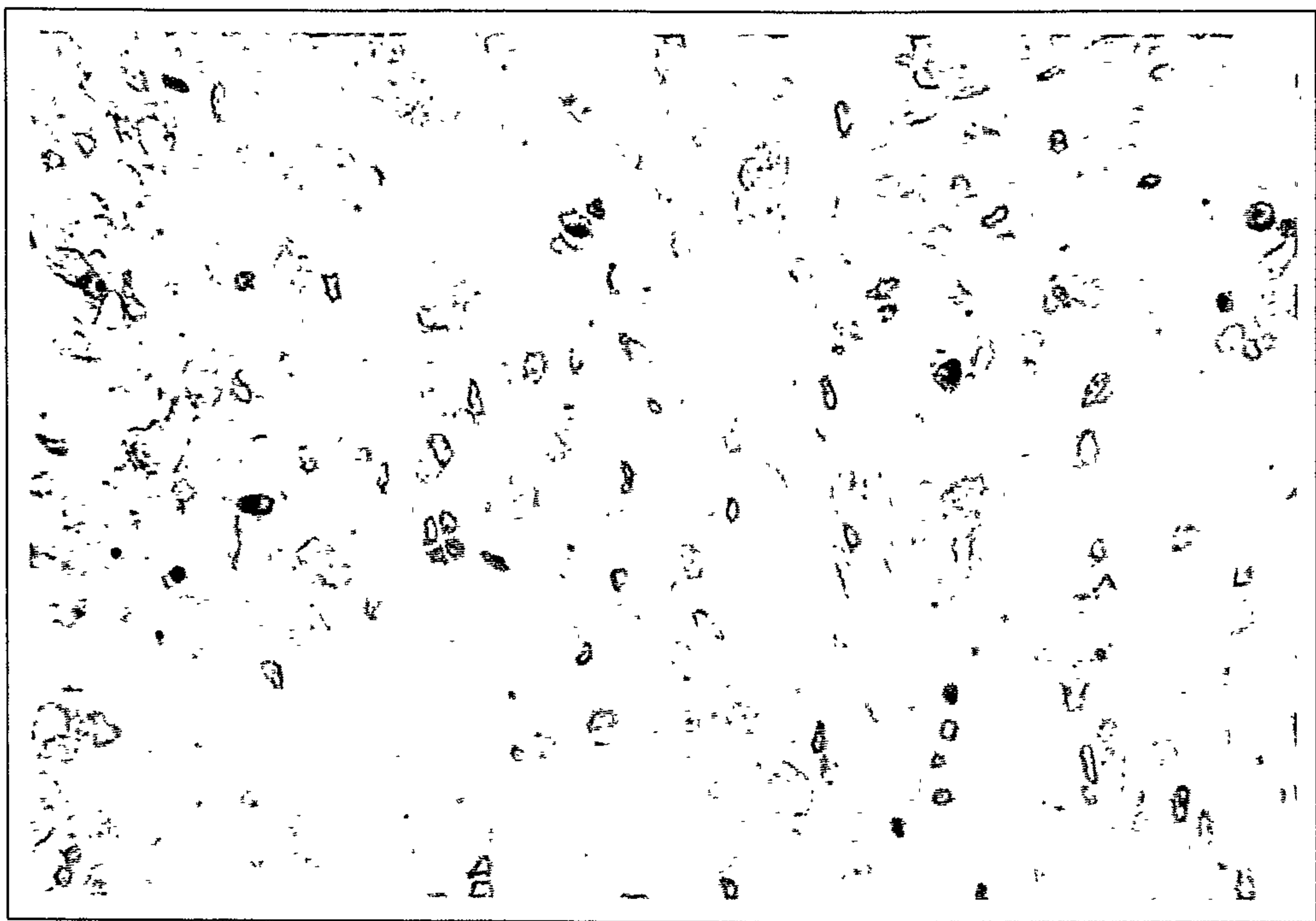


Figure 6.9 Histomicrograph illustrating the differential immunoperoxidase staining of blood vessels in the giant cell granuloma. Intense staining of the blood vessels on the periphery of the lesion is demonstrated with little evidence of reaction product deeper within the lesion adjacent to the aggregations of the giant cells (X100)

Figure 6.10 Histomicrograph illustrating heavy uptake of Factor VIII Related Antigen in the blood vessels on the periphery of the lesion with progressively less evidence of staining deeper within the lesion towards the giant cell aggregations (X200)

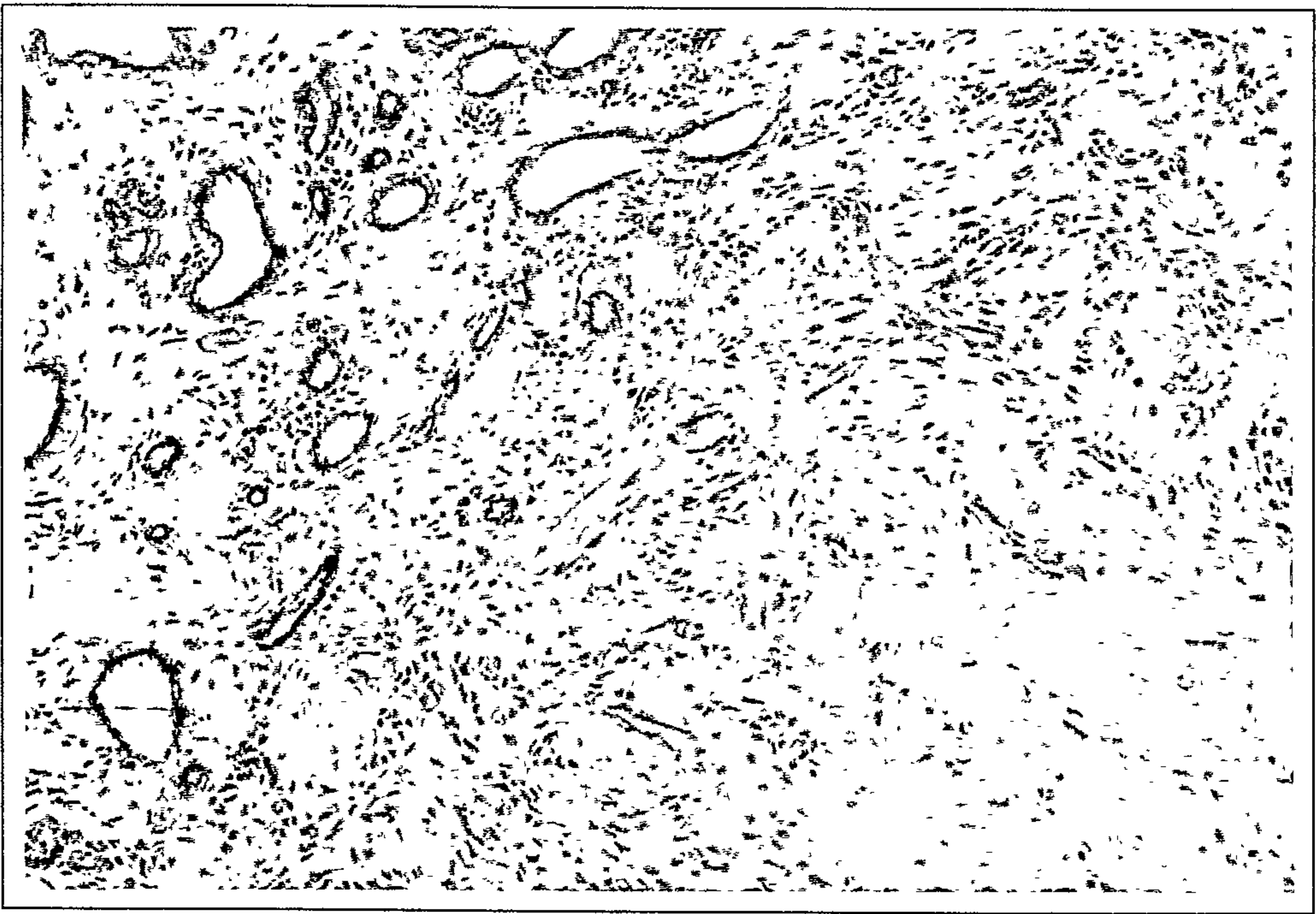
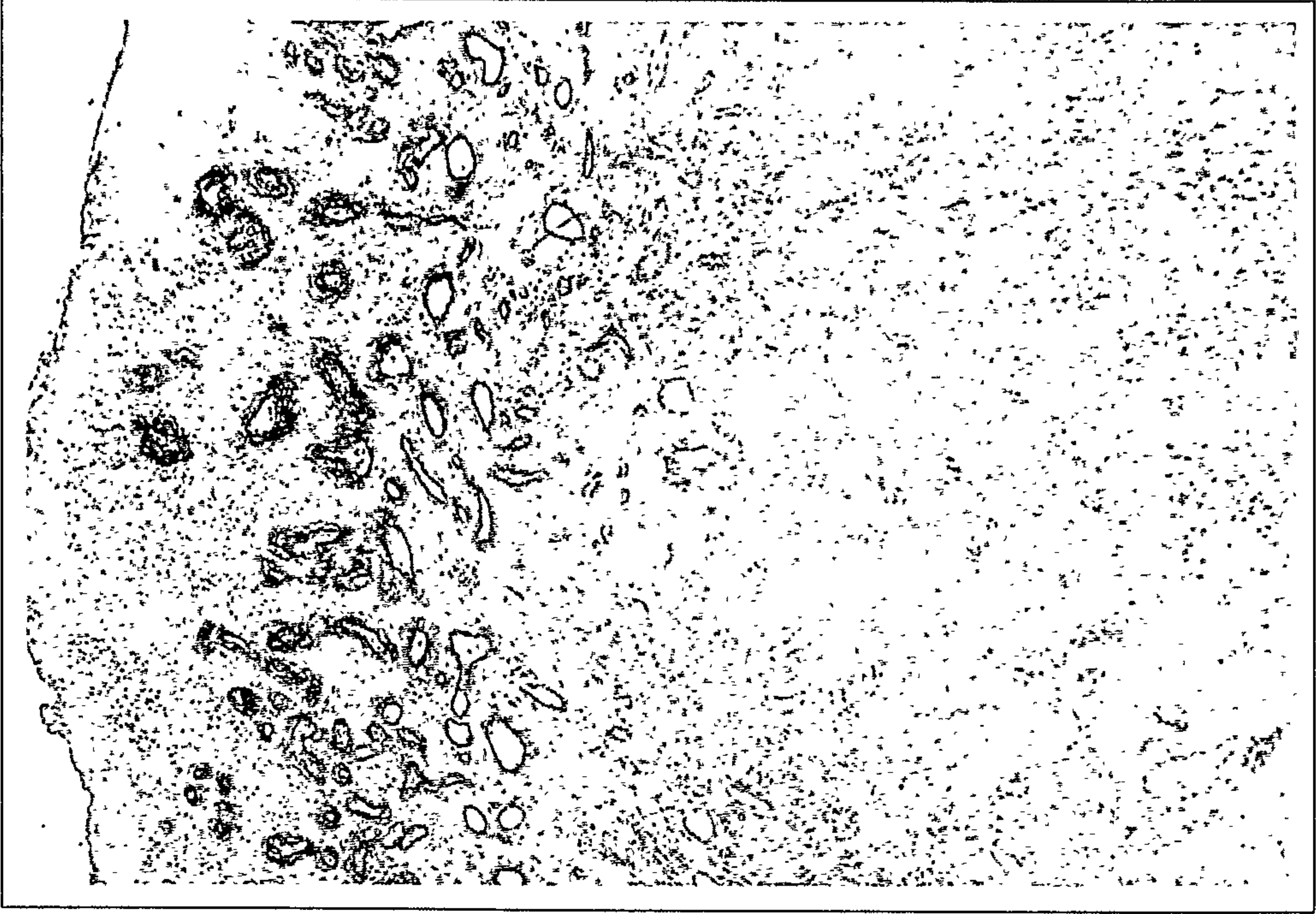


Figure 6.11 Histomicrograph illustrating the intense immunoperoxidase staining of the blood vessels on the periphery of the lesion to Factor VIII Related Antigen (X400)

Figure 6.12 Histomicrograph illustrating the differential immunoperoxidase staining of the blood vessels to Factor VIII Related Antigen (X200)

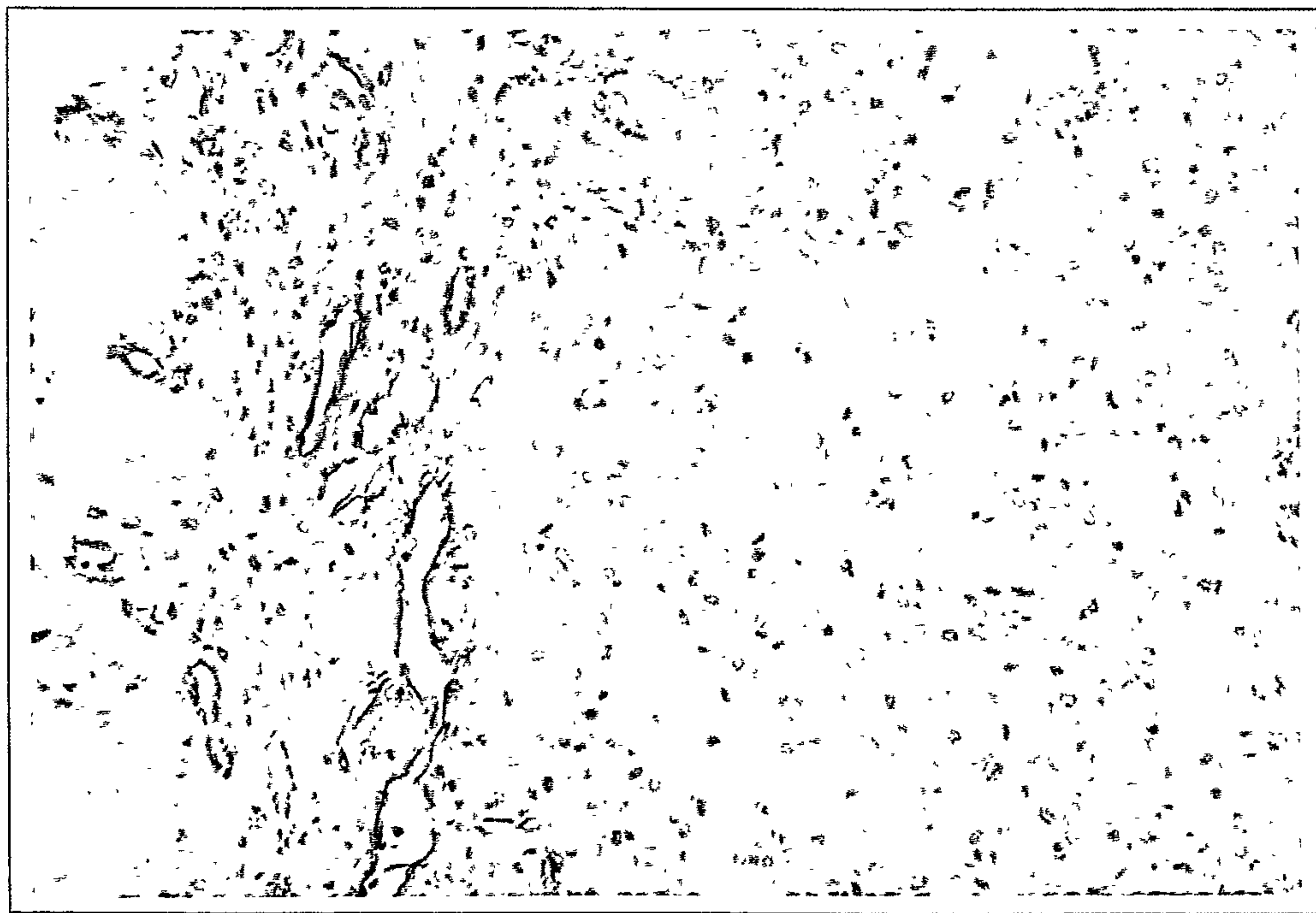
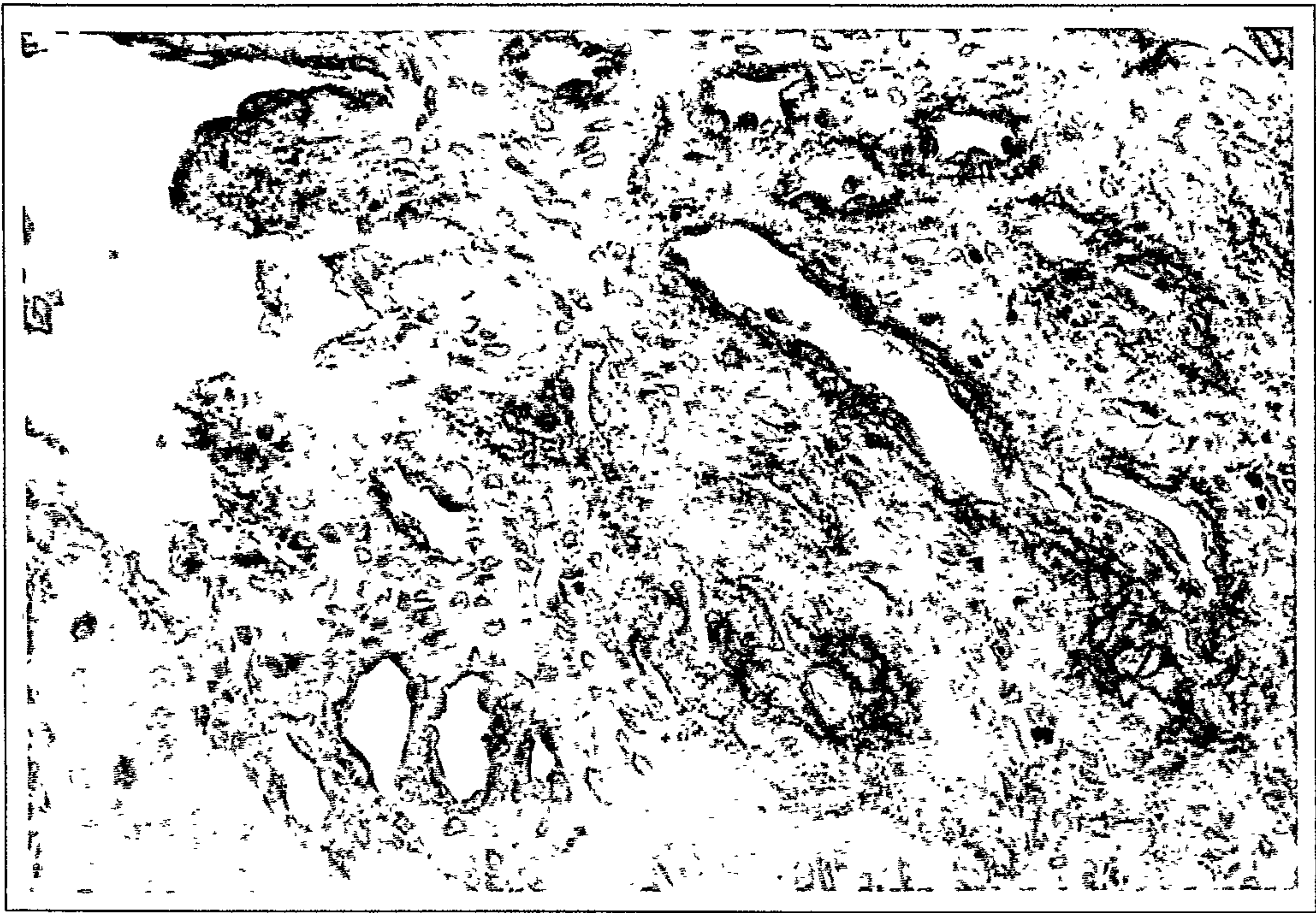


Figure 6.13 Histomicrograph illustrating the differential immunoperoxidase staining of the blood vessels to Factor VIII Related Antigen (X100)

Figure 6.14 Histomicrograph illustrating the differential immunoperoxidase staining of the blood vessels to Factor VIII Related Antigen (X200)

