Virchows Arch [Pathol Anat] (1982) 397:261–285

Virchows Archiv A

Pathology of a New Toxic Syndrome Caused by Ingestion of Adulterated Oil in Spain

F.J. Martinez-Tello, J.J. Navas-Palacios, J.R. Ricoy, R. Gil-Martín, J.M. Conde-Zurita, F. Colina-Ruiz Delgado, I. Tellez, A. Cabello, and S. Madero-García

Departamento de Anatomía Patológica, Ciudad Sanitaria "1° de Octubre" Carretera de Andalucía Km, 5,400, Madrid-26, Spain

Summary. The Toxic Syndrome (TS) caused by ingestion of adulterated rapeseed oil in Spain is a new disease of multisystemic character whose aetiology and pathogenesis remains unknown. The most prominent pathological feature is a peculiar non-necrotizing vasculitis, that affects mainly the intima and involves vessels of every type and size in practically every organ. The TS begins with an acute clinical picture with pleuropneumopathy, fever, headaches, exanthems and eosinophilia. In these early clinical phases the main pathological findings were observed in the lungs and consisted of intense pulmonary interstitial oedema with scanty inflammatory mononuclear infiltrates. Ultrastructural study revealed hydropic degeneration of pneumocytes types I and II with desquamation of type I. The patients in this phase died of respiratory failure, later deaths were due to thromboembolic complications. Later still the patients developped a neuromuscular syndrome, sclerodermiform skin lesions and severe weight loss and died predominantly of infectious complications and respiratory failure. The anatomopathological picture in the peripheral nerves was that of inflammatory neuropathy with a lymphocytic perineuritis that led to perineural fibrosis with secondary axonal degeneration. The muscle presented an interstitial inflammatory myopathy at first followed by a neurogenic muscular atrophy. The skin lesions in the late phases consisted in dermal or dermal and subdermal fibrosclerosis, with vasculitis of the small arteries in the lower dermis. The salivary glands and pancreas showed vasculitis and interstitial inflammation which progressed to interstitial fibrosis and parenchymal atrophy.

Key words: Toxic-oil syndrome – Vasculitis – Inflammatory myopathy – Perineuritis – Scleroderma

Offprint request to: F.J. Martínez-Tello at the above address

0340-1227/82/0397/0261/$05.00

In Spain, since May 1981, a new toxic syndrome (TS) has caused 271 deaths and affected around eighteen thousand people who have required hospitalization. At first the syndrome was considered to be an infection (mycoplasma pneumoniae) despite the considerable epidemiological evidence against this assumption. On June 10, 1981, the epidemic was traced to the ingestion of a particular lot of rapeseed cooking oil (Tabuenca 1981). This oil was sold fraudulently, door to door, as pure olive oil. The Goverment seized 3,000,000 l of oil, and thereby averted the presentation of new cases. However, by that time, a certain number of patients had had to be readmitted to hospital.

The illness was initially called "atypical pneumonia" because clinical and X-Ray findings were compatible with that diagnosis. Most commonly reported symptoms were: fever (37.5–39° C); respiratory distress; nausea and vomiting, various skin eruptions (morbilliform, papular), general discomfort, headaches, abdominal pain, myalgias, lymphadenopathies and marked eosinophilia. The radiological picture was consistent with intense pleuropulmonary transudation, with a striking interstitial pattern. Often there were Kerley septal lines with interlobular pleural swelling. Also, a certain number of patients presented bilateral alveolar involvement and had pleural effusions. All these manifestations were short-lived, especially the respiratory symptoms; the radiographic signs usually disappeared after 2–6 days (Tabuenca 1981). By this time some patients had died of respiratory failure. In the second week of June, patients began to present with thrombosis of large vessels and died because of thrombosis of the mesenteric arteries and veins or pulmonary thromboembolism (Report of the Pathology Commision 1981 a).

