

**CLINICO - PATHOLOGY AND ULTRASTRUCTURAL
STUDY OF NEPHROPATHY CHANGES DUE TO LUPUS
ERYTHEMATOSIS DISSEMINATUS ☼**

By

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INTRODUCTION

Knowledge concerning normal kidney structure and pathological changes has increased as the tools utilized in such studies have increased in sophistication. A nephropathic change of great concern has been that accompanying lupus erythematosus. Alterations in kidney structure accompanying this disease has been described as swelling, degeneration, fibrinoid changes, hyalization of glomerular cells and changes in the basement membrane (1). With the advent of the electron microscope, however, many limitations of magnification and resolution were obviated and many cellular and subcellular changes previously in doubt were clarified. Clinical diagnoses, also, were enhanced by the new dimensions of magnification and resolutions offered by the use of the electron microscope.

The visible manifestation of this disease may be described as cutaneous lesions especially on the nose, cheeks, and manubrium area. The visible manifestations may be accompanied by neurological and psychiatric symptoms.

Previous definitive studies by Klemperer and others (1-2), in their investigations on connective tissue degeneration in lupus erythematosus first focused attention to the fact that this disease is not only manifested externally but also internally in the abdominal organs specifically the kidney.

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Jassar et al (3) and Meurcke (4) also contributed to present knowledge on acute nephrosis due lupus and on accompanying damage to abdominal organs.

These works have laid a foundation for further, more extensive, knowledge of the ultrastructural changes which may occur during lupus erythromatosus as revealed by the electron microscope. This report comprises a study of a patient suffering from this disease.

MATERIALS AND METHODS

For light microscopic study kidney tissue taken by biopsy was fixed in 10% neutral formol and the paraffin embedded sections 5 microns in thickness were stained with hematoxylin and eosin. Other sections were stained with P.A.S. and Trichrome stain.

For electron microscopy additional biopsy tissue was cut into small pieces less than 2 mm², fixed in 2% buffered glutaraldehyde, for 1 hour and postfixcd in 2% buffered osmium tetroxide at pH 7.5, for an additional hour. Epoxy embedded sections 90-120 mmu taken with the Porter Blum ultramicrotome were lifted on copper grids, stained with lead citrate and examined with Simens Elmiskop 1 A.

CASE HISTORY

A nineteen year old woman from Tehran attended Pahalavi Hospital Medical Department complaining of pains in her knees and wrists. One year ago, she had swollen knees but after receiving penicillin she improved temporarily. Three months ago she came back again with swollen and painful knees, wrists and fingers. There was no significant history of disease in her family. The patient looked normal except that she was a little pale and had a slight redness of the skin around her nose. Her temperature was 40° C.

The patient passed a large amount of urine 7 to 8 times a day. This daily increase had started about two months previously. Results of the urinalysis were:

Albumin	5.5 gms P/L
W.B.C.'s	10-15 m/cm
R.B.C.'s	8-20 m/cm

Granular cylinders were found.

Intravenous Pyelography was instituted but at the end of one hour there was no excretion of the ultra-pack solution. Further tests revealed:

Blood urea	1.2 gm/L
Uric acid	73.5 mg/L

L. E. cells were found in peripheral blood.

Blood electrophoresis (5) (6).

Albumin	29.6%
a-1-a-globulin	5.1%
a-2-a-globulin	13.6%
b-1-a-globulin	10.9%
b-2-a-globulin	10.9%
g-globulin	40.8%
Protein (complete)	7.622 gm/100 ml blood serum

RESULTS

Light Microscopy - The capsule was generally thicker than ordinarily but this varied depending on the extent of progress of glomerular damage. Proliferation was found in endothelial, epithelial and mesenchymal cells. Epithelial adherence (parietal and visceral) resulting from glomerular proliferation was seen.

Epithelial cells were more voluminous than usual and showed a specific vacuolar degeneration. Endothelial cells showed more morphological changes along with inflammation and fibrinoid degeneration of the base of the endothelium.

Epithelial cells contained hematoxylin bodies and condensed granulations. In glomerular capillaries thrombohyalin was found; in the basement membrane wire loop formations with fibrinoid changes and hyaline were seen. This phenomenon is substantiated by examination with the PAS and Trichrome stains. There was some inflammation of epithelial cells of Bowman's capsule and condensation bodies were in the urinary spaces. Also, in the vessels associated with the capsule was a general thickening of hyaline and fibrinoid along with cellular infiltration of inflammatory intercytic tissue.