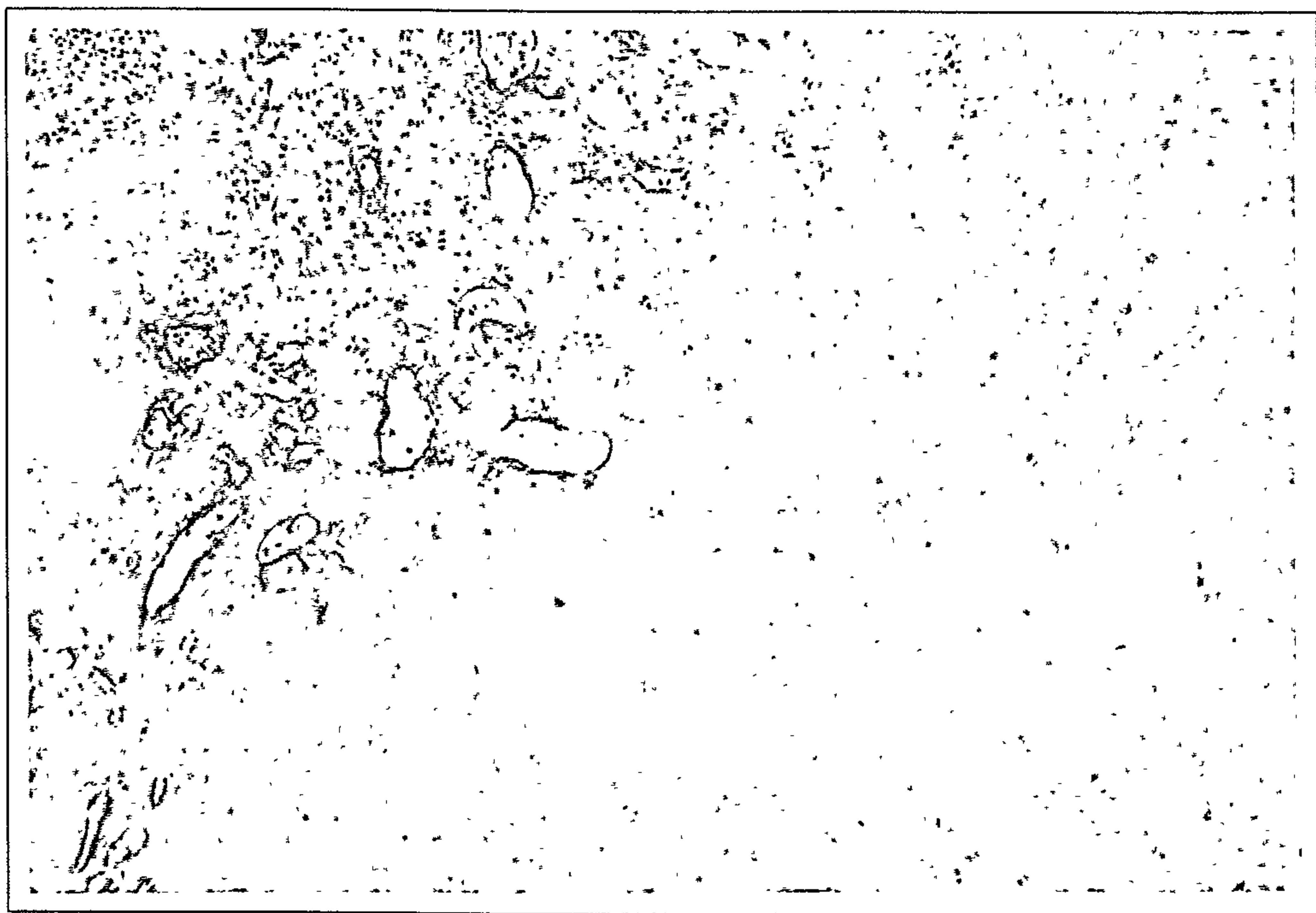
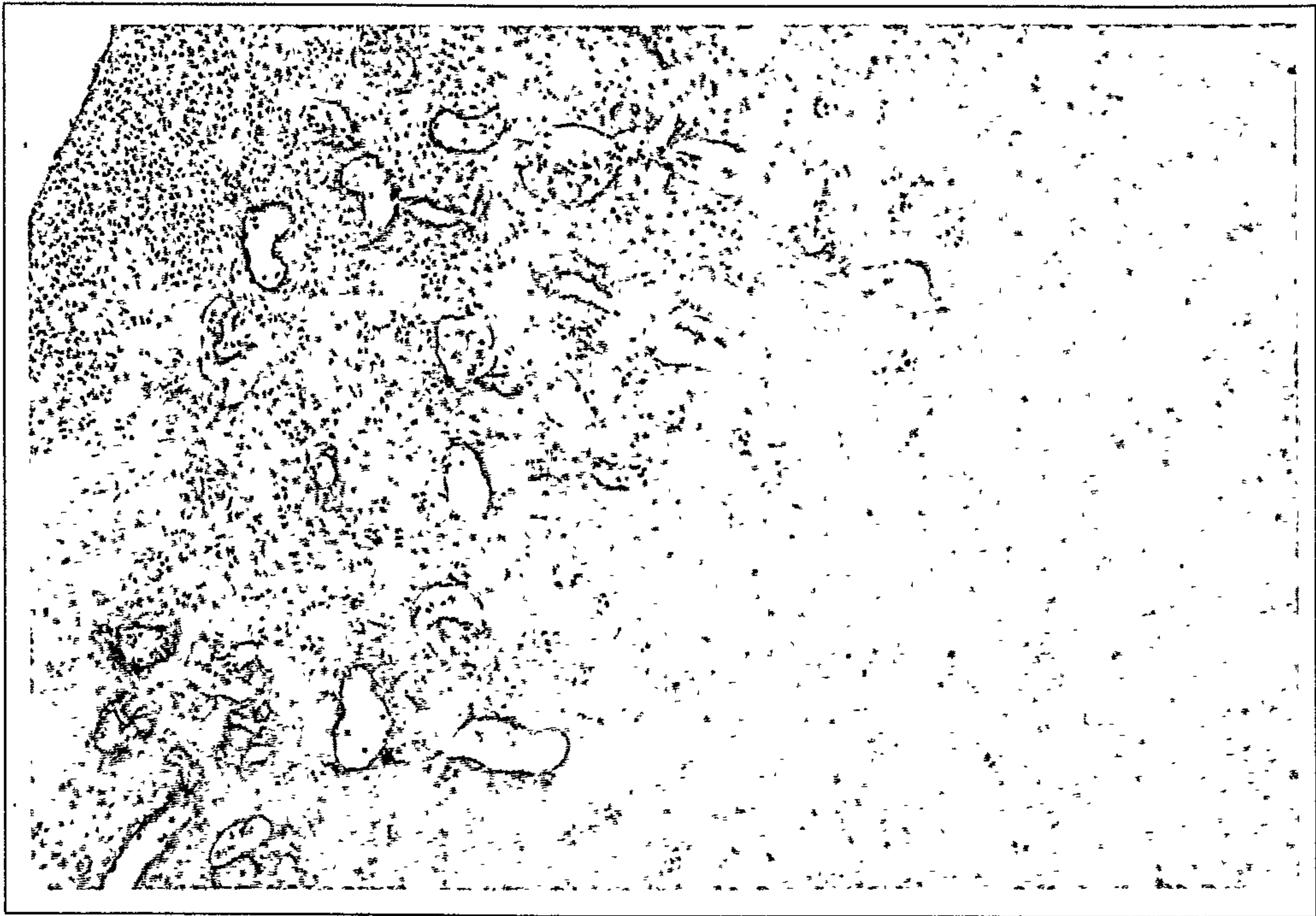
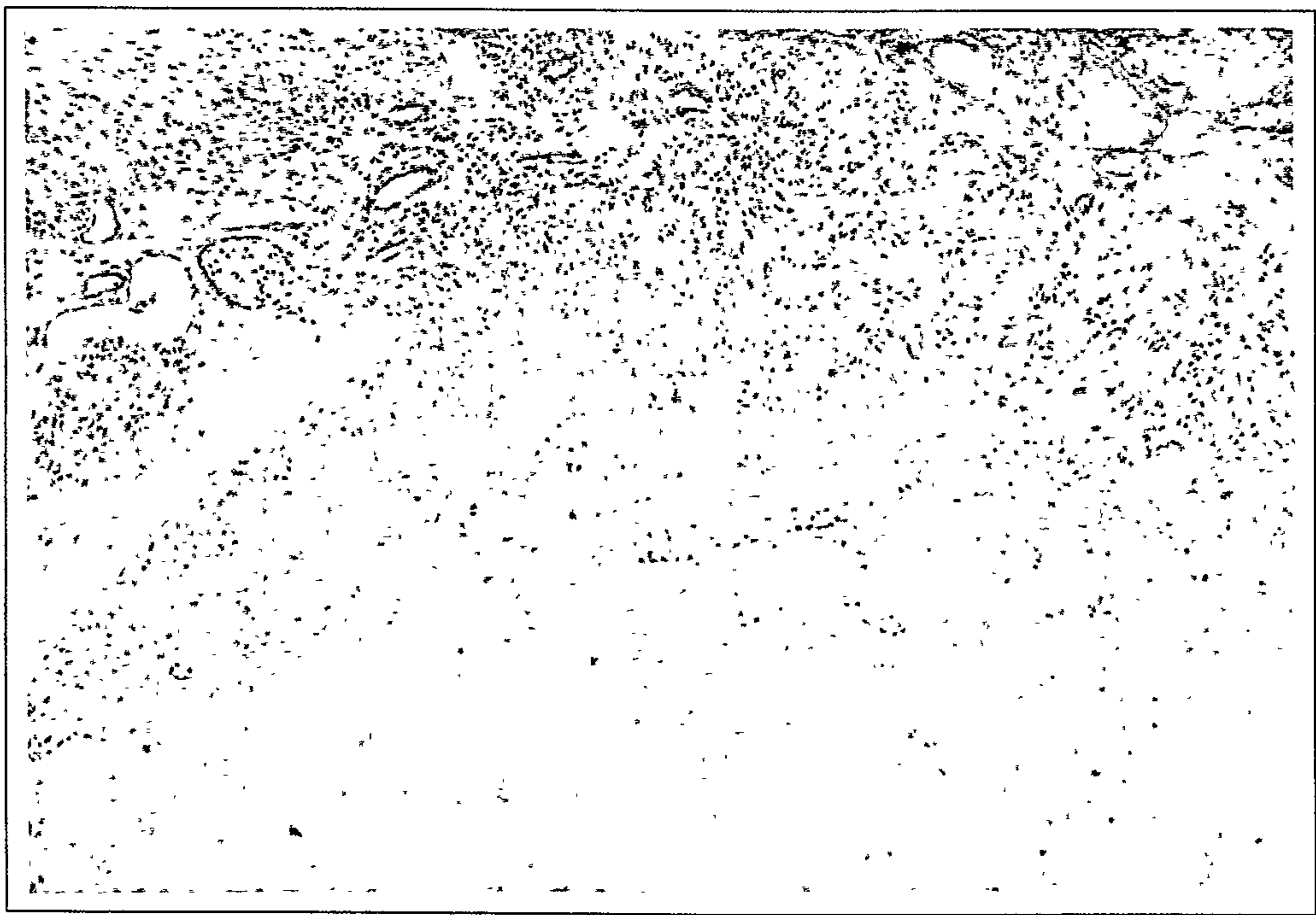
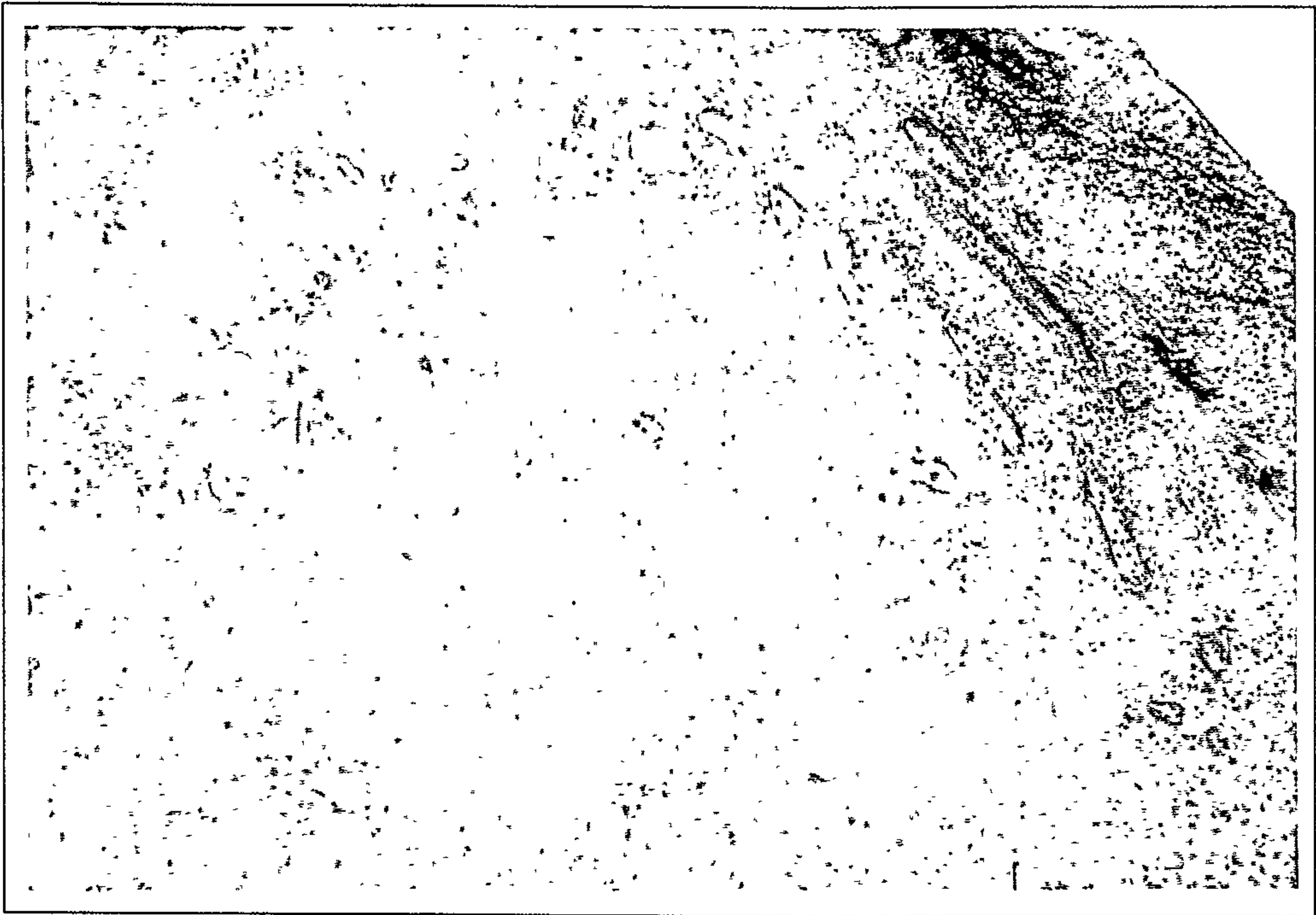


Figure 6.15 Histomicrograph illustrating the differential immunoperoxidase staining of the blood vessels to Factor VIII Related Antigen (X100)

Figure 6.16 Histomicrograph illustrating the differential immunoperoxidase staining of the blood vessels to Factor VIII Related Antigen (X100)



CHAPTER 7

DISCUSSION (PART 1)

7.1 Comparison of the Staining Pattern between the Peripheral and Central Giant Cell Granuloma and the Giant Cell Tumour of Bone

There was no significant difference in the immunoperoxidase staining pattern between the lesions. Therefore, the immunohistochemical markers used in this study have not been able to differentiate between these lesions.

7.2 Leukocyte Common Antigen

Leukocyte Common Antigen was present in the round mononuclear cells. This is consistent with the belief that these cells are macrophages.

These findings were consistent with the work of several studies. Aqel *et al* (1988) found the round mononuclear cells in giant cell tumour of bone to be positive for Leukocyte Common Antigen as well as being reactive to the macrophage markers EBM 11, 10.1 and HLA-DR(CA2). Brecher *et al* (1986) showed that the round mononuclear cells contain phagocyte associated antigens Leukocyte Common Antigen, EBM11, KB90, 60.1, FMC32, UCHM1.

The multinucleate giant cells did not react with Leukocyte Common Antigen. The presence or absence of Leukocyte Common Antigen on the giant cells is interesting in relation to the debate surrounding the origin of the giant cells. It is most likely that they arise from the same precursor cell as the macrophage. However, as Leukocyte Common Antigen is widely expressed in the majority of cells of lymphoid origin, it would seem unusual for the giant cells to fail to express this antigen if they originate from the monocytic stem cell line.

The results of this study support the findings of Horton *et al* (1985) and Athanosou *et al* (1986). These investigators used mouse monoclonal antibodies to Leukocyte Common Antigen and failed to demonstrate this antigen on the giant cells in the giant cell tumour of bone.

In contrast, variable and weak expression of Leukocyte Common Antigen on the giant cells have been reported by Brecher *et al* (1986). This discrepancy in results was attributed by these workers to the addition of an amplification step in the immunoperoxidase technique where the second and third stage incubations were repeated.

Aqel *et al* (1988) found that 4 out of 9 cases of giant cell tumour of bone were reactive to rat, but not mouse, antibody to Leukocyte Common Antigen. They observed that the degree of reactivity was proportional to the degree of infiltration with macrophages and therefore commented on the possibility of whether Leukocyte Common Antigen (CD45) is synthesised by the giant cells or merely cytophylically bound. Subsequently, CD45 molecular probes in Northern analysis were used in giant cell tumour of bone and it was noted that CD45 mRNA levels were extremely low and could be accounted for by contaminating macrophages.

However, Athanasou *et al* (1987) reported that in contrast to previous studies, Leukocyte Common Antigen was present in osteoclasts in foetal and adult bone specimens.

It is evident that with regard to the presence of Leukocyte Common Antigen on the giant cells, the literature reveals conflicting results. A possible explanation for the variability in results may be due to the fact that Leukocyte Common Antigen does not represent a single molecule but a family of structurally related proteins. Differences in specificity may be due to recognition of similar but distinct Leukocyte Common Antigen molecules. Variations in technical methods and tissue fixation could also be significant.

The previous studies cited used Leukocyte Common Antigen in giant cell tumours of bone. This is the first study to use Leukocyte Common Antigen in the giant cell granuloma. This study found a similar uptake of antibody in both the giant cell granuloma and the giant cell tumour lesions.

In summary, the findings in this study support the results of Horton *et al* (1985) and Athanasou *et al* (1986). The round mononuclear cells expressed Leukocyte Common Antigen and are therefore likely to be macrophages.

The giant cells did not react with Leukocyte Common Antigen in this study. Although this may be interpreted as evidence to support the view that they do not originate from a monocyte precursor, it is possible that the giant cells may have down-regulated some of their antigenic specificities in the process of maturation and differentiation from monocytic precursors.

7.3 MB1

The multinucleated giant cells stained strongly with MB1.

These findings are consistent with the work of Chilosì *et al* (1988) which showed that MB1 was strongly expressed by osteoclasts in foetal bone and by the multinucleated giant cells in giant cell tumour of bone. They also confirm and support the findings of Bonetti *et al* (1990) where MB1 was found to be present in 9 cases of peripheral giant cell granuloma.

As discussed previously, the work of Flanagan *et al* (1988) provided convincing evidence that the giant cells in the giant cell granuloma possess similar characteristics to osteoclasts including the ability to excavate bone *in vitro*, responsiveness to calcitonin and expression of osteoclast specific antibodies 13C2 and 23C6. The studies of Chambers *et al* (Chambers and Magnus, 1982) and Goldring *et al* (Goldring, Roelke, 1987) strongly supporting the osteoclast-like nature of the giant cells has been discussed in a previous chapter.

The results of this study, in conjunction with the findings of the studies cited above, favour the view that the giant cells in the giant cell granuloma are osteoclasts.

It is now well accepted through studies such as the parabiosis experiments of Gothring and Ericsson (1972) and Walker (1975) that the osteoclast has a haemopoietic origin with precursor cells arriving via the circulation.

However, the precursor cell remains to be identified. It is most likely that they arise from the monocyte-macrophage cell line. However, it has not been possible to demonstrate the formation of osteoclasts from macrophages (Anderson *et al*, 1979) and unlike macrophages, osteoclasts do not possess C3 and Fc receptors (Chambers,

1979), cannot phagocytise opsonised sheep red blood cells and do not contain non-specific esterase properties (Wood *et al*, 1977).

The findings of this study demonstrated that the giant cells failed to express Leukocyte Common Antigen which further undermines the possibility that the giant cell originates from the macrophage. However, it can be argued that during the process of differentiation the giant cells may lose and acquire specific surface alterations and the fact that they do not share certain phenotypic properties does not necessarily negate the possibility of their origin from the macrophage cell line.

If the giant cells are osteoclasts, then their role in the pathogenesis of the giant cell granuloma remains to be elucidated. They are most probably responsible for the destruction of bone often seen in these lesions, particularly in the central giant cell granuloma.

The fact that the giant cells are now considered to be osteoclasts may have some important implications in the clinical management of these lesions. An interesting clinical study by Harris (1993) which was influenced by the findings of Flanagan *et al* (1988) demonstrated that daily subcutaneous injections of calcitonin as an alternative to surgery resulted in total regression of 3 cases of central giant cell granuloma.