Electron Microscopy. Ultrastructural characteristics of lupus erythromatosus are described as follows:

1. Subcellular deposits - This is one of the most important ultrastructural changes in L. E. It was characterized in this tissue by a thickening of the basal lamina and wire loop formation resulting from changes of γ -globulin complement, fibrin, and numerous nuclear proteins. This deposit was seen to be concentrated primarily in the intercellular spaces of endothelial cells of the viens and in basal lamina. In many parts the accumulation of fibrinoid was on both sides of the basal lamina below the endothelial cells, epithelial cells and also the mesenchymal cells. The condition in which the deposits are formed in the endothelial celled cytofold.

2. Hematoxylin Bodies - These are fairly large granules which are visible by ordinary microscopy (6). They are situated in the cytoplasm of some epithelial and mesenchymal cells and a few in endothelial cells. Two types were found. One type apparently formed by myelin degeneration due to intense osmiophily. They may be degenerated lysosomes or the remains of some pycnotic nuclei affected by myelin degeneration. The second type was round or elliptical elements and were found only in epithelial cells. They were slightly osmiophilic unilaminar membranes and most were situated in the cytoplasm near the surface of the epithelial cells adjacent to the urinary space. This type appears to be the one originally described from light microscopy. This type of hematoxylin body may be derived from nuclei and contain high percentate of protein and some γ -globulin. Some large osmiophilic elements were seen inside the urinary space of the glomerula which resembled hematoxylin bodies.

3. Endothelial cells - Most of these cells were swollen and were characterized by cytofold.

4. Glomerular epithelial cells - These cells like other glomerulocytes were larger than normal (7). There were many small vesicles containing osmiophilic material in the cytoplasm. Some vesicles contained multivesicular bodies. The basal cytoplasm was irregular; the lumen surface had villi. Myelinic degeneration of lysosomes was readily evident in these cells. The lysosomes were larger than normal conditions. Glycogen deposits were found as tiny granulations, condensed (8). Glycogen could be seen in normal cells as well but not in the same pattern as in the degenerate cells.

5. Blood vessel damage - Fibrinoid deposits (9) were seen in the walls of the blood vessels of the kidney. These deposits were found also in the reticulum of the wall of the urinary tubules especially in proximal areas.

6. Changes in urinary tubules - There was an increased number of cytosegrosomes (10) in the proximal urinary tubules. These condensed granules surrounded by membranes, eventually develop into urinary cylinders and are discharged into the tubules. Mitochondria maintained a normal except for the presence of excessive granulation.

DISCUSSION

The diagnostic picture in relation to lupus is not clear or complete. Whereas there is a great deal known about causative or related factors, and specifics known about the clinical manifestations there remains yet to be known all related factors. There have been differences in opinion as to the cause of lupus. Some causative or affecting factors are sunlight (11) drugs (12) and perhaps heredity. Unfortunately neither of these singly or in combination have been pinpointed specifically as the etiological agent; however, the aggravating factors are known.

The disease, once incurred, may be diagnosed clinically very readily and there are several well-defined clinical manifestations. However, here again, the symptoms taken singly may be diagnostic of any one of many types of kidney nephropathy (13,14). Taken in combination these symptoms may essentially point lupus erythromatosus. The histological picture may confirm kidney nephropathy not lupus specifically. Histological findings, supporting the clinical findings may be sufficient for definitive diagnosis.

Electron microscopy, on the other hand, has afforded an even further check on the original clinical diagnosis in that there may exist several ultrastructural characteristics in lupus kidney which are not completely characteristic of other types of kidney nephropathy. This is not necessarily a recommendation that the electron microscope be universally employed as a clinical tool. On the other hand the availability of this tool would serve as a means of definitely confirming that which has so far been revealed to have been correctly assessed. Much more important, however, is the availability of this instrument as a research tool hopefully revealing hitherto unknown aspects of certain pathologies which will point to basic causes and hopefully to corrective or preventive action.

Diagnostically the presence of kidney disease and the presence of L.E.

SUMMARY AND CONCLUSIONS

Differences exist as to the physical manifestation of the L. E. syndrome however certain basic symptoms appear in all cases of the disease. These basic symptoms may not be accompanied by other peripheral manifestations.

Urine albumin - The appearance of urine albumin to the extent of 8-10 mgm.mil is a sign of kidney damage. This may be accompanied by acute kidney inadequacy, cyrtitis with fever and pain. There may be a varying globulin excretion which is taken by some as a precursor of ensuing damage. This point, of course, has been debated.

Nephrotic syndrome - Nephrotic syndrome is found in 30% of all of the cases of systematic L.E.

The disease occurs more frequently in women than in men and especially among young people. More than one person in a family may be affected leading to the assumption that the trait is inherited. The hereditary nature of the disease, however, has not been definitely established. Basically the disease appears to original as an abnormal immunological reaction to external or internal causes or perhaps even auto-immunological. Causative or aggravating factors may be long exposure to sunlight, and ultraviolet rays which may free lysozymes or other proteolytic enzymes which attack the ground substance of the cell membranes of the endothelium. Drugs such as penicillin, sulfonamide and hydralyzin have caused allergic reactions resulting in lupus although differences of opinions exist as to the roles these drugs may play as a causative agent since in some patients a withdrawal of medication results in accessation of the symptoms but in other patients there is no apparent change. It has been proposed that the above mentioned drugs react with body proteins to form the antibodies causative of the disease. Infections also have been mentioned as a causative factor as well as rheumatic factors and rheumatic arthritis.

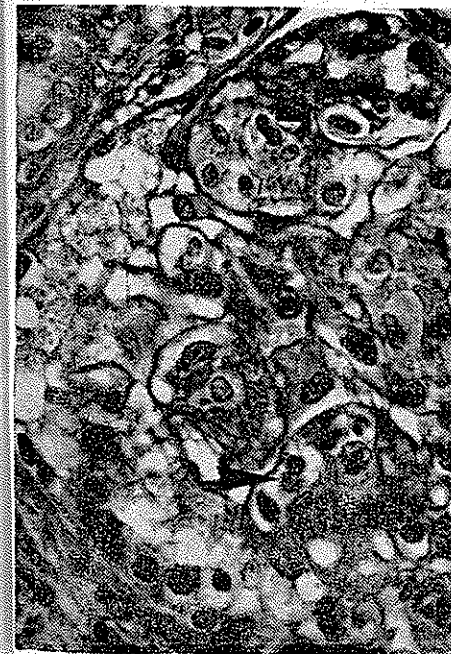
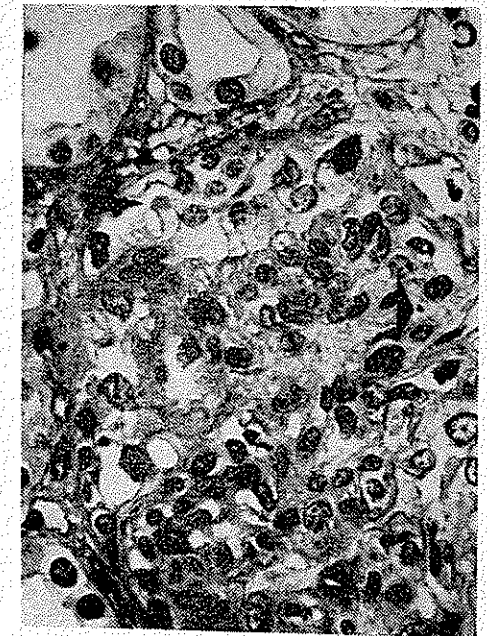
When clinical and paraclinical signs indicate L.E. definitive conclusions may be reached utilizing electron microscopy.

Cells (15,16) in peripheral blood may confirm definitively lupus erythematosus. Additional serological evidence such as hyper g-globulinemia, 7-s-g-globulin and 19-s-g-globulin may then positively confirm the diagnosis (17). Supporting evidence of a specific type of glomerular damage substantiates prior clinical and paraclinical evidence.

The foregoing ultrastructural characteristics are then seen to represent an additional check of routine diagnostic procedures. In addition the irregularities observed permits an opportunity to correlate minute ultrastructural changes with known biochemical changes previously demonstrated.

Legend of figures.