7.4 S-100 Protein

The lesions were negative for S-100 Protein. This finding was validated by the positive staining in the myo-epithelial cells (and ductal epithelial cells) in the salivary gland tissue section which served as the positive control.

As mentioned previously, the selection of this antibody was influenced by the desire to explore the possibility of this marker of cells of neural crest origin to be a diagnostic indicator for the differences between the giant cell granuloma and the giant cell tumour.

As the facial structures embryologically originate from the neural crest, the hypothesis was that S-100 Protein may be present or re-expressed in the cells of the giant cell granuloma of the jaws in contrast with those of the giant cell tumour of long bones.

Unfortunately, the lesions were found to be negative for S-100 Protein and therefore this antibody did not prove to be a useful diagnostic indicator for the differences between the lesions of the jaws and those of long bones.

This finding also confirmed the results of Regezi *et al* (1987). Interestingly, these workers also observed the presence of several S-100 Protein positive cells which had dendritic processes in 50% of the lesions studied. They considered these cells to be Langerhan cells. These findings were not observed in the lesions in this study. It should be noted however that these dendritic cells in the study of Regezi *et al* (1987) were only observed in 11 out of 22 peripheral giant cell granulomas and 13 out of 22 central giant cell granulomas and were reported to possess "moderate immunoreactivity" with less than 25% of the cells reacting positively.

7.6 Smooth Muscle Specific Actin

This antibody was directed in particular at the stromal spindle-shaped cells which are believed to be myofibroblasts (Dayan *et al*, 1989; El-Labban and Lee, 1983). These cells were found to be negative for this marker while smooth muscle cells in the walls of blood vessels in the connective tissue of the lesions stained positively.

This finding is interesting in the light of the results of Tsukada *et al* (1987) which showed the presence of Smooth Muscle Specific Actin in the myofibroblasts of healing wounds. It was also shown to be present in the hyperplastic fibrous tissue response of Dupuytren's contracture and in the stroma of breast carcinoma.

The concept of the myofibroblast was introduced by Gabbiani *et al* (1971) to describe the modified fibroblast with smooth muscle-like features present in granulation tissue with a possible role in wound contraction. These workers comment that the fibroblast and smooth muscle cell are more closely related than previously believed and speculate that "either cell may be capable of modulating towards an intermediate type" (Gabbiani *et al*, 1971).

Gabbiani and co-workers subsequently analysed the intermediate filament protein composition of these myofibroblast cells using immunohistochemistry and found them to express vimentin and not desmin which supports the concept of the derivation of these cells from fibroblasts and not smooth muscle cells (Schurch *et al*, 1984).

However, in another study involving Gabbiani (Kocher *et al*, 1984) it was shown that intimal smooth muscle cells following injury can present with a vimentin-positive and desmin-negative immunophenotype. In cases of advanced pulmonary fibrosis, cells with ultrastructural features of smooth muscle have been demonstrated to be desmin-negative (Adler *et al*, 1981; Woodcock-Mitchell *et al*, 1984).

Therefore, it is suggested that smooth muscle cells may be able to develop organelles associated with synthesis and secretion of connective tissue proteins when appropriately stimulated. Fibroblasts may also develop cellular modifications similar to smooth muscle cells in tissues such as granulation tissue of healing wounds and play an active role in the process of wound contracture.

The spindle-shaped cells in the giant cell granuloma and giant cell tumour are ultrastructurally similar to the myofibroblasts described by Gabbiani *et al* (1971). However, they differ immunohistochemically in their expression of Smooth Muscle Specific Actin which was found to be negative in this study in contrast to the myofibroblasts in healing wounds in the study of Tsukada *et al* (1987). Significantly, Tsukada *et al* (1987) found this antibody to be negative in two specimens of giant cell tumour of bone which supports and confirms the results of this study.

There is generally very little other information at present on the nature of the spindle-shaped cells in these giant cell lesions. Apart from their ultrastructural features, studies have demonstrated their ability to undergo active proliferation in culture (El-Mofty and Osdoby, 1985; Goldring *et al*, 1987) and transplantation (Cohen *et al*, 1988) which has not been observed to be the case for the multinucleated giant cells.

The spindle-shaped cells were unreactive to all the antibodies used in this study. They therefore not of haemopoietic or endothelial origin. This study has also demonstrated that while these cells ultrastructurally resemble myofibroblasts they do not express Smooth Muscle Specific Actin. They are therefore phenotypically different to the myofibroblasts in the study by Tsukada *et al* (1987). The nature and histogenesis of the spindle-shaped cells and their role in the pathogenesis of the giant cell granuloma remains to be elucidated.

7.7 Factor VIII Related Antigen

The most interesting finding in this screening study was the differential reactivity of the blood vessels to Factor VIII Related Antigen.

There are three published immunohistochemical studies where the antibody to Factor VIII related antigen was used to study the giant cell granuloma and the giant cell tumour (Aqel *et al*, 1988; Brecher *et al*, 1986; El-Mofty and Osdoby, 1985). These studies found that the giant cells, the macrophage cells and the spindle-shaped cells were negative for this endothelial cell marker and these findings were confirmed in this study.

In relation to the blood vessels within the lesion, these studies provided very little information. El-Mofty and Osdoby (1985) reported that "the endothelial cells of the blood capillaries outside the lesion were strongly positive" but failed to define the meaning of the statement "outside the lesion" and did not comment any further on this finding.

Aqel *et al* (1988) stated that they "are negative for markers of endothelial cell origin" and Brecher *et al* (1986) commented that "only endothelial cells reacted with antibody to Factor VIII" and did not describe or continue to discuss this finding.

It is not surprising that these studies placed minimal attention on the vasculature in these giant cell lesions as it was assumed that the multinucleated giant cells were the all important component in the lesion. The studies were primarily directed at analysing the giant cells and their cellular origins.

This study found the blood vessels on the periphery of the lesion to stain intensely with this antibody. However, deeper within the lesions and within the aggregations of the multinucleated giant cells, reaction product was not evident.

The concept that endothelial cells are a homogenous group of cells lining a variety of vascular spaces is no longer held and it is now well recognised that endothelial cells represent a phenotypically diverse group of cells that vary morphologically and functionally from site to site. The recent advances in the understanding of the

biology of endothelial cells is summarised in reviews by Fajardo *et al* (1989) and Thorgeirsson and Robertson (1978).

Turner *et al* (1987) demonstrated the phenotypic diversity of endothelial cells in situ with immunohistochemistry. They found that endothelial cells possess heterogeneous phenotypes that correlate with vessel type. Therefore, not all endothelial cells express Factor VIII Related Antigen. This antigen has been shown to be absent in splenic sinusoids, lymphatics and glomerular capillaries and known to be present in arterioles, capillaries, venules and high endothelial venules (Turner *et al*, 1987).

The expression of Factor VIII Related Antigen also appears to be related to the degree of differentiation of endothelial cells. Mukai *et al* (1980) found that Factor VIII staining was strongest in small vessels and Wilson (1984) demonstrated variable staining for Factor VIII Related Antigen in vascular neoplasms. Burgdorf *et al* (1981) showed that poorly differentiated vascular tumours in particular, angiosarcomas and the proliferating spindle cells in Kaposi's sarcoma were frequently negative for Factor VIII Related Antigen.

The differential reactivity of the blood vessels to Factor VIII Related Antigen in this screening study led to the development of Part 2 of the study where the vasculature in these lesions were further investigated.

CHAPTER 8

THE STUDY (PART 2)

8.1 Introduction

The significant finding from Part 1 of the study was the differential staining of the blood vessels to Factor VIII Related Antigen in the giant cell granulomas. Further study of the vasculature of the lesion was considered worthwhile for several reasons. Clinically, these giant cell lesions appear to be vascular in nature. In addition, the intimate relationship of the blood vessels with the multinucleated giant cells histologically and ultrastructurally has also been observed in several studies (Adkins *et al*, 1969a; Katsikeris *et al*, 1988) and has even prompted the speculation for the endothelial origin of the giant cells (Drepper *et al*, 1961).

These giant cell lesions could possibly be reactive in nature. As the process of neo-vascularisation is a significant stage in the event of healing and repair, the blood vessels in these lesions could perhaps play an important role in this regard.

Finally, the giant cells are now believed to be osteoclasts (Flanagan *et al*, 1988). Osteoclasts have been shown to originate from bone marrow precursors (Gothlin and Ericsson, 1972) and by implication, must therefore arrive on site via the circulation. The vasculature may therefore be an important link in understanding the pathogenesis of these lesions.

The interpretation of the findings with Factor VIII Related Antigen is influenced by the unreliable expression of this antigen reported in a variety of pathological entities (Burgdorf *et al*, 1981; Wilson, 1984).

Two other endothelial cell markers, Ulex Europae 1 Lectin and QBend 10 were used to extend the study of the blood vessels in the lesion and an investigation of the ultrastructure of the vasculature using transmission electron-microscopy was undertaken.

8.2 Aims

The aim of Part 2 of this study was to obtain more information on the nature of the vasculature of the giant cell granuloma of the jaws using Immunohistochemistry and Transmission Electron-Microscopy.

1. Immunohistochemistry

To use other specific endothelial cell markers, Ulex Europae 1 lectin and QBend 10. These markers were chosen as they have been reported to be more reliable in the staining of vascular and neoplastic proliferations when compared with Factor VIII Related Antigen (Miettinen *et al*, 1982; Ordonez and Batsakis, 1984; Ramani *et al*, 1990).

2. Transmission Electron-Microscopy

To investigate and describe the ultrastructure of the endothelium of the giant cell granuloma using transmission electron-microscopy.

8.3 Materials and Methods

8.3.1 Immunohistochemistry

8.3.1.2 Tissue specimens

10 archival tissue specimens were selected from the group obtained in Part 1 of the study.

These included:

- 4 central giant cell granulomas
- 4 peripheral giant cell granulomas
- 2 giant cell tumours of bone

8.3.1.3 Endothelial Cell Markers

The endothelial cell markers chosen were:

1. Ulex Europae Agglutinin Type 1 (DAKO, California, U.S.A.)
2. QBend 10 (Novacastra, Newcastle upon Tyne, U.K.)

8.3.1.4 Immunoperoxidase Procedure

The three-stage indirect technique (Sternberger *et al*, 1970) was used for Ulex Europae Agglutinin Type 1 and the avidin-biotin complex method for QBend 10.

The methods used are similar to those described in Part 1 of the study with minor differences. Therefore, only these alterations in the techniques used will be described in this section.

1. Ulex Europaes 1 Lectin

The sections were incubated with Ulex Europaes 1 Lectin (DAKO, California, U.S.A.) in a dilution of 1:100 at room temperature for 30 minutes.

A rabbit antibody to Ulex Europaes 1 Lectin (DAKO, California, U.S.A.) was then used at a dilution 1:200 at room temperature for a further 30 minutes.

Swine-anti-rabbit Immuglobulin conjugated to peroxidase (DAKO, California, U.S.A.) was used at a dilution of 1:100 at room temperature for 30 minutes.

2. QBend 10

The primary monoclonal antibody QBend 10 (Novacastra, Newcastle upon Tyne, U.K.) was used at a dilution of 1:25 and the sections incubated for 30 minutes at room temperature. The second and third antibody procedure was identical to that described in Part 1 of the study.

8.3.1.5 Controls

The positive controls consisted of formalin-fixed paraffin-embedded specimens of endometrium and angiosarcoma.

Negative controls consisted of omitting the primary antibody.

8.3.2 Transmission Electron-Microscopy

8.3.2.1 Tissue Specimens

Two fresh specimens of peripheral giant cell granuloma were obtained. The first was a recurrent mandibular lesion from a 6 year old male and the second was a large lesion on the posterior edentulous mandibular ridge from an 80 year old male patient.

8.3.2.2 Fixation and Embedding

The specimens were:

1. Trimmed with a scalpel into small blocks of approximately 5 mm by 3 mm and fixed in Karnovsky's solution for 3 hours.
2. Rinsed several times in buffer for 60 seconds and then placed in the buffer solution overnight.
3. Fixed in Osmium Tetroxide for 3 hours.
4. Dehydrated in a series of Alcohols (50%, 70%, 95%, 100% and 100%) with 0.1% Sodium Chloride for a period of 10 minutes each.
5. Placed in 100% Acetone for 10 minutes. This step was repeated once.
6. Placed in Acetone-Resin mixture in a 1:1 ratio for 1 hour and then infiltrated with Spurs Resin at 70 degrees Celsius for 10 minutes. This step was repeated 3 times.

7. Embedded in easy moulds and polymerised at 70 degrees Celsius for 8 hours.

8.3.2.3 Preparation of Semi-Thin Sections

The embedded sections were:

1. Sectioned with an ultramicrotome (Reichert-Jung) using a glass knife at 0.5 μm and placed on a glass slide.
2. Counterstained with Methylene Blue.
3. Examined under a conventional light microscope and a diagram was drawn to show the area of interest. The areas with more abundant blood vessels were chosen.

8.3.2.4 Sectioning for Transmission Electron-Microscopy

1. The specimens were sectioned in accordance with the area selected from the semi-thin sections using an ultramicrotome (Reichert-Jung). A diamond knife was used to cut the specimens into sections of 90 nm.
2. The sections were placed on a circular copper grid.

8.3.2.5 Counter-Staining

The sections on the grid were:

1. Placed in 2% ethanolic uranyl acetate for 15 minutes.
2. Washed in distilled water for a few seconds.
3. Placed in a solution of Lead Citrate for 4 minutes.

8.3.2.6 Analysis

The ultrastructure of the endothelium was examined with a Phillips CM 10 Transmission Electron-Microscope.

CHAPTER 9

RESULTS (PART 2)

9.1 Immunohistochemistry

The immunohistochemical findings with respect to the blood vessels in the giant cell granuloma was similar to that of Factor VIII Related Antigen. The endothelial cells on the periphery of the lesion were observed to stain positively with Ulex Europae 1 Lectin (Figures 9.1.1, 9.1.2 and 9.1.3) and QBend 10 (Figures 9.1.5 and 9.1.6) whilst deeper within the lesion adjacent to the giant cell aggregates no reaction product could be identified (9.1.4, 9.1.7 and 9.1.8). Ulex Europae 1 Lectin was also found to strongly stain the epithelial cells in the peripheral giant cell lesions (Figures 9.1.1 and 9.1.3) and this was not seen in the sections immunostained with QBend 10.

9.2 Transmission Electron-Microscopy

The micro-circulation on the periphery of the lesions showed distinct ultrastructural differences relative to deeper within the lesion amongst the aggregates of the multinucleated giant cells.

The blood vessels on the periphery of the lesion were lined with well-formed plump endothelial cells with basal lamina and intercellular junctions (Figure 9.2.1). There were consistent signs of acute inflammation with a striking abundance of

polymorphonuclear leukocytes and macrophages associated with the endothelial cells (Figure 9.2.2). These inflammatory cells are seen both in the lumen of the vessel (Figure 9.2.3) and in the soft tissue stroma of the lesion (Figure 9.2.4). They were also observed to be fixed in the process of migrating through the inter-endothelial cell junctions (Figure 9.2.5).

The basal lamina of some of the endothelial cells on the periphery of the lesion were observed to be split or "reduplicated" (Figure 9.2.6).

Deeper within the lesion associated with the aggregations of giant cells, blood vessels with normal ultrastructure were not readily found (Figures 9.2.7 to 9.2.12). The endothelium in these deeper lesions were difficult to recognise and identify despite an abundance of erythrocytes. Frequently, erythrocytes were aligned in rouleaux formation as though travelling within the lumen of a vessel but with no evidence of adjacent endothelial cell lining (Figures 9.2.11 and 9.2.12).

Figure 9.1.1 Histomicrograph illustrating differential reactivity of the blood vessels to Ulex Europaes 1 Lectin showing the positive staining of the blood vessels on the periphery of the lesion while in the deeper areas of the lesion closer to the giant cell aggregations minimal reaction product was evident. The positive staining of the epithelial cells is also illustrated (X200)

Figure 9.1.2 Histomicrograph showing the gradual transition of reactivity of the blood vessels to Ulex Europaes 1 Lectin (X400)

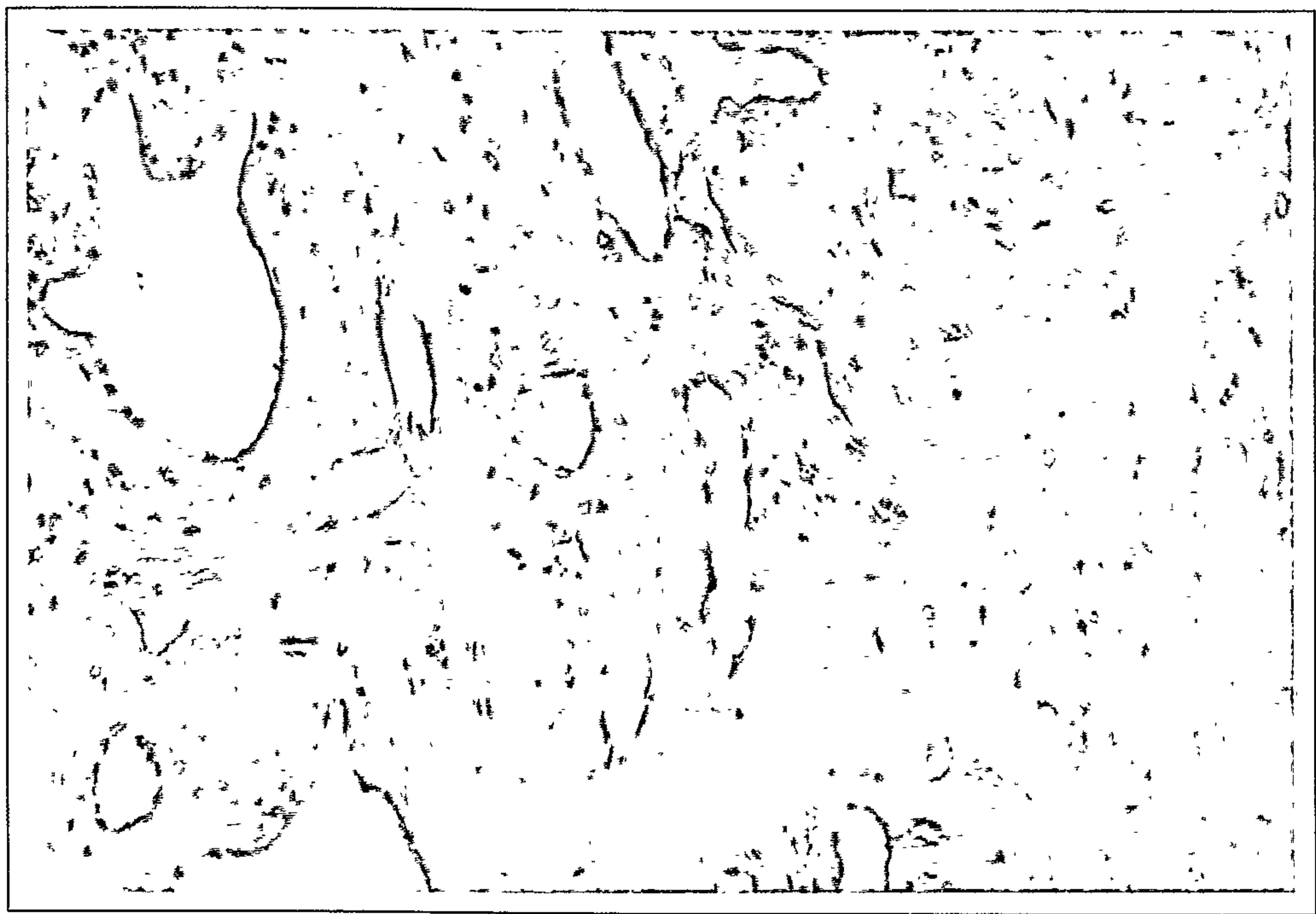
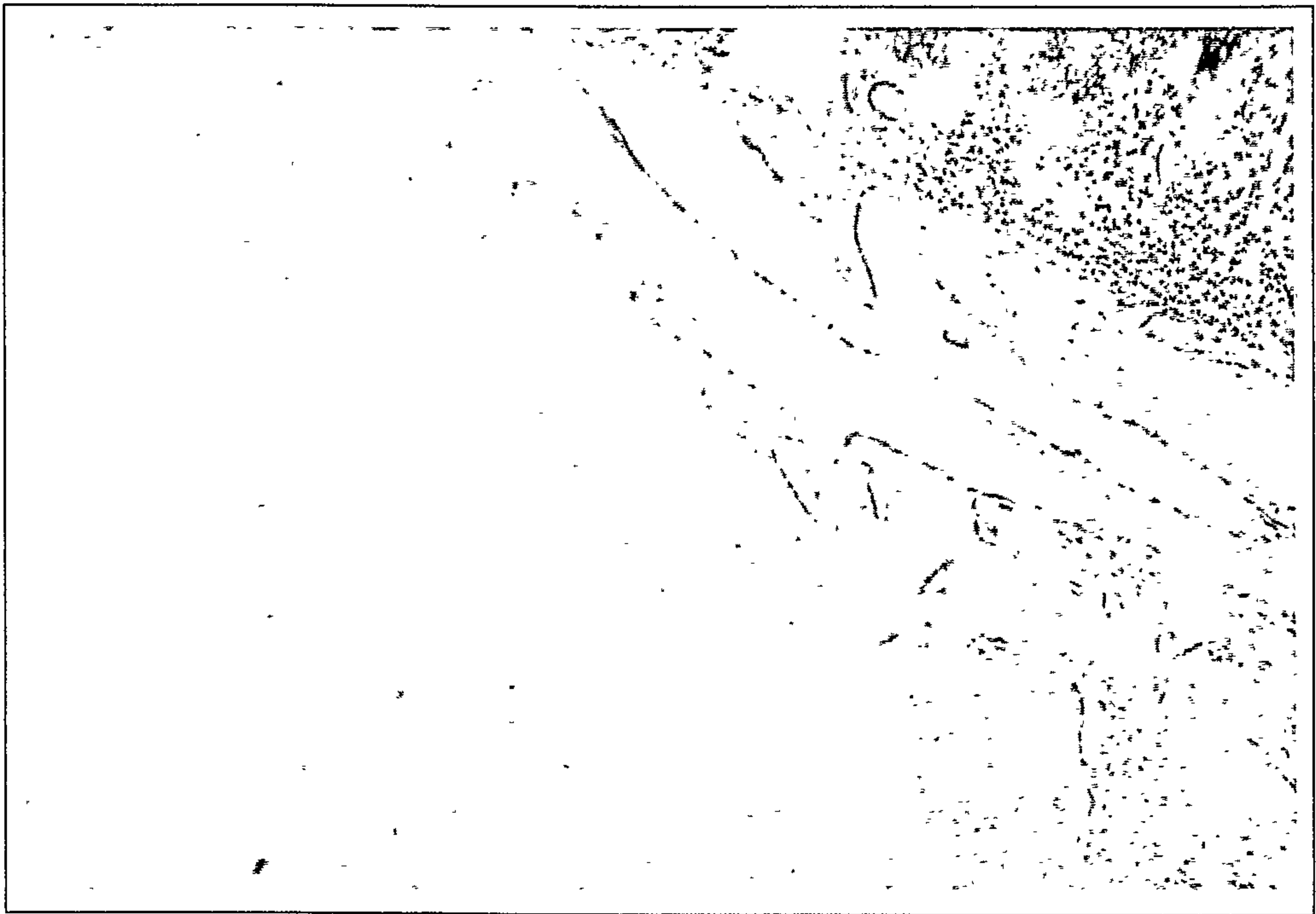


Figure 9.1.3 Histomicrograph illustrating the intense staining of blood vessels (and epithelial cells) on the periphery of the lesion to Ulex Europaes 1 Lectin (X400)

Figure 9.1.4 Histomicrograph of the deeper areas of the lesion illustrating the negative staining of the blood vessels to Ulex Europaes 1 Lectin (X400)

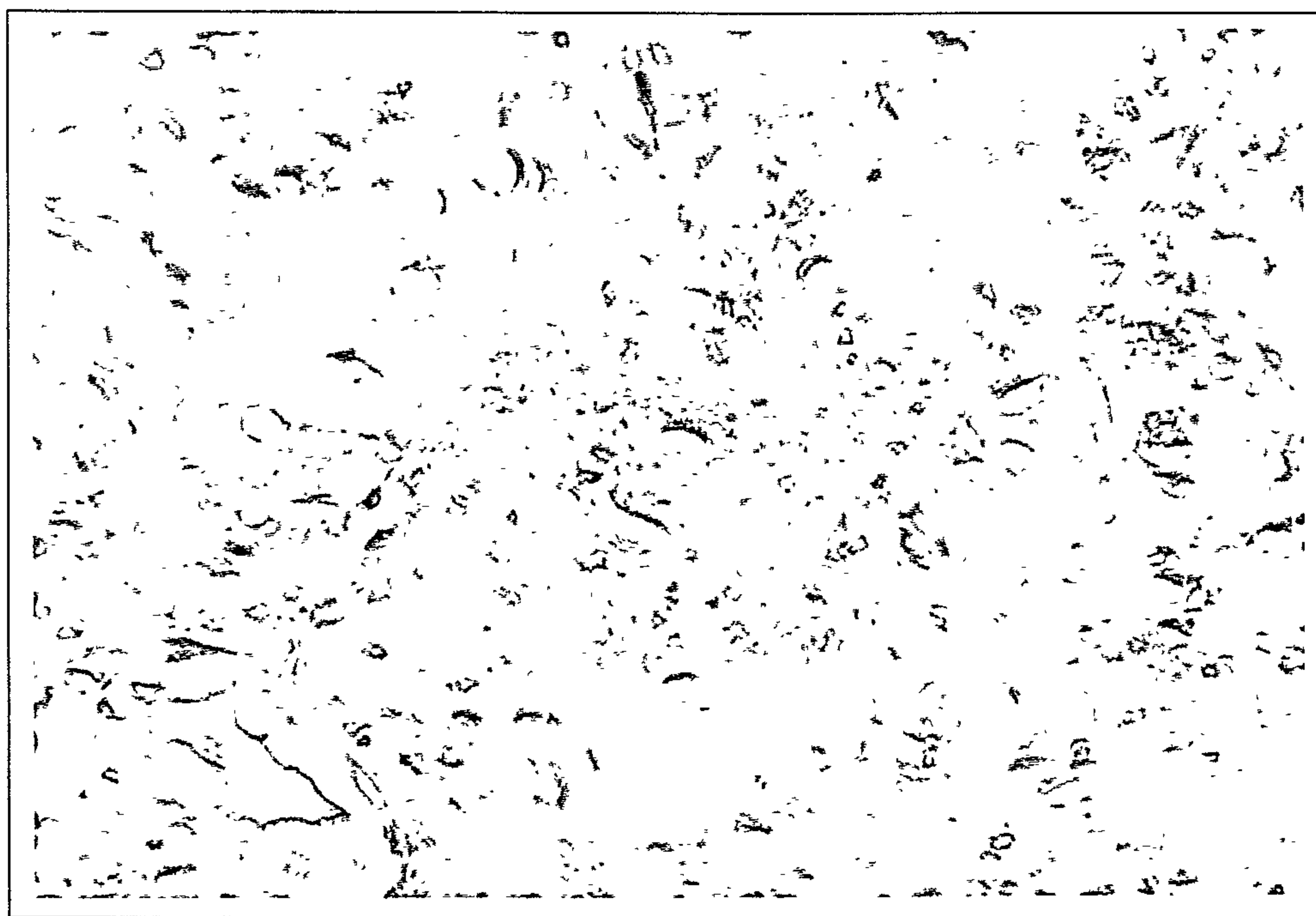
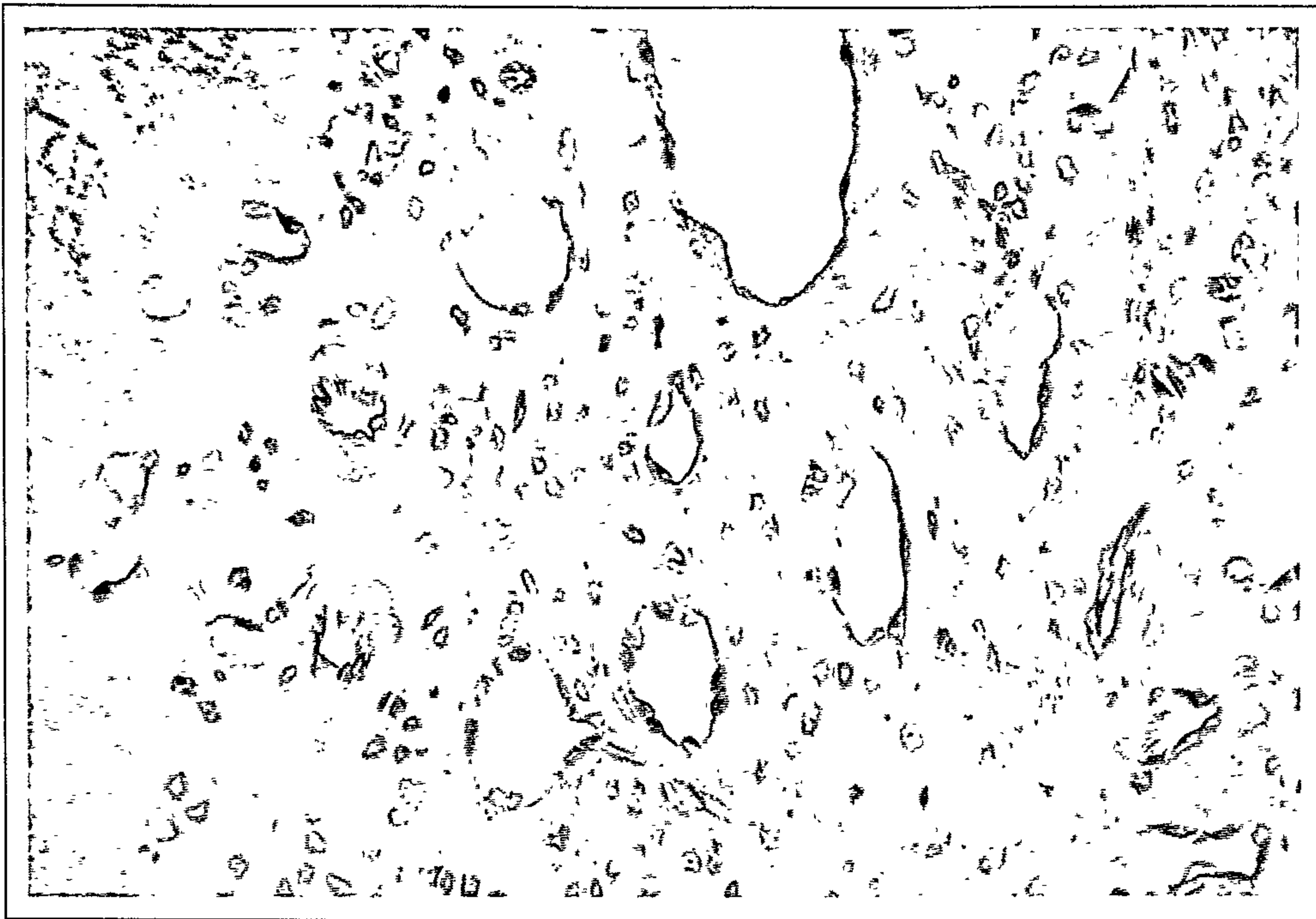


Figure 9.1.5 Histomicrograph illustrating the intense staining of blood vessels on the periphery of the lesion to QBend 10 (X400)

Figure 9.1.6 Histomicrograph illustrating the intense staining of blood vessels on the periphery of the lesion to QBend 10 (X400)

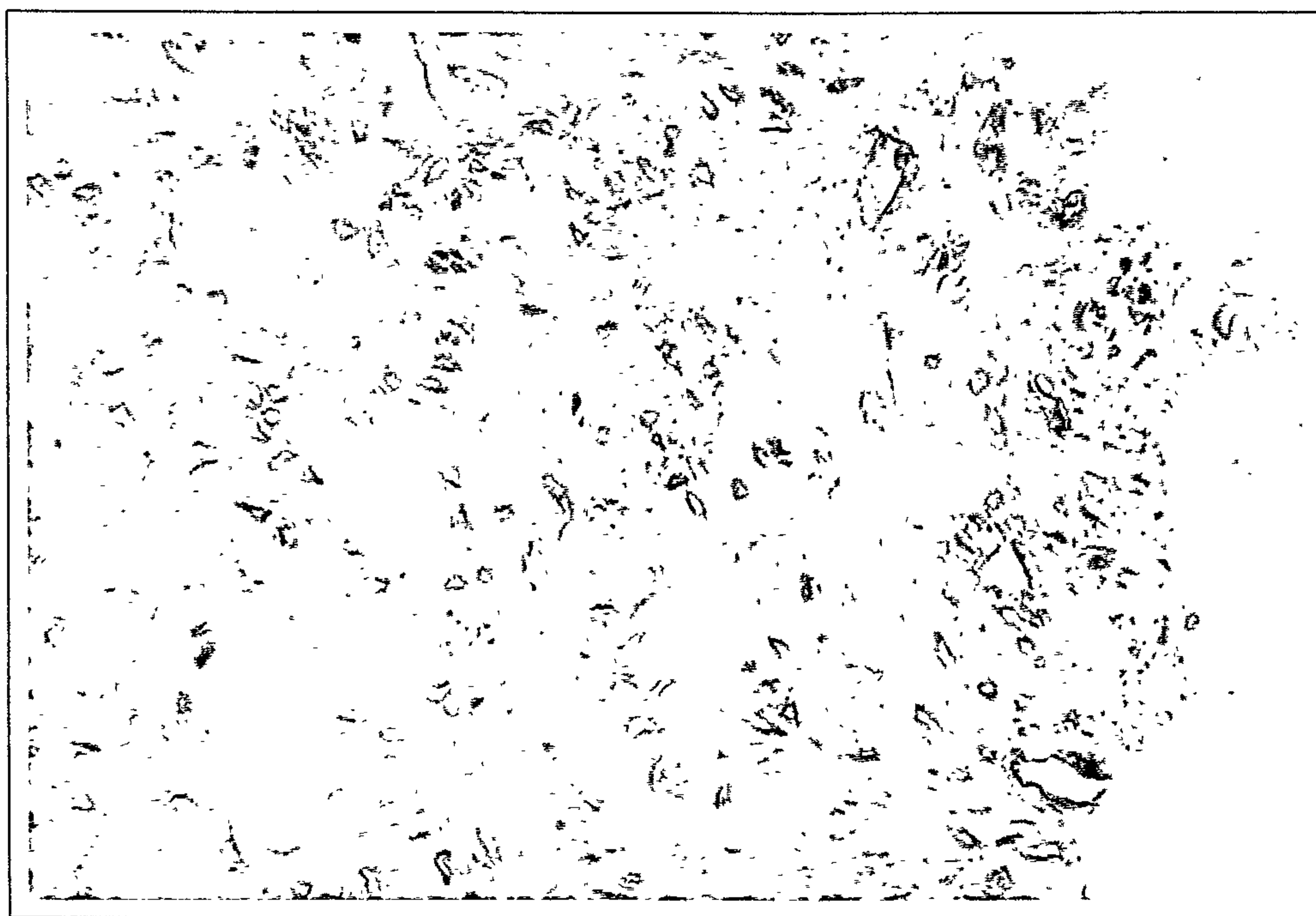
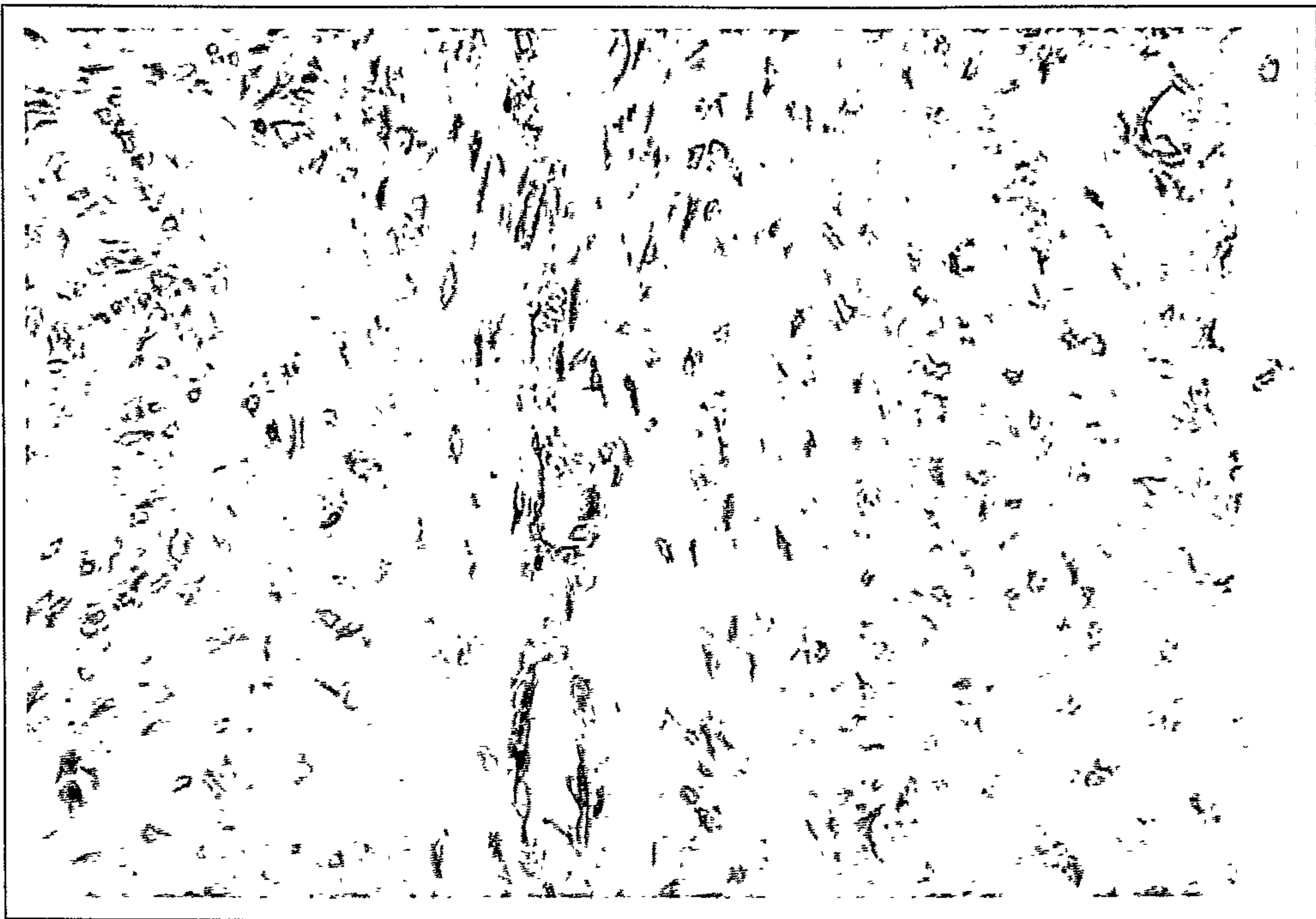


Figure 9.1.7 Histomicrograph of the deeper areas of the lesion illustrating the negative staining of the blood vessels adjacent to the giant cell aggregations to QBend 10 (X400)

Figure 9.1.8 Histomicrograph of the deeper areas of the lesion illustrating the negative staining of the blood vessels adjacent to the giant cell aggregations to QBend 10 (X400)