- Fig: 1, A, B, C. Disseminated lupus erythematosus. This photomicrographs shows various degrees of glomerular alteration.
 Fig. A and B (arrow) shows the hamatoxylin body and hyalin thrombi.
 Fig. C shows cell proliferation (arrow) hyalin thrombi.
 Fig. D PAS stain. arrow shows wire loops formation.
- Fig: 2 Electron micrography showing the epithelial cell proliferation. Numerous microvesicular body are seen in the cytoplasm of the epithelial cells. Presence of cytofold in the endothelial cells. Mag. 10000X.
- Fig: 3 Electron micrography of epithelial cell and capillary loops. Many dense granule and micro-vesicular body are seen in the epithelial cell. Micro-villous proliferation in the epithelial cells are seen. Mag. 5000X.
- Fig: 4 Presence of fibrinoid deposits in the basement membrane and glycogene granules Mag. 5000X.
- Fig: 5 Electron micrography showing the thickness of basement membrane and micro-villous proliferation of epithelial cell. Mag. 10000X.
- Fig: 6 Presence of hyalin granule in the Bowman's space. Mag. 10000X.
- Fig: 7 Electron micrography showing the hamtoxylin body and myelinic degeneration. HB Hamatoxylin body, MD myelinic degeneration, Mag: Fig.7 5000X and Fig. A. - 15000X.
- Fig: 8 Electron micrography showing the thickness of basement membrane with fibrinoid deposits. Presence of cytofold and microvillous proliferation of endothelial and epithelial cell. Mag. 5000X.
- Fig: 9 Dense granule and vesicular degeneration of the epithelial cells. Mag. 10000X.
- Fig: 10 Electron micrography showing the fibrinoid deposit in the capillary wall. Mag. 10000X.
- Fig: 11 Electron micrography showing a portion of proximal tubule containing many cytosome, cytoseresoms and dense granule. Mag. 5000X.
- Fig: 12 Electron micrography showing a portion of distal tubule. Arrow showing fibrinoid deposit in the basement membrane. Mag. 10000X.