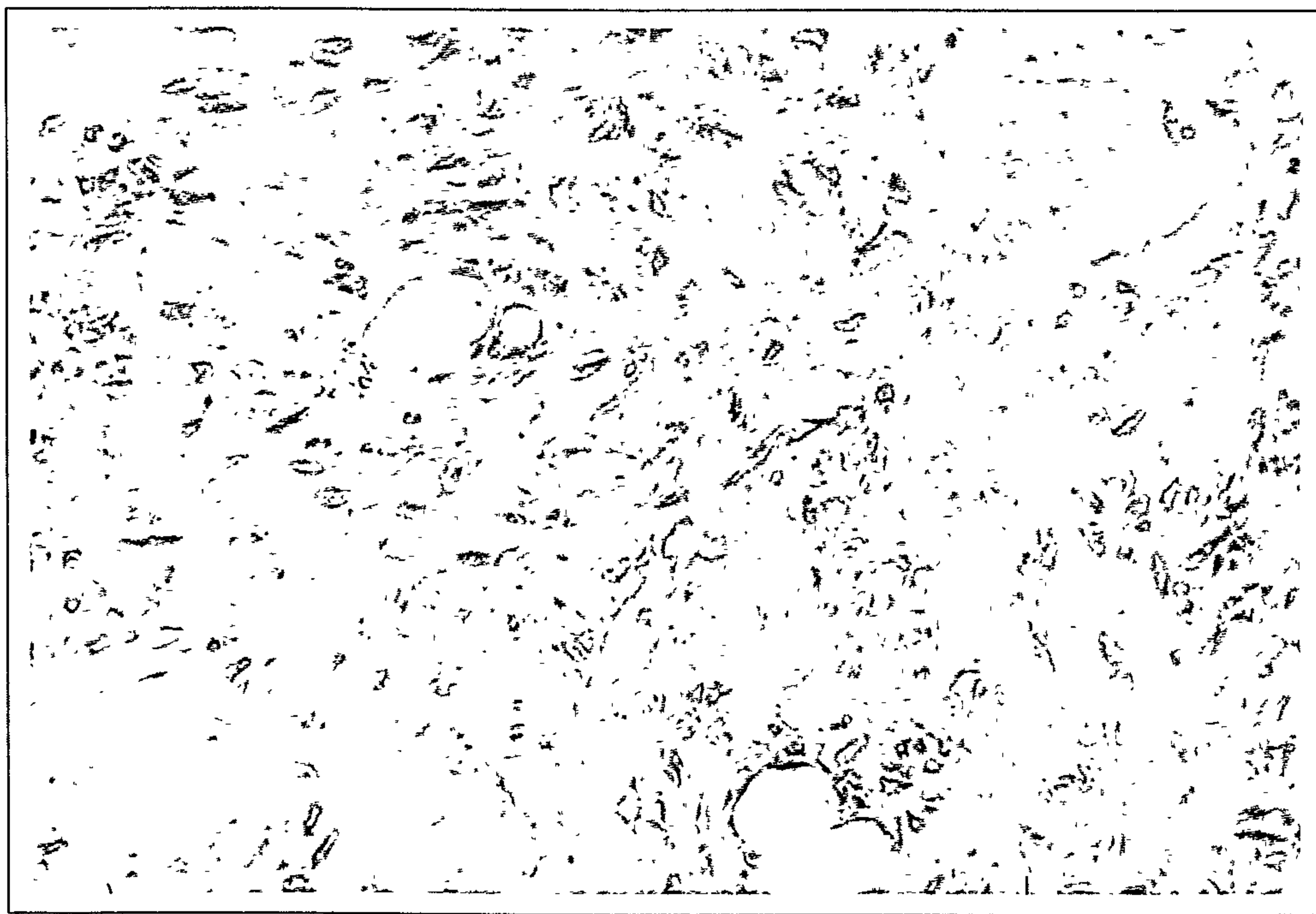
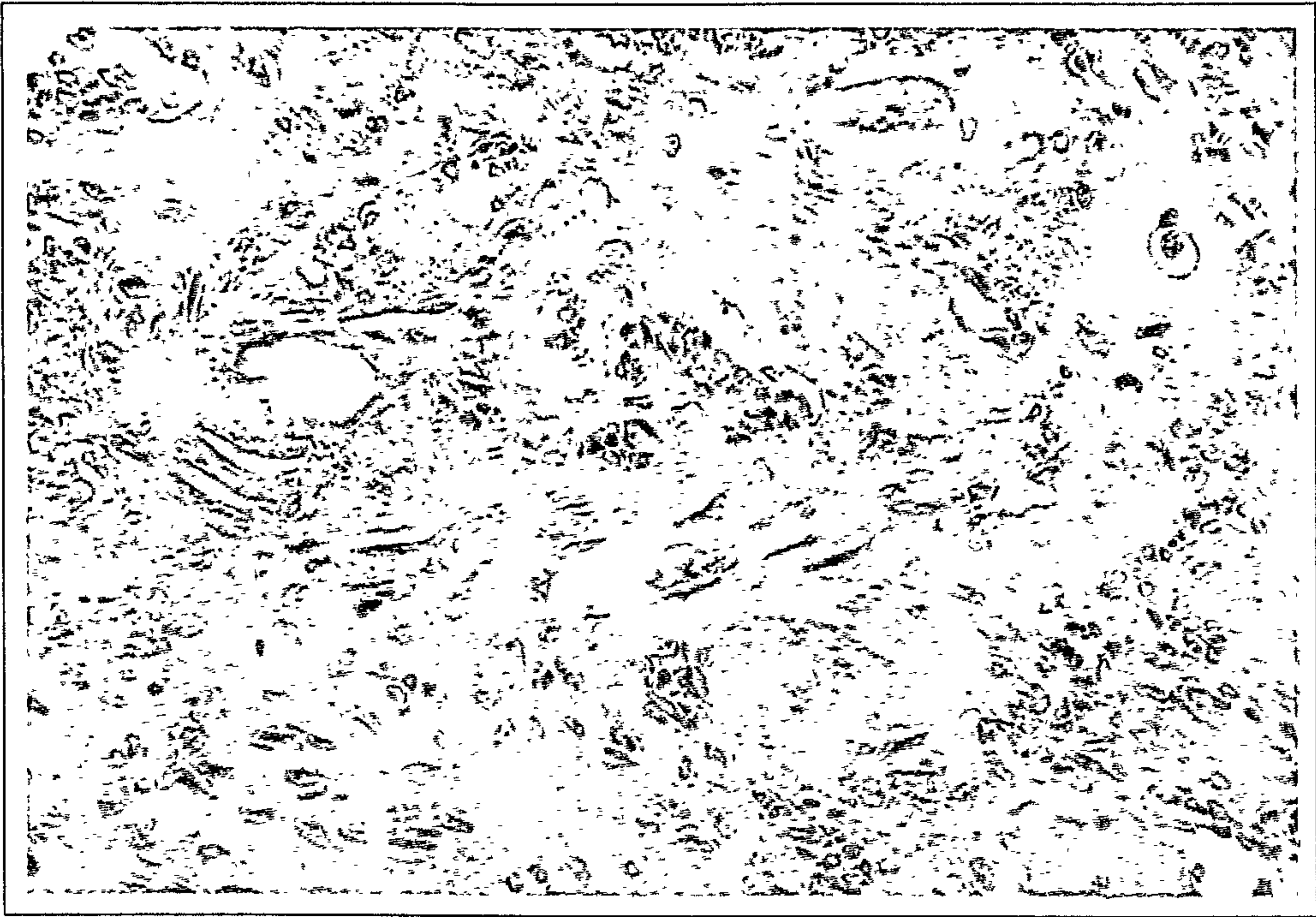


Figure 9.2.1 Transmission electron-micrograph of blood vessels on the periphery of the lesion exhibiting high endothelial cells with well developed intercellular junctions (X9750)

Figure 9.2.2 Transmission electron-micrograph of the peripheral areas of the lesion illustrating endothelial cells associated with inflammatory cells (X4350)

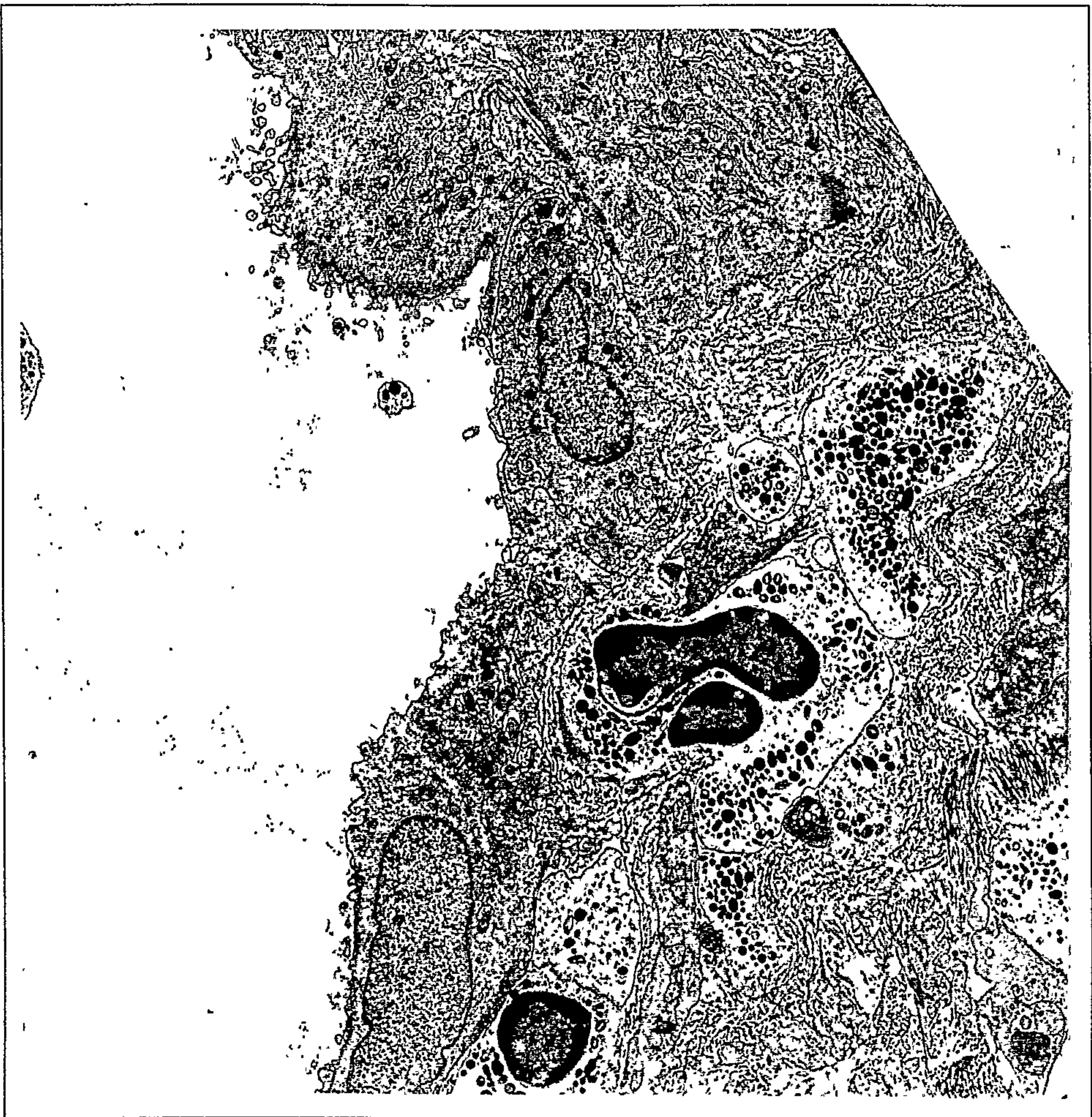
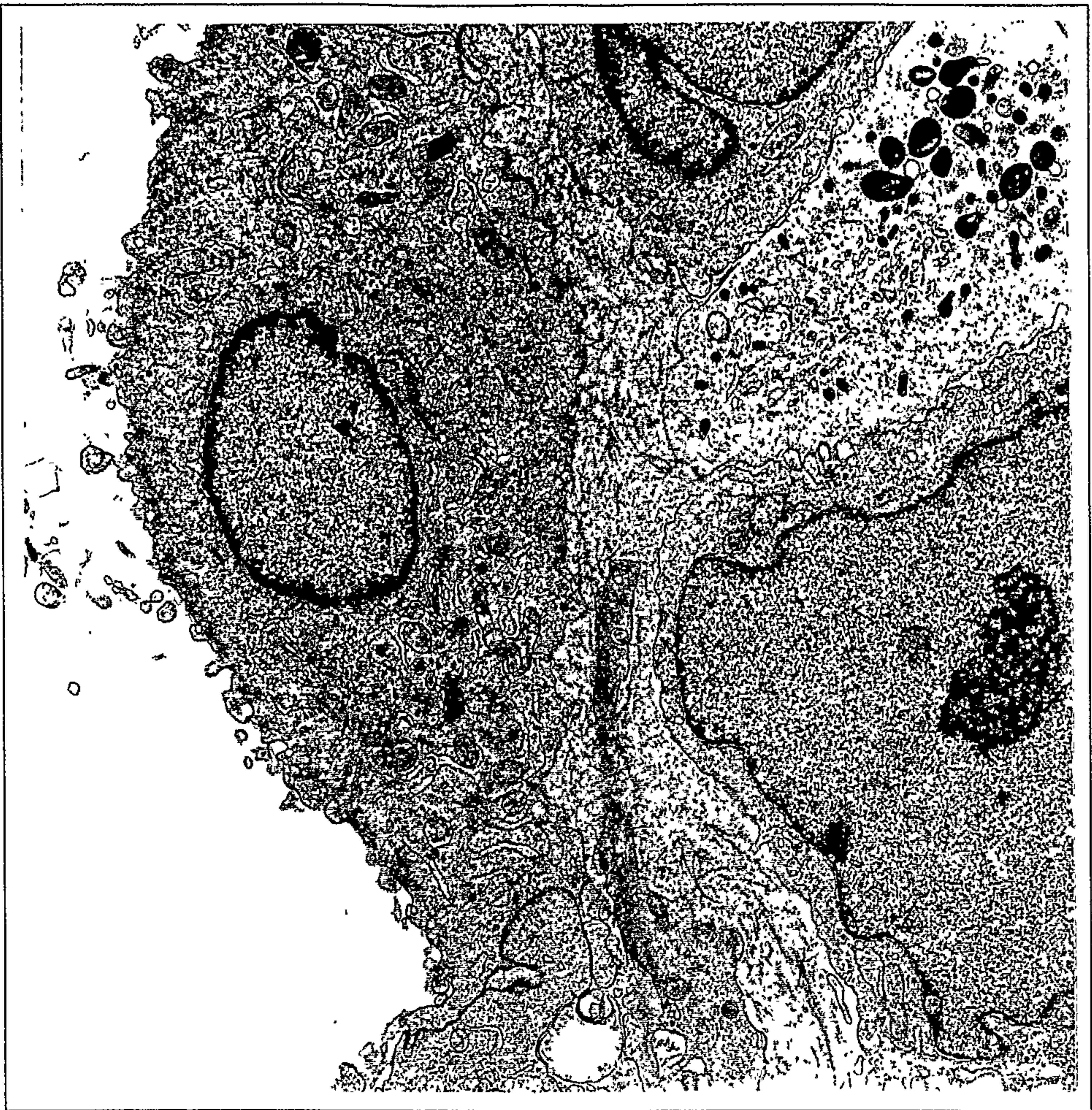


Figure 9.2.3 Transmission electron-micrograph of endothelial cells on the periphery of the lesion illustrating the abundance of inflammatory cells within the lumen of the vessel (X4350)

Figure 9.2.4 Transmission electron-micrograph illustrating the blood vessels on the periphery of the lesion with an abundance of inflammatory cells and a marginating macrophage (X3250)

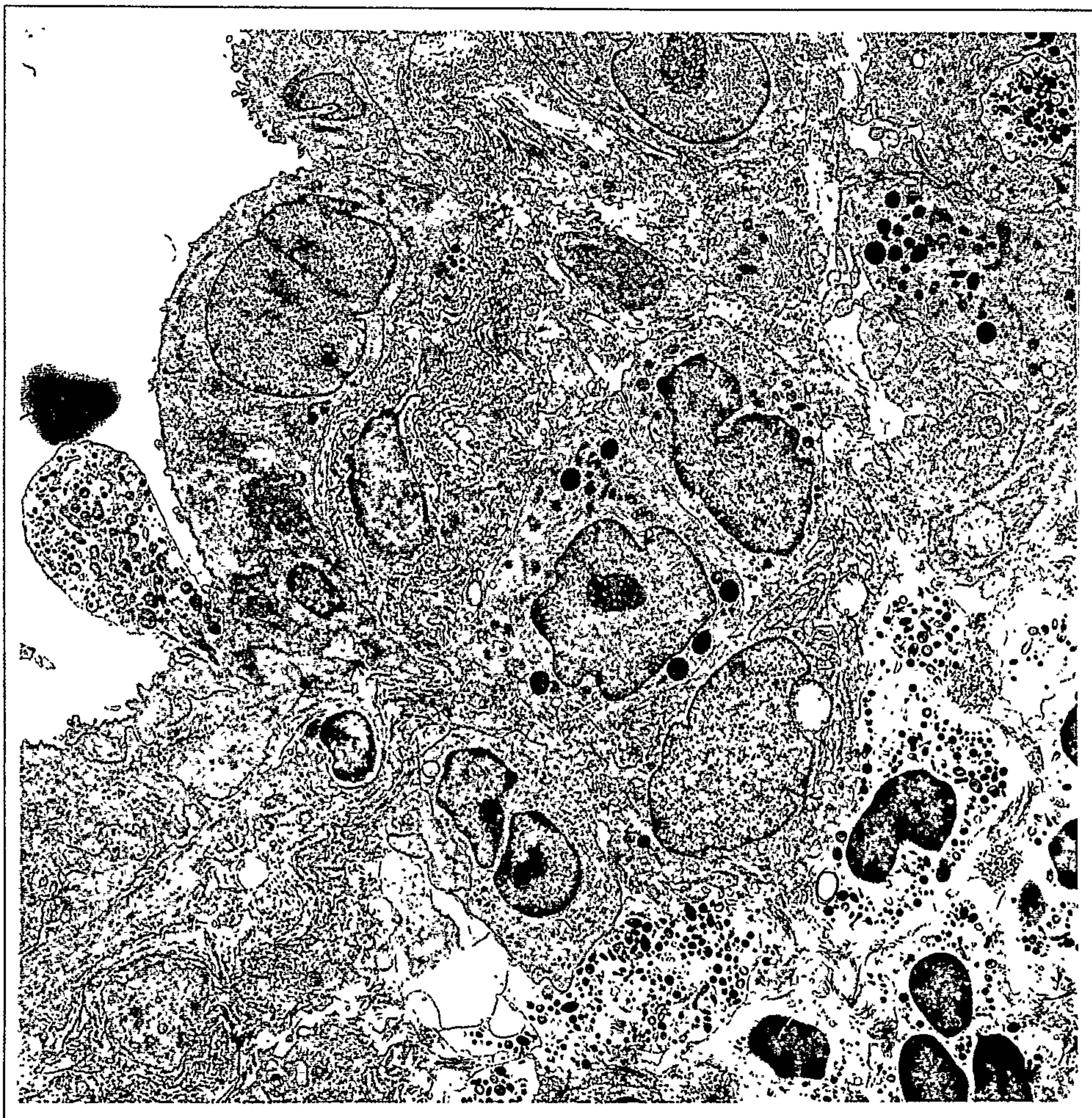
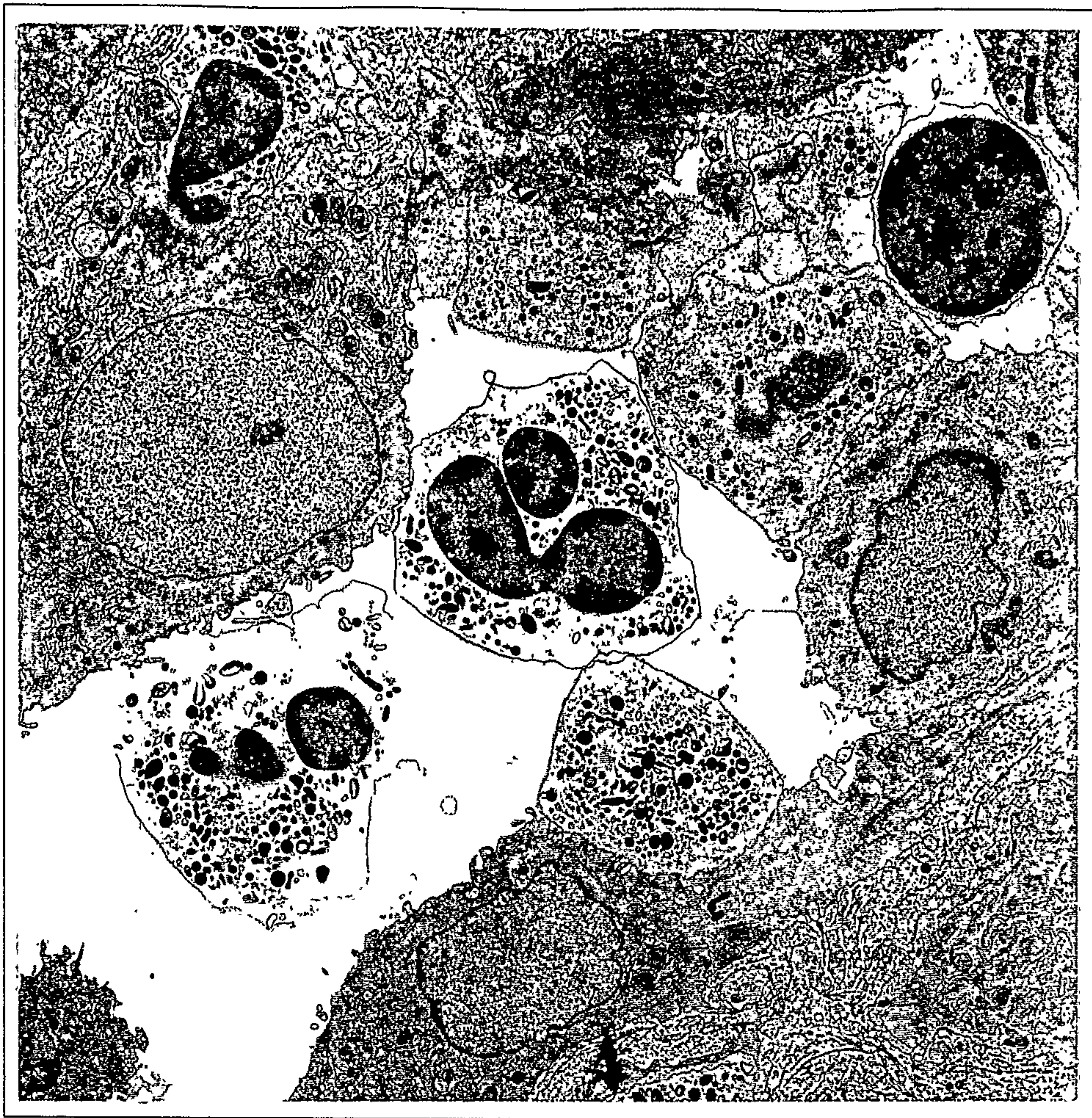


Figure 9.2.5 Transmission electron-micrograph of endothelial cells on the periphery of the lesion with an inflammatory cell in the process of diapedesis (X9750)

Figure 9.2.6 Transmission electron-micrograph of an endothelial cell on the periphery of the lesion illustrating "splitting" or reduplication of the basal lamina (X9750)

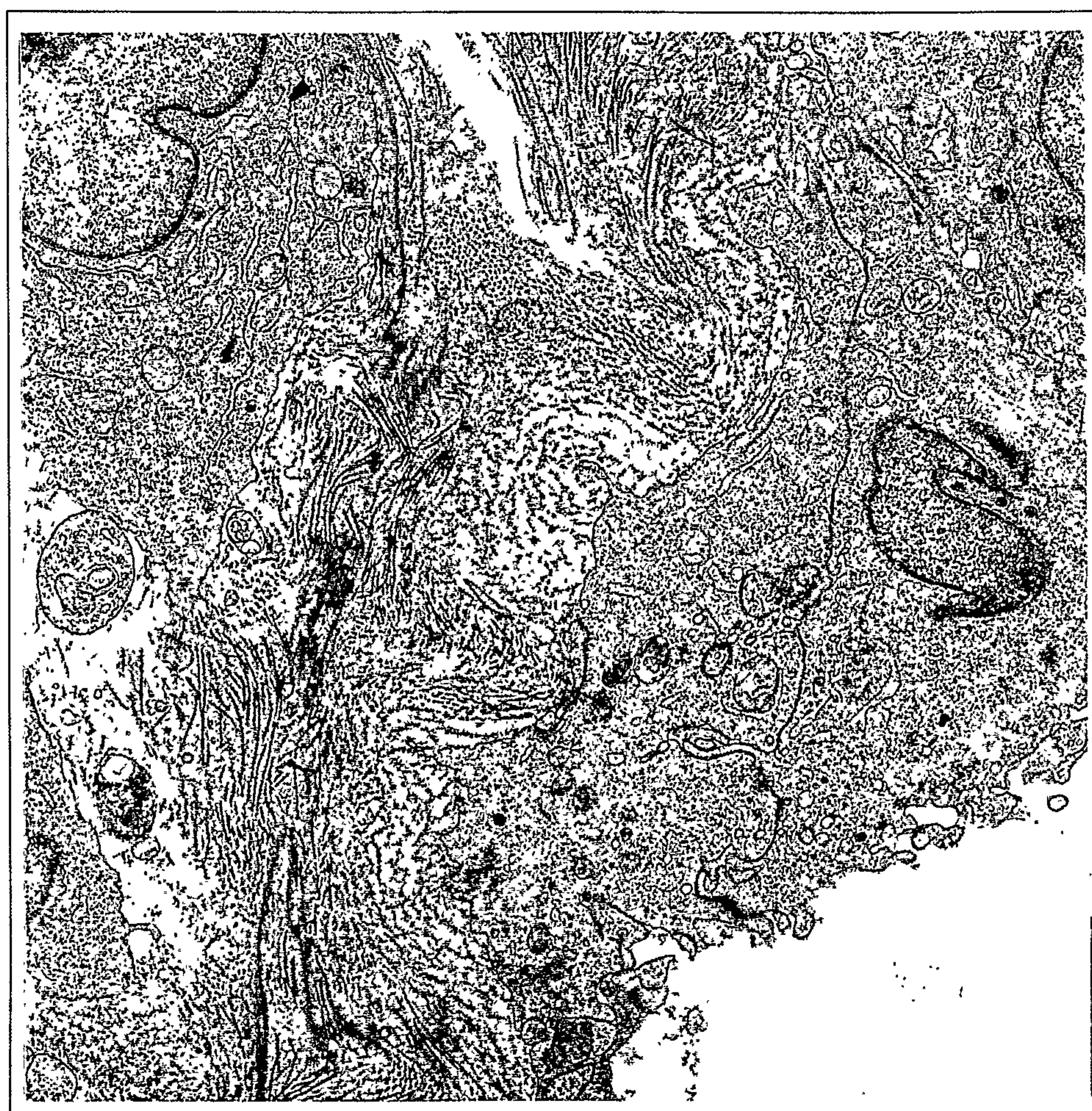
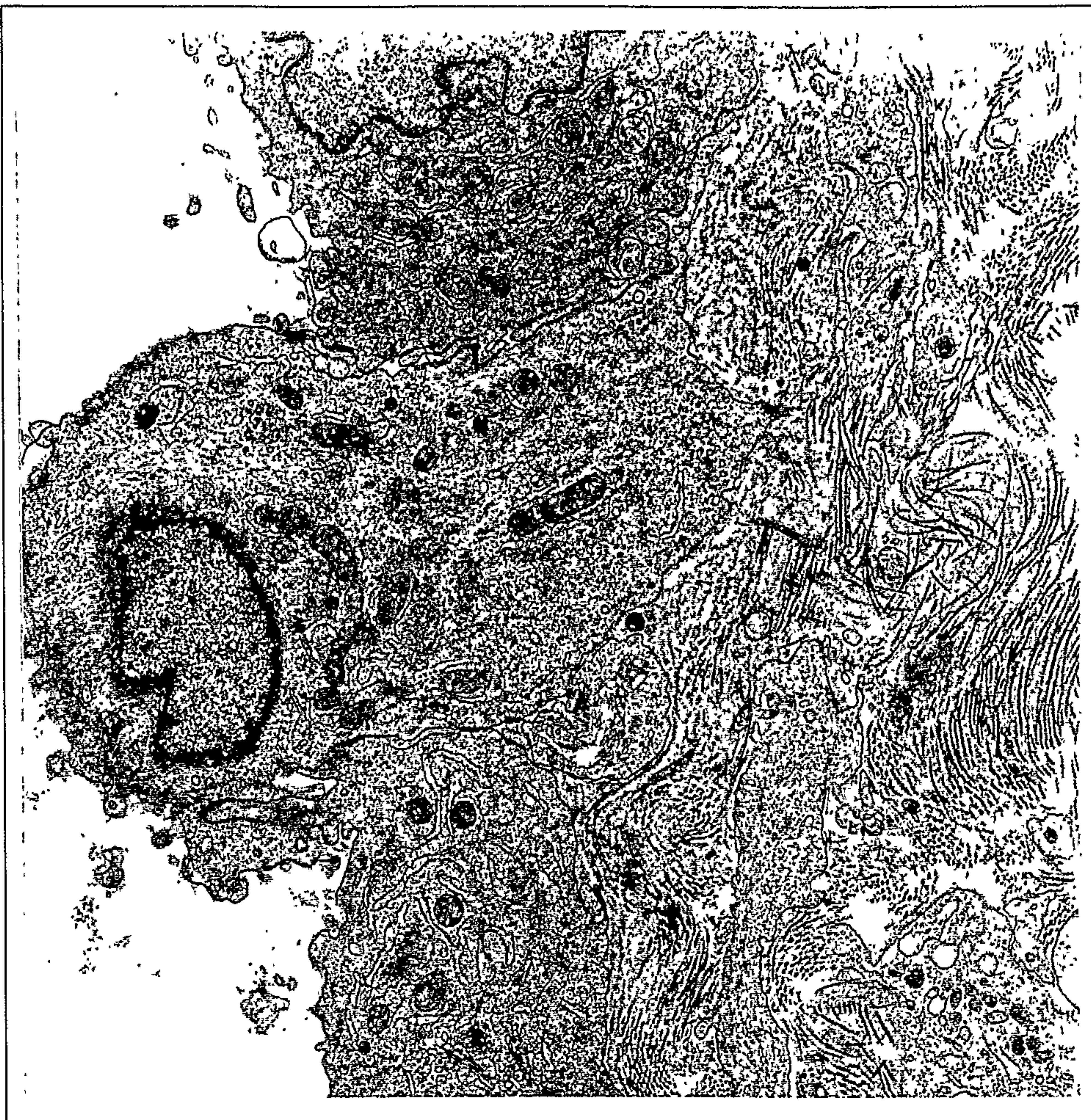


Figure 9.2.7 Transmission electron-micrograph illustrating the deeper areas of the lesion where structurally complete blood vessels could not be identified (X5750)

Figure 9.2.8 Transmission electron-micrograph illustrating the deeper areas of the lesion where structurally complete blood vessels could not be identified (X3250)

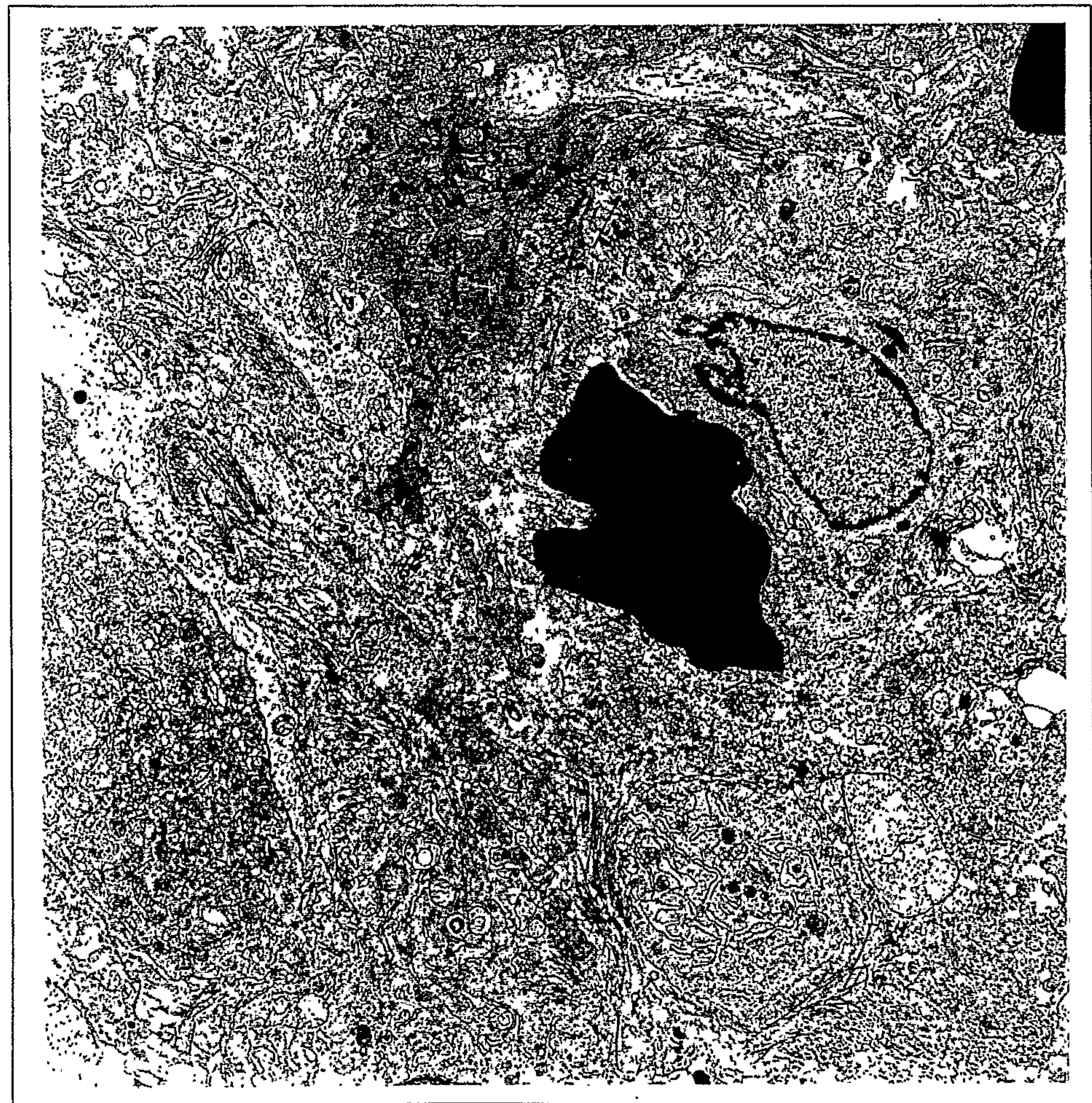
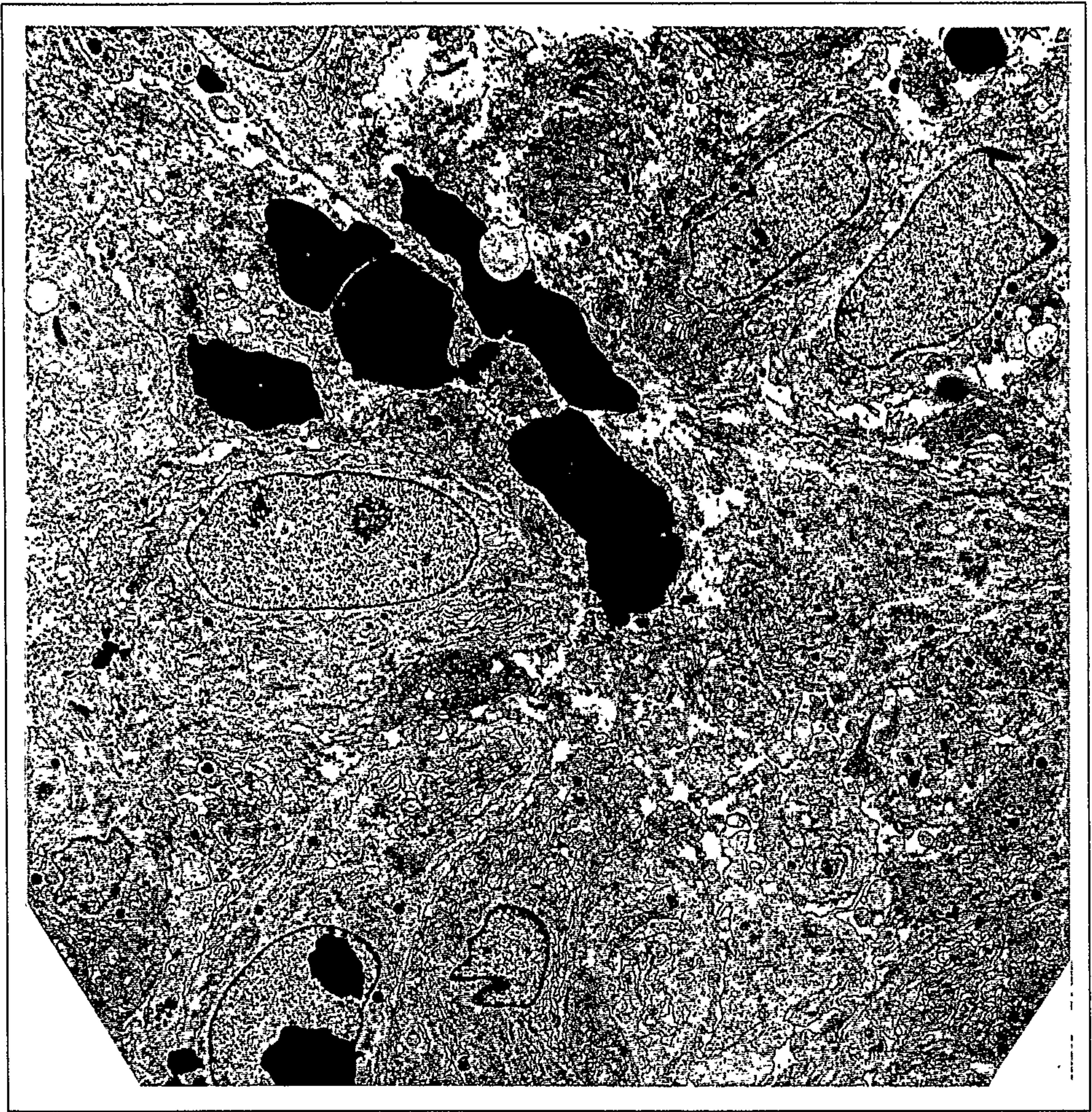


Figure 9.2.9 Transmission electron-micrograph illustrating the characteristic finding in the deeper areas of the lesion where areas with abundant erythrocytes were observed but normal endothelial structure could not be identified (X5750)

Figure 9.2.10 Transmission electron-micrograph illustrating the characteristic finding in the deeper areas of the lesion where areas with abundant erythrocytes were observed but normal endothelial structure could not be identified (X3250)