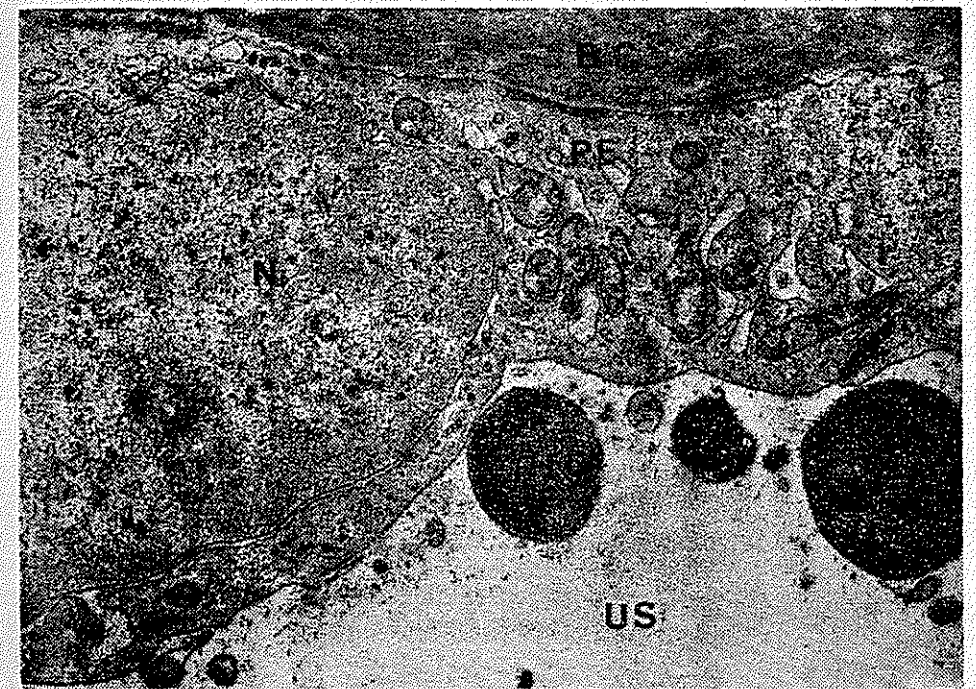
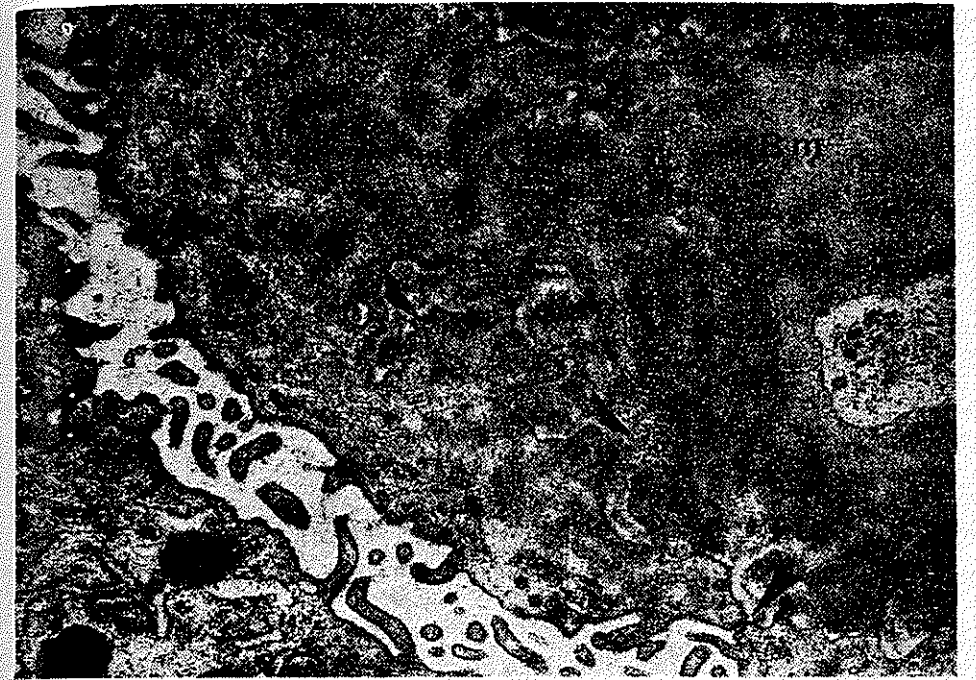