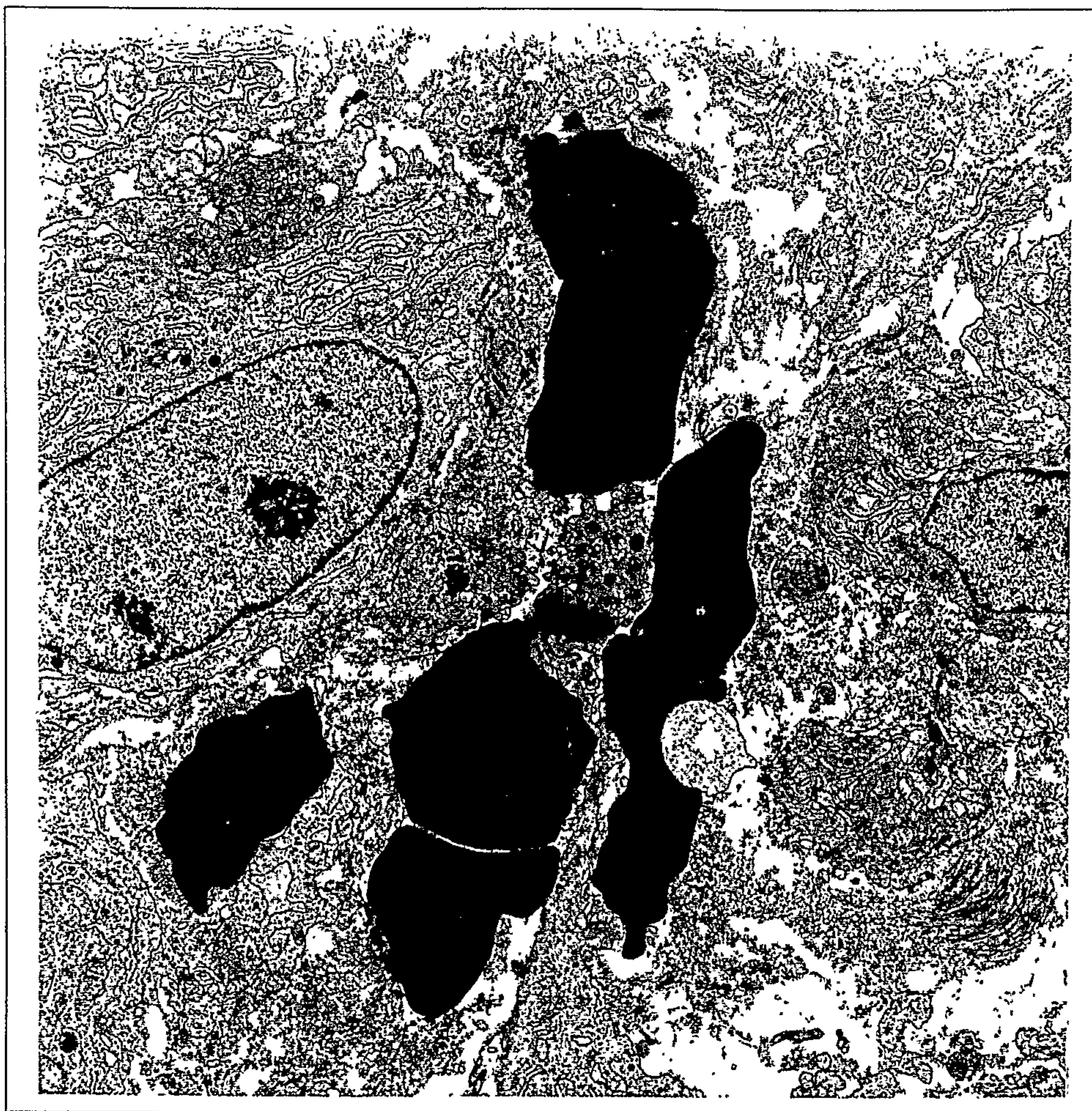
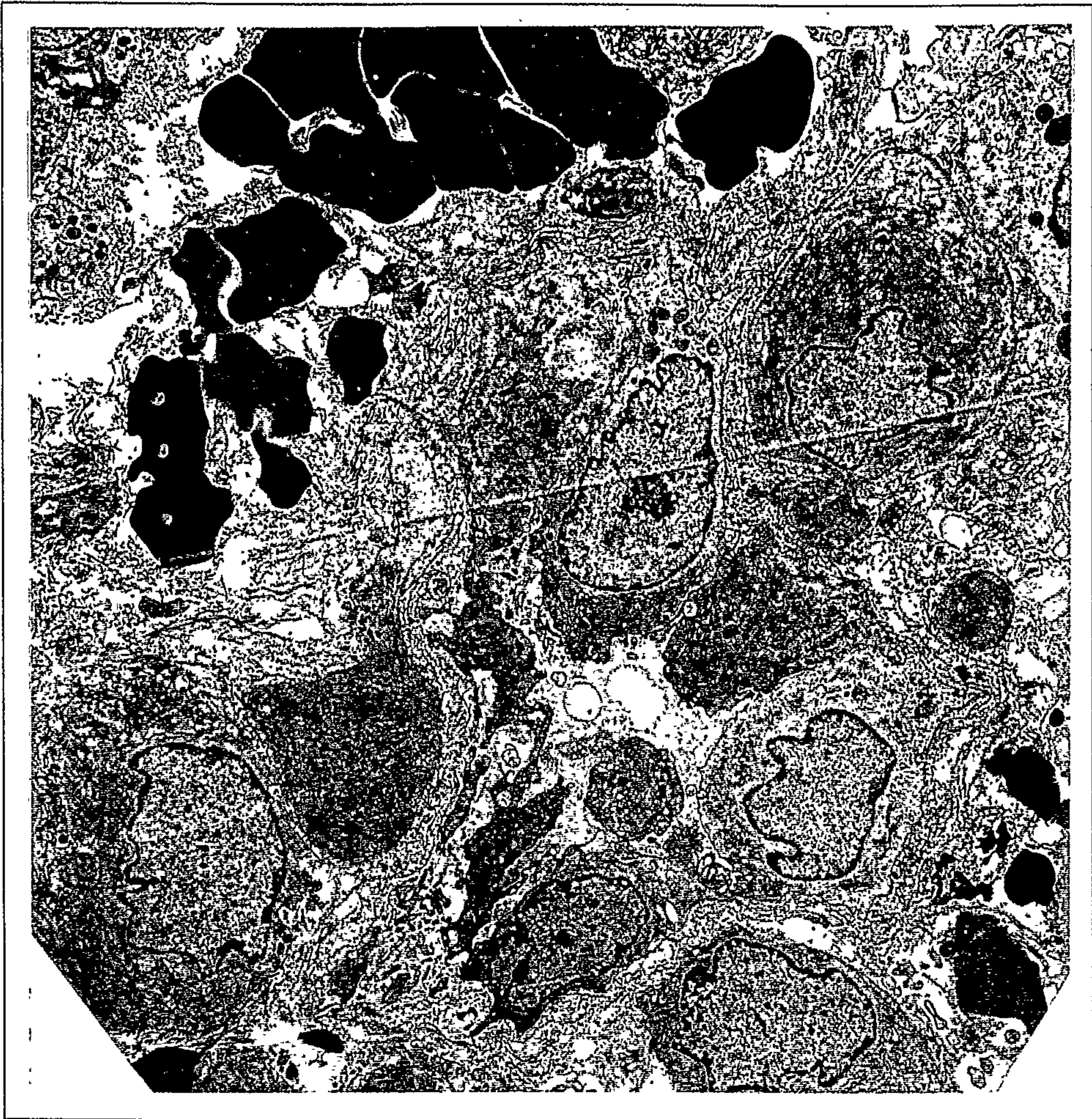
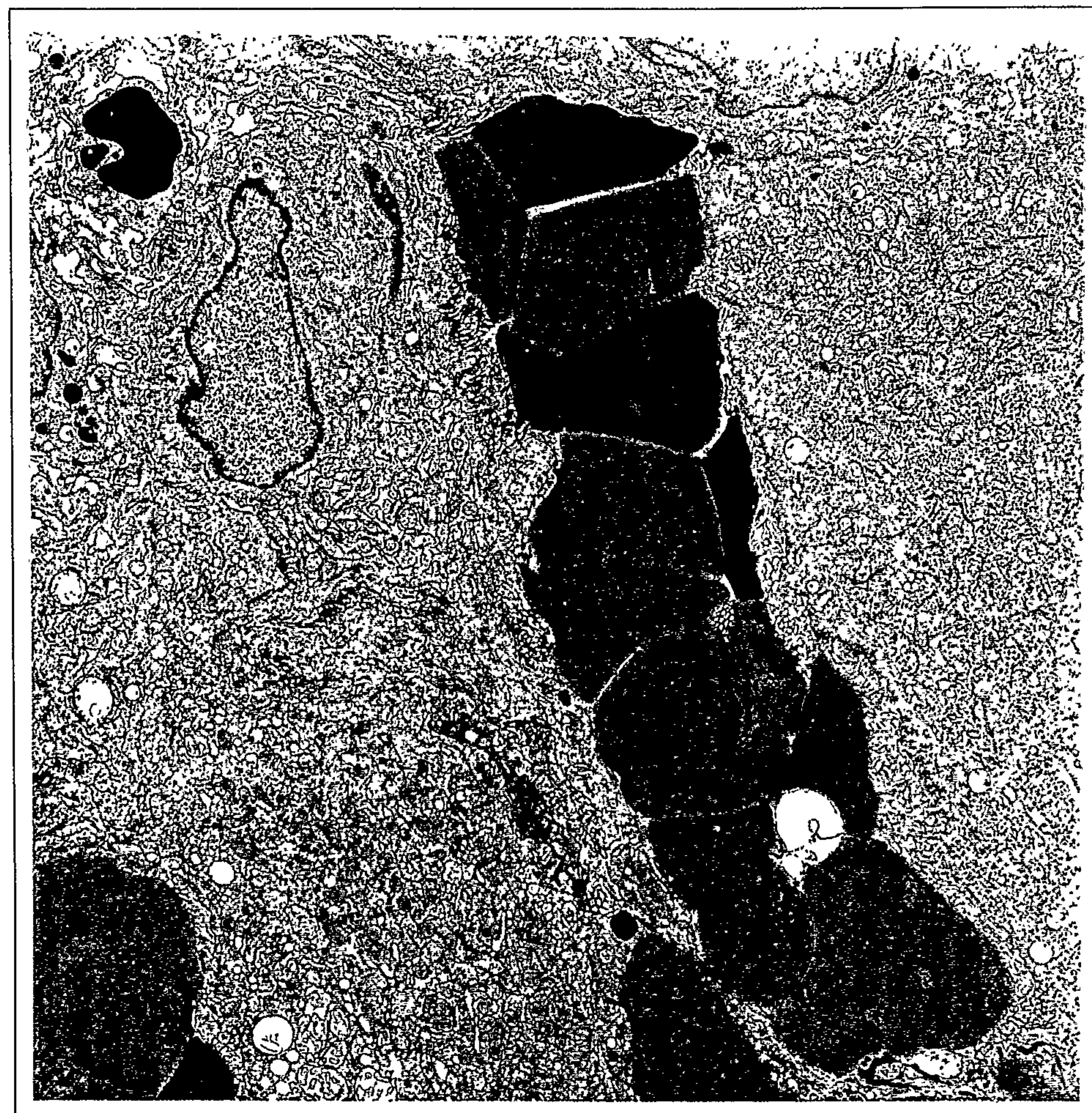
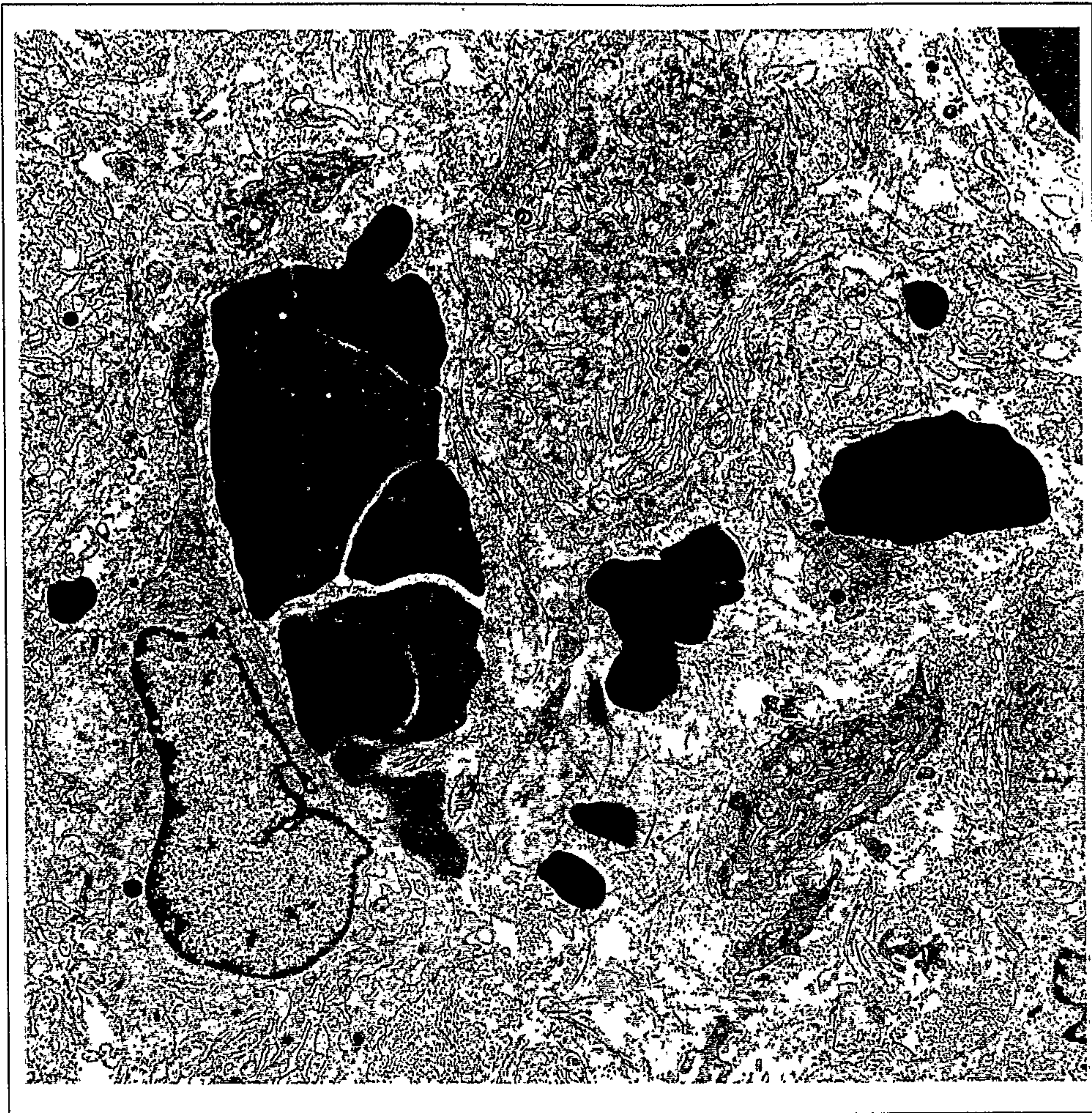


Figure 9.2.11 Transmission electron-micrograph illustrating erythrocytes in rouleaux formation but with no evidence of adjacent endothelial cell lining (X5750)

Figure 9.2.12 Transmission electron-micrograph illustrating erythrocytes in rouleaux formation but with no evidence of adjacent endothelial cell lining (X4350)



CHAPTER 10

DISCUSSION (PART 2)

10.1 Immunohistochemistry: Ulex Europaes 1 Lectin and QBend10

Ulex Europaes 1 is a lectin specific for alpha-L-fucose residues of sugar moieties (Allen *et al*, 1977; Debray *et al*, 1981) and reacts preferentially with vascular endothelium irrespective of the blood group type of the tissue donor (Holthofer *et al*, 1981, Holthofer *et al*, 1982).

Its introduction as an endothelial cell marker was a result of the finding that Factor VIII Related Antigen was unreliable in the detection of endothelial cells in some benign vascular proliferations and in malignant endothelial cells (Burgdorf *et al*, 1981).

Miettinen *et al* (1982) compared the staining pattern of Factor VIII Related Antigen to that of Ulex Europaes in a variety of tissues and tumours and found that Ulex Europaes was a more reliable marker. In particular, they found that whilst Factor VIII Related Antigen was negative in certain small capillaries such as those of the pyogenic granuloma, Ulex Europaes showed markedly stronger staining.

Other studies (Little *et al*, 1986, Ordonez and Batsakis, 1984) confirmed these findings. Ulex Europaes 1 Lectin was found to be a more sensitive marker than

Factor VIII Related Antigen and was strongly positive for angiosarcomas and the spindle-shaped cells in Kaposi's sarcoma.

QBend 10 is a monoclonal antibody recently introduced as a marker for endothelium (Ramani *et al*, 1990, Sankey *et al*, 1990). The advantages associated with the use of this antibody is attributed to its ability to stain the spindle-shaped cells of Kaposi's sarcoma more reliably as compared with Factor VIII Related Antigen and Ulex Europae 1 Lectin (Sankey *et al*, 1990). Another advantage of this antibody over Ulex Europae 1 Lectin is the lesser degree of background staining and also the lack of cross-reactivity with epithelial cells and in certain carcinomas (Ramani *et al*, 1990). QBend 10 recognises an epitope different to that of Factor VIII Related Antigen and Ulex Europae 1 Lectin (Watt *et al*, 1987).

Both Ulex Europae 1 Lectin and QBend 10 were found to produce a similar staining pattern to that observed with Factor VIII Related Antigen. The similar staining pattern observed with all these endothelial markers could have a trivial explanation such as loss of antigen deeper in the lesions due to poor penetration of fixative. This is considered unlikely as the reactivity of the macrophages and the giant cells in these regions for the other markers used in this study were not affected similarly.

10.2 Transmission Electron-Microscopy

The electron microscopic study of these lesions indicated that the reason for the differential staining observed may be in the differences in the structure of the microcirculation in the periphery of the giant cell aggregates relative to the deeper regions.

The endothelial cells on the periphery exhibited high endothelial cells and margination of neutrophils and macrophages and reduplication of basal lamina.

The endothelial cells on the periphery were closely associated with an abundance of polymorphs and macrophages. There was also evidence of cell margination and diapedesis. These observations indicate that the majority of the peripheral blood vessels were essentially normal blood vessels involved in the inflammatory process.

However, some vessels on the periphery also showed signs of basal lamina reduplication. This interesting phenomenon is reported to be present beneath endothelial cells of several disease states such as in tissues of diabetics (Osterby and Lundbaek, 1970) and in the synovial membrane and skin of patients with rheumatoid arthritis (Ghadially, 1983).

Reduplication of the basal lamina has also been described in some benign and less aggressive tumours. It is thought that a reduplicated lamina in a tumour is usually an indicator of innocence and regression or a relatively low level of clinical malignancy (Ghadially, 1988).

In contrast, structurally complete blood vessels in the deeper areas of the lesion could not be identified readily. Areas with abundant erythrocytes but with no signs of normal endothelial structure in the adjacent tissue were characteristic. Therefore, the ultrastructural findings have supported the immunohistochemical observations.

The significance of these observations with regard to the pathogenesis of the lesion remains to be elaborated but this preliminary study suggests that further analysis of the microvasculature may be rewarding. It is believed that the multinucleated giant cells are simply reactionary in nature (Cohen *et al*, 1988, El-Mofty and Osdoby, 1985) and that the so called "principal tumour cell" may lie within the stromal components of the lesion. The multinucleated giant cells have been demonstrated to be osteoclasts (Flanagan *et al*, 1988) and their presence is thought to be responsible for much of the osteolytic destruction seen in the central giant cell lesions. While the stimulus for their presence in the lesion has not yet been determined, it is thought

that they may be responding to a stimulus from the stroma. Osteoclasts have been shown to be of haemopoietic origin (Gothlin and Ericsson, 1972) and they must therefore arrive via the circulation. It may be interesting to speculate that there may be a link between the anomalies demonstrated in the vasculature of this lesion and the presence of the multinucleated giant cells or osteoclasts.

SUMMARY AND CONCLUSIONS

This study has been able to confirm and support some of the current evidence regarding the histogenesis of the giant cell granuloma. The osteoclastic nature of the multinucleated giant cells was supported by their reactivity with the monoclonal antibody MB1. The round mono-nuclear cells were reactive to Leukocyte Common Antigen which supports the evidence for their being macrophages. The spindle cells were unreactive to all the antibodies chosen and in particular, were unreactive to Smooth Muscle Specific Actin although they are believed to be myo-fibroblastic in nature. Their histogenesis remains uncertain.

Significantly, this study has demonstrated the relative lack of reactivity of the deep areas of the lesion to three commonly used endothelial cell markers namely, Factor VIII Related Antigen, Ulex Europae 1 Lectin and QBend 10. Transmission electron microscopy indicated that the immunohistochemical observations may be due to an absence of a mature functional microvasculature in these regions. Further study will be required to clarify the significance of these findings with respect to the controversies and uncertainties surrounding the pathogenesis of these giant cell lesions.

REFERENCES

1. Abrams B, Shear M. A histological comparison of the giant cells in the central giant cell granuloma of the jaws and the giant cell tumour of bone. *J Oral Pathol* 1974;3:217-223.
2. Adkins KF, Martinez MG, Hartley MW. Ultrastructure of giant cell lesions. *Oral Surg Oral Med Oral Path* 1969a;28:713-723.
3. Adkins KF, Martinez MG, Robinson LH. Cellular morphology and relationships in giant cell lesions of the jaws. *Oral Surg Oral Med Oral Pathol* 1969b;28:216-222.
4. Adler KB, Craighead JE, Vallyathan NV, Evance JN. Actin containing cells in pulmonary fibrosis. *Am J Pathol* 1981;102:427-437.
5. Allen HJ, Johnson EAZ, Matta KL. A comparison of the binding specificities of lectins from *Ulex Europaes* and *Lotus tetragonolobus*. *Immunol Commun* 1977;6:585.
6. Andersen L, Arwill T, Ferjerskov O, Heyden G, Philipsen HP. Oral giant cell granulomas : An enzyme histochemical and ultrastructural study. *Acta Path Microbiol Scand* 1973;81:617-629.
7. Andersen L, Ferjerskov O, Philipsen HP. Oral giant cell granulomas: A clinical and histological study of 129 new cases. *Acta Path Microbiol Scand* 1973;81:606-616.

8. Anderson ND, Coyler RA, Riley LH. Skeletal changes during prolonged external irradiation : alterations in marrow, growth plate and osteoclast populations. *Johns Hopkins Med J* 1979;145:73-83.
9. Aqel NM, Pringle JAS, Horton MA. Cellular heterogeneity in giant cell tumour of bone (osteoclastoma): an immunohistological study of 16 cases. *Histopathol* 1988;13:675-685.
10. Athanasou NA, Heryet A, Quinn J, Gatter KC, Mason DY, McGee JO. Osteoclasts contain macrophage and megakaryocyte antigens. *J Pathol* 1986;147:153-158.
11. Athanasou NA, Quinn J, McGee JO. Leucocyte Common Antigen is present on osteoclasts. *J Pathol* 1987;153:121-126.
12. Auclair PL, Cuenin P, Kratochvil FJ, Slater LJ, Ellis GL. A clinical and histomorphologic comparison of the central giant cell granuloma and the giant cell tumour. *Oral Surg Oral Med Oral Pathol* 1988;66:197-208.
13. Austin LT, Dahlin DC, Royer RQ. Giant cell reparative granuloma and related conditions affecting the jaw bones. *Oral Surg* 1959;12:1285-1295.
14. Avrameas S. Immunoenzyme techniques: Enzymes as markers for the localisation of antigens and antibodies. *Int Rev Cytol* 1970;27:349.
15. Bartel H, Piatowska D. Electron microscopic study of peripheral giant cell reparative granuloma. *Oral Surg* 1977;43:82-96.
16. Baudier J, Briving C, Deinum J, Haglid K, Sorskog L, Wallin M. Effect of S-100 proteins and calmodulin on Ca²⁺ induced disassembly of brain microtubule proteins in vitro. *FEBS Lett* 1982;147:165-167.
17. Bernier JL, Cahn LR. The peripheral giant cell reparative granuloma. *J Am Dent Assoc* 1954;49:141-148.