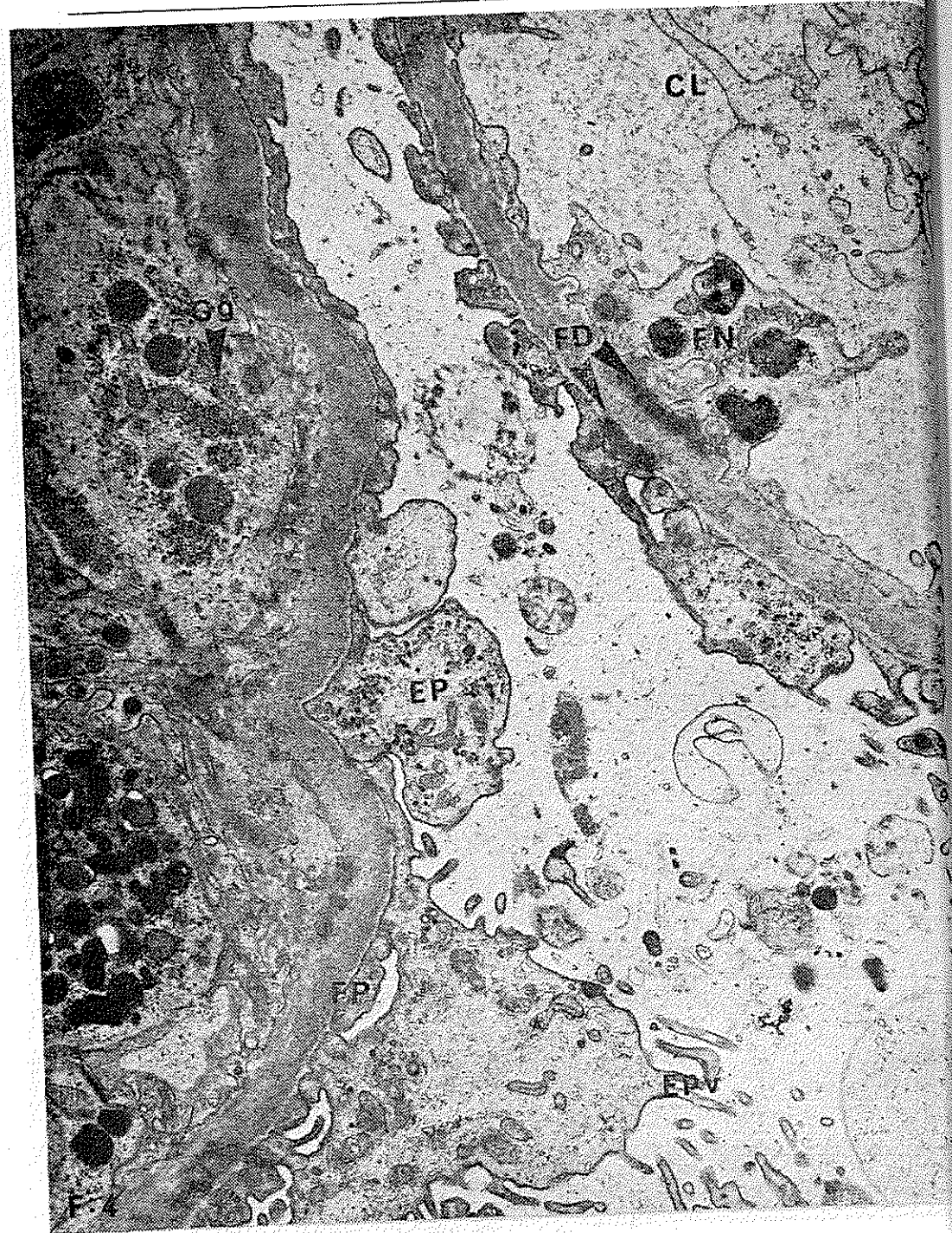


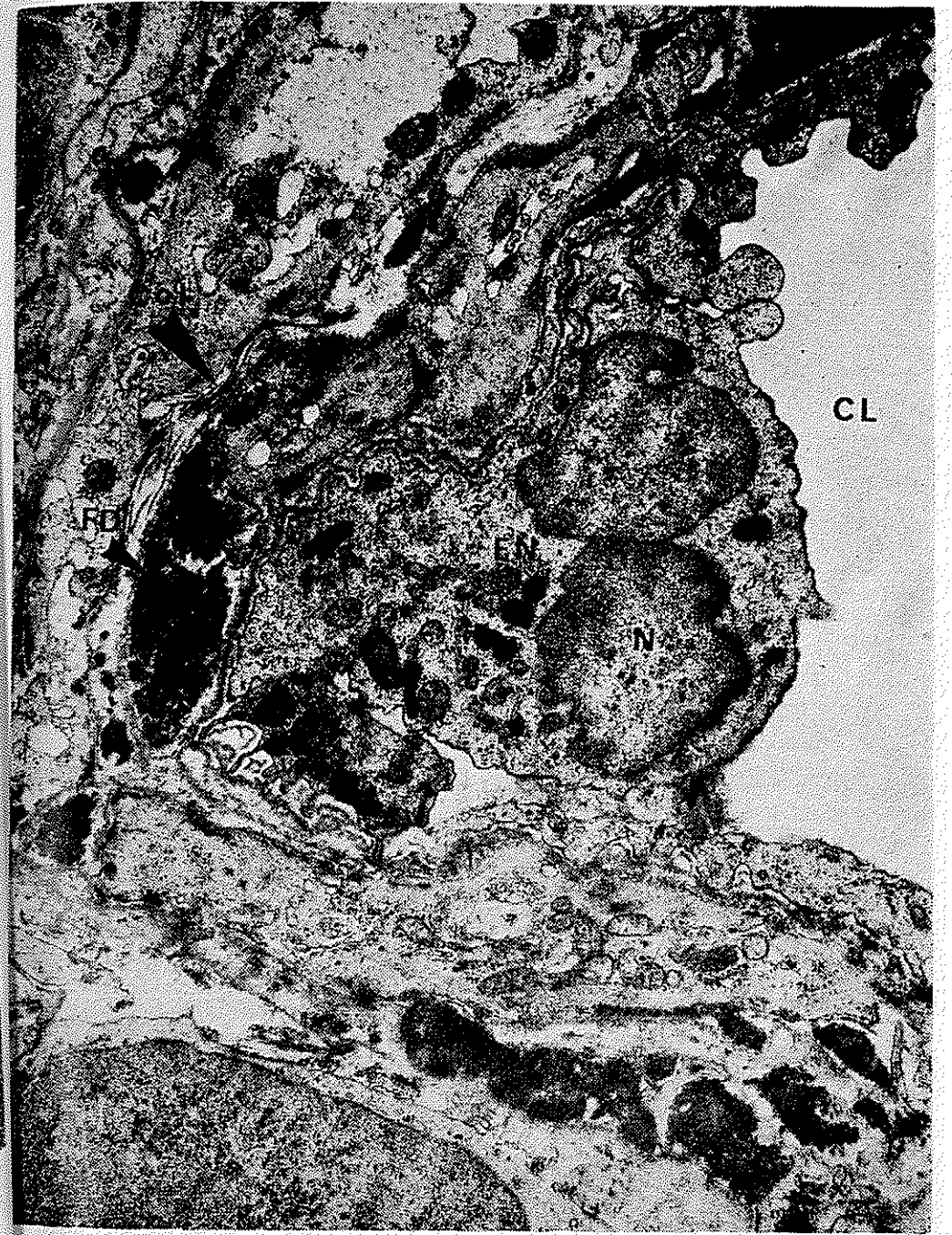
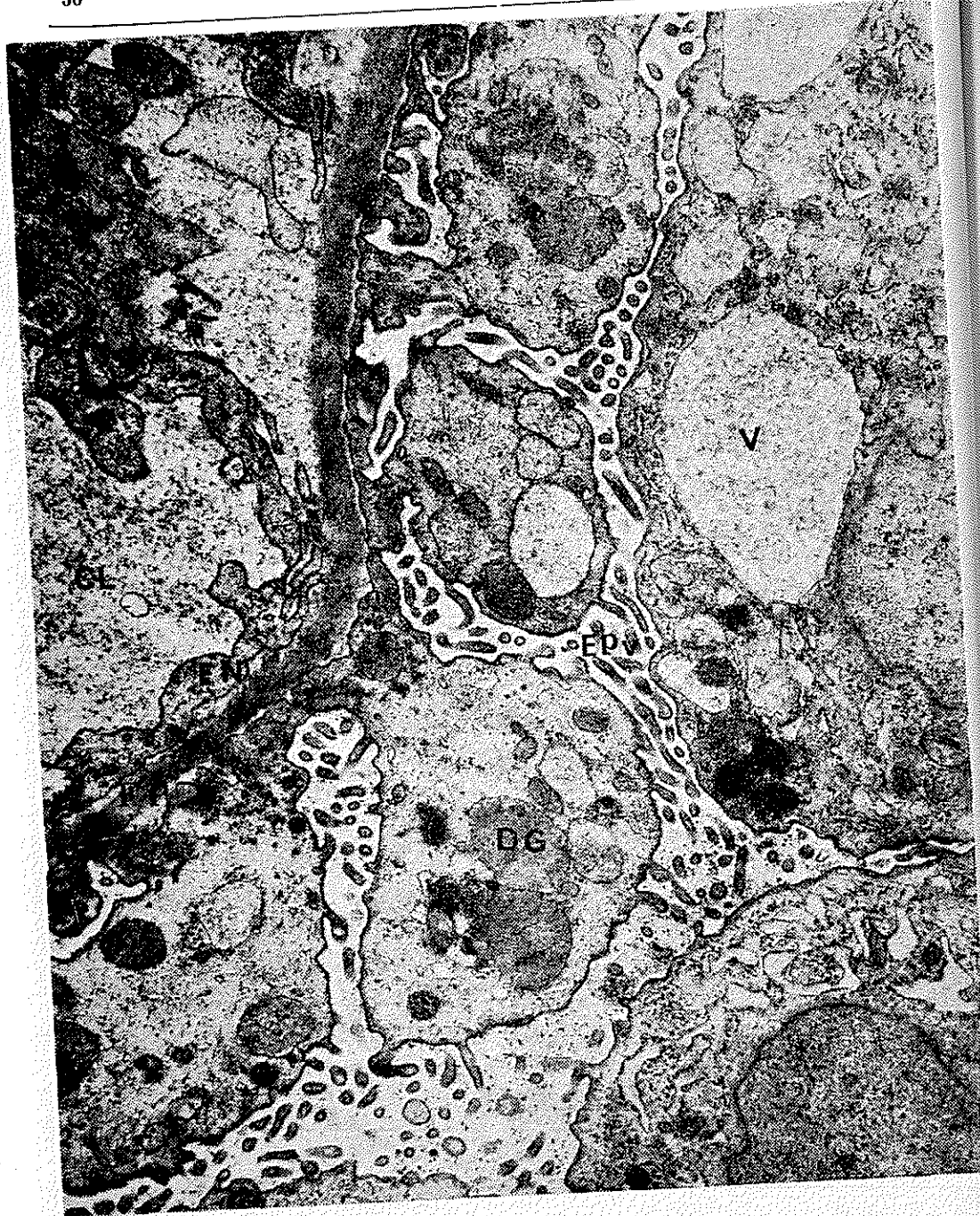