18. Bhaskar SN, Bernier JL, Godby F. Aneurysmal bone cyst and other giant cell lesions of the jaws : report of 104 cases. *Oral Surg* 1958;34:781.
19. Bhasker SN, Cutright DE, Beasley JD, Perez B. Giant cell reparative granuloma (peripheral): Report of 50 cases. *J Oral Surg* 1971;29:110-115.
20. Bonetti F, Pelosi G, Martignoni G, et al. Peripheral giant cell granuloma : Evidence for osteoclastic differentiation. *Oral Surg Oral Med Oral Pathol* 1990;70:471-475.
21. Brecher ME, Franklin WA, Simon MA. Immunohistochemical study of mononuclear phagocyte antigens in giant cell tumour of bone. *J Pathol* 1986;125:252-257.
22. Bridge JA, Neff JR, Bhatia PS, Sanger WG, Murphey MD. Cytogenetic findings and biologic behaviour of giant cell tumours of bone. *Cancer* 1990;65:2697-2703.
23. Burgdorf WHC, Mukai K, Rosai J. Immunohistochemical identification of Factor VIII Related Antigen in endothelial cells of cutaneous lesions of alleged vascular nature. *Am J Clin Pathol* 1981;75:167-171.
24. Calissano P, Bangham AD. Effect of two brain specific proteins (S100 and 14-3-2) on cation diffusion across artificial lipid membranes. *Biochem Biophys Res Commun* 1971;43:504-509.
25. Chambers TJ. Phagocytosis and trypsin-resistant glass adhesion by osteoclasts in culture. *J Pathol* 1979;127:55-60.
26. Chambers TJ, Magnus CJ. Calcitonin alters behaviour of isolated osteoclasts. *J Pathol* 1982;136:27-39.

27. Chilosì M, Gilioli E, Lestani M, Menestrina F, Fiore-Donati L. Immunohistochemical characterisation of osteoclasts and osteoclast-like cells with monoclonal antibody MB1 on paraffin embedded tissues. *J Pathol* 1988;156:251-254.
28. Chuong R, Kaban LB. Diagnosis and treatment of jaw tumours in children. *J Oral Maxillofac Surg* 1985;43:323-332.
29. Chuong R, Kaban LB, Kozakewich H, Perez-Atayde A. Central giant cell lesions of the jaws: A clinicopathologic study. *J Oral Maxillofac Surg* 1986;44:708-713.
30. Clark EA, Ledbetter JA. Leukocyte cell surface enzymology: CD45 (LCA, T200) is a protein tyrosine phosphatase. *Immunol Today* 1989;10:225-228.
31. Cohen MA. Management of a huge central giant cell granuloma of the maxilla. *J Oral Maxillofac Surg* 1988;46:509-513.
32. Cohen MA, Grossman ES, Thompson SH. Features of central giant cell granuloma of the jaws xenografted in nude mice. *Oral Surg Oral Med Oral Pathol* 1988;66:209-217.
33. Cohen MA, Hertzanu Y. Radiologic features, including those seen with computed tomography, of central giant cell granuloma of the jaws. *Oral Surg Oral Med Oral Pathol* 1988;65:255-261.
34. Cones DMT. An unusual bone tumour complicating Paget's disease. *J Bone Joint Surgery* 1953;35B:101-105.
35. Cooke BED. Giant cell epulis: Histogenesis and natural history. *Brit Dent J* 1952;93:12-16.
36. Coons AH, Creech HJ, R.N. J. The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. *J Immunol* 1942;42:159-170.

37. Dahlin DC, Cupps RE, Johnson EW. Giant cell tumour : A study of 195 cases. *Cancer* 1970;25:1061-1070.
38. Dalchau R, Fabre JW. Studies on the distribution among T lymphocytes of the high molecular weight form of the human leukocyte antigen. In: Bernard A, Boumsell L, Dausset J, Milstein C, Schlossman SF, ed. *Leukocyte Typing*. New York: Springer-Verlag, 1984: 507. vol 2).
39. David H, Korth I. Elektronenmikroskopische und histochemische untersuchungen menschlicher riesenzellepulvis. *Frankfurt Z. Pathol* 1959;70(89-97).
40. Davis JMG. The ultrastructural changes that occur during the transformation of lung macrophages to giant cells and fibroblasts in experimental asbestosis. *Br J Exp Pathol* 1963;44:568-575.
41. Dayan D, Buchner A, David R. Myofibroblasts in peripheral giant cell granuloma : Light and electron microscopic study. *Int J Oral Maxillofac Surg* 1989;18:258-261.
42. Debray H, Decout D, Strecker G, Spik G, Montreuil J. Specificity of twelve lectins towards oligosaccharides and glycopeptides related to N-glycosylproteins. *Eur J Biochem* 1981;117:41.
43. Drepper H, Themann H, Wannenmacher E. Electron and microscopic and histochemical studies on giant cell epulis of man. *Frankfurt Z Pathol* 1961;71:202-220.
44. Eckardt A, Pogrel MA, Kaban LB, Chew K, Mayall BH. Central giant cell granulomas of the jaws: Nuclear DNA analysis using image cytometry. *J Oral Maxillofac Surg* 1989;1989:3-6.

45. Eisenbud L, Stern M, Rothberg M, Sachs SA. Central giant cell granuloma of the jaws : Experiences in the management of thirty- seven cases. *J Oral Maxillofac Surg* 1988;46:376-384.
46. Eisenstein R. Giant cell tumour of tendon sheath. *J Bone Joint Surg* 1969;50:476-486.
47. El-Labban NG, Lee KW. Myofibroblasts in central giant cell granuloma of the jaws: an ultrastructural study. *Histopathol* 1983;7:907-918.
48. El-Mofty SK, Osdoby P. Growth behaviour and lineage of isolated and cultured cells derived from giant cell granuloma of the mandible. *J Oral Pathol* 1985;14.
49. Fajardo LP. The complexity of endothelial cells. *Am J Clin Pathol* 1989;92:241-250.
50. Ficarra G, Kaban LB, Hansen LS. Central giant cell lesions of the mandible and maxilla: A clinicopathologic and cytometric study. *Oral Surg Oral Med Oral Pathol* 1987;64:44-49.
51. Finley JCW, Grossman GH. Somatostatin-containing neurons in the rat brain : Widespread distribution revealed by immunocytochemistry after pretreatment with pronase. *Am J Anat* 1978;153:483-488.
52. Finley JCW, Petrusz P. The use of proteolytic enzymes for improved localisation of tissue antigens with immunocytochemistry. New York: Academic Press, 1982(Bullock GR, Petrusz P, ed. *Techniques in Immunocytochemistry*; vol 1).
53. Flanagan AM, Tinkler SMB, Horton MA, Williams MD, Chambers DJ. The multinucleate cells in giant cell granulomas of the jaw are osteoclasts. *Cancer* 1988;62:1139-1145.

54. Franklin CD, Craig GT, Smith CJ. Quantitative analysis of histological parameters in giant cell lesions of the jaws and long bones. *Histopathol* 1979;3:511-522.
55. Gabbiani G. The myofibroblast : A key cell for wound healing and ficrocontractive diseases. *Progress Clin Biol Res* 1981;54:183-194.
56. Gabbiani G, Ryan GB, Majno G. Presence of modified fibroblasts in granulation tissue and their role in wound contracture. *Experientia* 1971;27:549-550.
57. Ghadially FN. *Fine Structure of Synovial Joints*. London: Butterworths, 1983
58. Ghadially FN. *Ultrastructural Pathology of the Cell and Matrix*. (Third ed.) London: Butterworths, 1988; vol 1).
59. Giansanti JS, Waldron CA. Peripheral giant cell granuloma : Review of 720 cases. *J Oral Surg* 1969;27:787-91.
60. Goldring SR, Roelke MS, Petrison KK, Bhan AK. Human giant cell tumours of bone identification and characterisation of cell types. *J Clin Invest* 1987;79:483-491.
61. Gothlin G, Ericsson JLE. Observations on the mode of uptake of thorium dioxide particles by osteoclasts in fracture callus. *Calcif Tissue Res* 1972;10:216-222.
62. Graham RCJ, Karnovsky MJ. The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney : Ultrastructural cytochemistry by a new technique. *J Histochem Cytochem* 1966;14:291-302.
63. Hamlin WB, Lund PK. "Giant Cell Tumours" of the mandible and the facial bones. *Arch Otolaryngol* 1967;86:658-665.
64. Hanaoka H, Friedman B, Mack P. Ultrastructure and histogenesis of giant cell tumour of bone. *Cancer* 1970;24:1408-1423.

65. Harris M. Central giant cell granulomas regress with calcitonin therapy. *Brit J Oral Maxillofac Surg* 1993;31:89-94.
66. Hattersley G, Chambers TJ. Calcitonin receptors as markers for osteoclastic differentiation: Correlation between generation of bone resorptive cells. *Endocrinology* 1989;125:1606-1612.
67. Hayward JR. Malignant giant cell tumour of the mandible: report of a case. *J Oral Surg* 1959;17:75-80.
68. Holthofer H, Virtanen I, Karinemi L, Hormia M, Linder E, Miettinen A. Ulex Europae 1 Lectin as a marker for vascular endothelium in human tissues. *Lab Invest* 1982;47:60-66.
69. Holthofer H, Virtanen I, Pettersson E, et al. Lectins as fluorescence microscopic markers for saccharides in the human kidney. *Lab Invest* 1981;45:391.
70. Horton MA, Lewis D, McNulty K, Pringle JAS, Chambers TJ. Monoclonal antibodies to osteoclastomas (Giant Cell Bone Tumours) : Definition of osteoclast specific antigens. *Cancer Res* 1985;45:5663-5669.
71. Horton MA, Lewis D, McNulty K, Pringle JAS, Fuller K, Chambers TJ. Human fetal osteoclasts fail to express macrophage antigens. *Brit J Expr Pathol* 1984;66:103-108.
72. Hoyer LW, de los Santos RP, Hoyer JR. Anti-haemophilic factor antigen: localisation in endothelial cells by immunofluorescent microscopy. *J Clin Invest* 1973;52:2737-2744.
73. Huang SN, Minassian H, Moore JD. Application of immunofluorescent staining on paraffin sections improved by trypsin digestion. *Lab Invest* 1976;35:383-390.
74. Hutter RVP, Worcester JN, Francis KC, Foote FW, Stewart FW. Benign and malignant giant cell tumours of bone. *Cancer* 1962;15:653-690.

75. Isobe T, Okuyama T. The amino-acid sequence of S-100 protein and its relation to the calcium binding proteins. *Eur J Biochem* 1978;116:79-86.
76. Jaffe HL. Giant cell reparative granuloma, traumatic bone cyst and fibrous (fibrous-osseous) dysplasia of jaw bones. *Oral Surg* 1953a;6:159-175.
77. Jaffe HL. Giant cell tumour (osteoclastoma) of bone : its pathologic delimitation and the inherent clinical implications. *Am Roy Coll Surg Engl* 1953b;13:343.
78. Jaffe HL, Lichtenstein L, Portis RB. Giant Cell Tumour of Bone. Its pathologic appearance, grading, supposed variants and treatment. *Arch Pathol* 1940;30:993.
79. Katsikeris N, Kakarantza-Angelopoulos E, Angelopoulos AP. Peripheral giant cell granuloma: Clinicopathologic study of 224 new cases and review of 956 reported cases. *Int J Oral Maxillofac Surg* 1988;17:94-99.
80. Kocher O, Skalli O, Bloom WA, Gabbiani G. Cytoskeleton of rat aortic smooth muscle cells: Normal condition and experimental intimal thickening. *Lab Invest* 1984;50:645-652.
81. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody. *Nature* 1975;256:495-497.
82. Lichtenstein L. Aneurysmal bone cyst. A pathological entity commonly mistaken for giant-cell tumour and occasionally for haemangioma and osteogenic sarcoma. *Cancer* 1950;3:279.
83. Lichtenstein L. Giant Cell Tumour of Bone. Current status of problems in diagnosis and treatment. *J Bone Joint Surg* 1951;33A:143.
84. Lichtenstein L. Aneurysmal bone cyst. Further observations. *Cancer* 1953;6:1228.

85. Little D, Said JW, Siegel RJ, Fealy M, Fishbein MC. Endothelial cell markers in vascular neoplasms: an immunohistochemical study comparing Factor VIII Related Antigen, blood group specific antigens, 6-Keto-PGFL Alpha, and Ulex Europaeus 1 Lectin. *J Pathol* 1986;149:89-95.
86. Lucas RB. Giant cell lesions. In: *Pathology of Tumours of the Oral Tissues*. Third ed. Churchill Livingstone, 1976: 263-278.
87. Mallory FB. Giant Cell Sarcoma. *J Med Res* 1911;49:141-48.
88. Marks SC. The origin of osteoclasts : Evidence, clinical implications and investigative challenges of an extra skeletal source. *J Pathol* 1983;12:226-256.
89. Mason DY, Gatter KC. The role of immunocytochemistry in diagnostic pathology. *J Clin Pathol* 1987;40:1042-1054.
90. McKee PA, Anderson JC, Switzer ME. Molecular structural studies of human factor VIII. *Ann NY Acad Sci* 1975;240:8-33.
91. Miettinen M, Holthofer H, Lehto V, Miettinen A, Virtanen I. Ulex Europaeus 1 Lectin as a marker for tumours derived from endothelial cells. *Am J Clin Pathol* 1982;79:32-36.
92. Mintz GA, Abrams A, Carlsen GD, Melrose R, Fister W. Primary malignant giant cell tumour of the mandible. *Oral Surg* 1981;51:1644-171.
93. Moore BW. A soluble protein characteristic of the nervous system. *Biochem Biophys Res Commun* 1965;19:739-744.
94. Mukai K, Rosai J, Burgdorf WHC. Localisation of Factor VIII Related Antigen in vascular endothelial cells using an immunoperoxidase method. *Am J Surg Pathol* 1980;4:273-276.

95. Nakajima T, Watanabe S, Sato Y, Kameya T, Hirota T, Shimosato Y. An immunoperoxidase study of S-100 protein distribution in normal and neoplastic tissues. *Am J Surg Pathol* 1982;6:715-727.
96. Nakane PK, Pierce GB. Enzyme labelled antibodies for the light and electron-microscopic localisation of tissue antigens. *J Cell Biol* 1966;33:307.
97. Ordonez NG, Batsakis JG. Comparison of Ulex Europae 1 Lectin and Factor VIII related antigen in vascular lesions. *Arch Pathol Lab Med* 1984;108:129-132.
98. Osterby R, Lundbaek K. The basement membrane morphology in Diabetes Mellitus. In: Ellenberg M, Rifkin H, ed. *Diabetes Mellitus: Theory and Practice*. New York: McGraw-Hill, 1970: 178.
99. Piovella F, Nalli G, Malamani GD, et al. The ultrastructural localisation of factor VIII related antigen in human platelets, megakaryocytes and endothelial cells utilizing a ferritin-labelled antibody. *Br J Haematol* 1978;39:209-213.
100. Poppema S, Hollema H, Visser L, Vos H. Monoclonal antibodies (MT1, MT2, MB1, MB2, MB3) reactive with leukocyte subsets in paraffin-embedded tissues sections. *Am J Pathol* 1987;127:418-429.
101. Ramani P, Bradley NJ, Fletcher CDM. A new monoclonal antibody to endothelium: assessment of its diagnostic utility in paraffin sections. *Histopathol* 1990;17:237-242.
102. Reading M. A digestion technique for the reduction of background staining in the immunoperoxidase method. *J Clin Pathol* 1977;30:88-90.
103. Regezi JA, Zarbo RJ, Lloyd RV. Muramidase, Alpha-1 Antitrypsin, Alpha-1 Antichymotrypsin, and S-100 Protein immunoreactivity in giant cell lesions. *Cancer* 1987;59:64-68.

104. Ridley M, Turk J, Badenoch-Jones P. In vitro modification of membrane receptors of cells of the mononuclear phagocyte system. *J Pathol* 1979;127:173.
105. Sankey EA, More L, Dhillon AP. QBEnd/10 : A new immunostain for the routine diagnosis of Kaposi's Sarcoma. 1990;*J Pathol*:267-271.
106. Sara AS, Ayala AG, El-Naggar RJY, Raymond AK, Murray JA. Giant cell tumour of bone: A clinicopathologic and DNA flow cytometric analysis. *Cancer* 1990;66:2186-2190.
107. Schajowicz F. Giant cell tumours of bone (osteoclastoma). *J Bone Joint Surg* 1961;43-A:1-27.
108. Schurch W, Seemayer TA, Lagace R, Gabbiani G. The intermediate filament cytoskeleton of myofibroblasts: An immunofluorescence and ultrastructural study. *Virchowa Arch [Pathol Anat]* 1984;403:323-336.
109. Schwartz HS, Jenkins RB, Dahl RJ, Dewald GW. Cytogenetic analyses on giant cell tumours of bone. *Clin Orthop Rel Res* 1989;240:250-260.
110. Shaklar G, Meyer I. Giant cell tumours of the maxilla and mandible. *Oral Surg Oral Med Oral Pathol* 1961;14:809-827.
111. Sternberger LA, Hardy PH, Cuculis JJ. The unlabelled antibody-enzyme method of immuno-histochemistry preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochaetes. *J Histochem Cytochem* 1970;18:315.
112. Streefkerk JF. Inhibition of erythrocyte pseudoperoxidase activity by treatment with hydrogen peroxide following methanol. *J Histochem Cytochem* 1972;20:829-831.
113. Streuli M, Morimoto C, Shreiber M, Schlossman SF, Saito H. Characterisation of CD45 and CD45R monoclonal antibodies using transfected mouse cell lines that

express individual human leukocyte common antigens. *J Immunology* 1988;141:3910-4.

114. Sutton JS, Weiss L. Transformation of monocytes in tissue culture into macrophages, epithelioid cells and multinucleated giant cells. *J Cell Biol* 1966;28:303-332.

115. Taylor CR, J. B. Immunohistological detection of intracellular immunoglobulin containing cells in formalin-fixed embedded tissues using peroxidase labelled antibody. *J Clin Pathol* 1974;24:14-20.

116. Thompson SH, Bischoff P, Bender S. Central giant cell granuloma of the mandible : Case report. *J Oral Maxillofac Surg* 1983;41(1983):743-746.

117. Thorgeirsson G, Robertson AL. The vascular endothelium - pathobiologic significance. *Am J Pathol* 1978;93:803-848.

118. Tsukada T, McNutt MA, Ross R, Gown AM. HHF35, A Muscle Actin-Specific Monoclonal Antibody. 2: Reactivity in Normal, Reactive, and Neoplastic Human Tissues. *Am J Pathol* 1987;127:389-402.

119. Tsukada T, Tippens D, Gordon D, Ross R, Gown AM. HHF35, A Muscle Actin Specific Monoclonal Antibody. 1. Immunocytochemical and Biochemical Characterisation. *Am J Pathol* 1987;126:51-60.

120. Turner RD, Beckstead JH, Warnke RA, Wood GS. Endothelial cell phenotypic diversity: In situ demonstration of immunologic and enzymatic heterogeneity that correlates with specific morphologic subtypes. *Am J Clin Pathol* 1987;87:569-575.

121. Valazquez A, Payne FE, Kroath RS. Viral induced fusion of human cells. I. Qualitative studies of the fusion of human diploid fibroblasts induced by sendai virus. *J Cell Physiol* 1971;78:93-110.

122. Waldron CA, Shafer WG. The central giant cell reparative granuloma of the jaws: An analysis of 38 cases. *Am J Clin Pathol* 1966;45:437-447.
123. Walker DG. Control of bone resorption by haemopoietic tissue. The induction and reversal of congenital osteopetrosis in mice through use of bone marrow and splenic transplants. *J Expr Med* 1975;142:651-663.
124. Watt SM, Karhi K, Gatter K. Distribution and epitope analysis of the cell membrane glycoprotein (HPCA-1) associated with human haemopoietic progenitor cells. *Leukaemia* 1987;1:417-426.
125. Wheathers DR, Campbell WG. Ultrastructure of the giant-cell fibroma of the oral mucosa. *Oral Surg Oral Med Oral Pathol* 1974;38:550-561.
126. Wilson AJ. Factor VIII Related Antigen staining by immunoperoxidase technic in smaller laboratories: A potential problem. *Am J Clin Pathol* 1984;81:117-120.
127. Wood GW, Neff JR, Gollahon KA, Gourley WK. Macrophages in giant cell tumours of bone. *J Pathol* 1977;125:53-58.
128. Woodcock-Mitchell J, Adler KB, Low RB. Immunohistochemical identification of cell types in normal and bleomycin-induced fibrotic rat lung. *Am Rev Respir Dis* 1984;130:910-916.