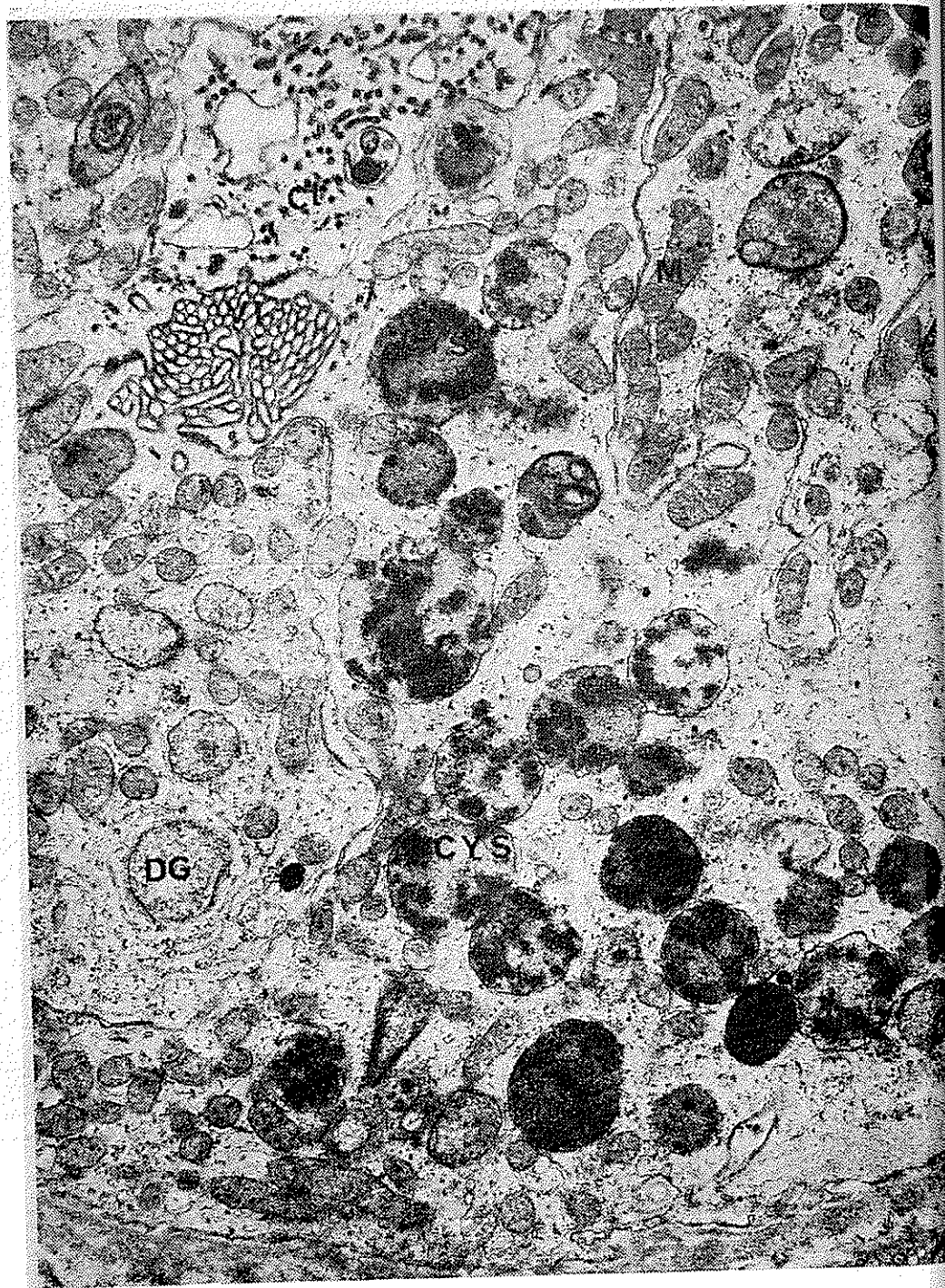
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EFFECTS OF THYROIDECTOMY ON THE SERUM PROTEIN FRACTIONS IN DOG

By:

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INTRODUCTION

Variations in the serum protein level in clinical hypothyroidism or after thyroidectomy have been reported by several authors, (3,6,9).

A number of investigators have observed a significant decrease in the albumin fraction (Abreu et al 1957, and Aschkensay 1960) and increase in gamma globulin (Moor 1945, and Boas 1955) after thyroidectomy.

Because of the role of plasma alpha globulin and albumin as the thyroid hormone-carrier (thyroxine-binding-protein) to the tissues (Anderoli et al 1961) and because of some discrepancies in the results reported so far on the alteration of the plasma protein fractions in the course of human spontaneous hypothyroidism (Atlas et al 1950), we decided to study the effect of thyroidectomy on the dog serum protein fraction.

METHODS

A total of 29 puppies of both sexes and weighing 2.5-7 kg were thyroidectomized under anesthesia induced by I.V. injection of 30-40 mg/kg Pentobarbital (Nembutal).

Serum electrophoresis was performed before and then every week after thyroidectomy until the spontaneous death of the animals.

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