The clinical features that patients presented in the arly stages, "1st. clinical phase" (1st CPh), differed from the clinical picture observed later in a number of patients that had to be readmitted to the hospitals, denominated "2nd. clinical phase" (2nd CPh). In the 2nd CPh of the disease the most prominent clinical features were: 1. Neuromuscular changes ranging from simple myalgia to muscular weakness and atrophy; 2. Severe weight loss and scleroderma-like skin manifestations; and 3. Pulmonary hypertension (Gilsanz 1982). About 1,000 patients have been admitted to the C.S.S.S. "1° de Octubre", Madrid, because of the TS (Toxic Epidemic Syndrome Study Group. Hospital 1° de Octubre, Madrid, 1982). In the Department of Pathology we had the oportunity to study a significant number of biopsies and autopsies of these patients. The aim of this publication is to report the pathology of this new and complicated disease.

Material and Methods

Material

Autopsies. Up to December 1981, 20 autopsies were studied; 11 corresponded to the 1st CPh and 9 to the 2nd CPh (Tables 1a, b). Tissue blocks (for histological study) were routinely taken from every organ, including the peripheral nervous system (sciatic, tibial, radial, and sural nerves) and skeletal muscles (gastrocnemius and quadriceps).

Biopsies. Biopsies were taken from the following organs: lungs, peripheral nerves, skeletal muscle, skin, liver and kidneys. The placenta was also studied (Table 2). Lung biopsies in

Table 1a. 1st CPh. Autopsic pathology in toxic oil syndrome

Case	A. Nr.	Date	Age	Sex	BW.Kg.	Specific pathologic	Fundamental disease	Cause of Death
1	42/81	23-V	56	M	80	VL; I. Pneumonitis NBTE (r. atrium + l. ventricle)	I. Pneumonitis	Respiratory failure
2	52/81	8-VI	66	M	59	VL; Pancreatitis	Bronchopneumonia	Respiratory failure
3	56/81	11-VI	16	F	56	VL; I. Pneumonitis	I. Pneumonitis	Respiratory failure
4	57/81	11-VI	47	F	70	VL; I. Pneumonitis Thrombosis (superior mesenteric vein)	Intestinal infarction	Hypovolemic shock
5	59/81	17-VI	47	M	55	VL; I. Pneumonitis Thrombosis (pulmonary + mesenteric artery)	Pulmonary infarcts intestinal infarcts	Pulmonary infarcts
6	66/81	19-VI	20	F	76	VL; I. Pneumonitis Thrombosis (pulmonary arteries + mesenteric veins)	Pulmonary infarcts intestinal infarcts	Hypovolemic shock
7	68/81	29-VI	31	F	80	VL; I. Pneumonitis Thrombosis (pulmonary arteries + mesenteric veins)	Pulmonary infarcts intestinal infarcts	DIC.; hypovolemic shock
8	70/81	30-VI	52	M	74	VL; NBTE (r. chambers) Miocarditis; Focal necrosis of the pancreas	Thrombophlebitis (r. femoral vein)	Pulmonary embolism
9	75/81	5-VII	67	F	50	VL; I. Pneumonitis Thrombosis (superior mesenteric artery) Miocarditis	Intestinal infarction	Hypovolaemic shock
10	85/81	13-VII	28	F	68	VL; I. Pneumonitis Thrombosis (pulmonary artery)	Pulmonary infarcts	Respiratory failure
11	86/81	14-VII	48	F	94	VL; Thrombosis (superior mesenteric vein + pulmonary artery)	Pulmonary infarcts	Respiratory failure

Table 1b. 2nd CPh. Autopsic pathology in toxic oil syndrome

Case	A. Nr.	Date	Age	Sex	BW.Kg.	Specific pathologic lesions	Fundamental disease	Cause of death
12	129/81	14-X	12	F	32	VL; Skin scl.L.; NML Ciocarditis; Parotid: Sclerosis + Atrophy	Bacterial endocarditis (r. atrium + mitral)	Sepsis
13	133/81	24-X	32	M		VL; Skin scl.L.; NML NBTE (mural l. ventricle) Fibrosis of pancreas	Bronchopneumonia	Pulmonary embolism
14	139/81	1-XI	37	F	48	VL; Skin scl.L.; NML Fibrosis + Atrophy of pancreas and thyroid	Pulmonary embolism	Cardiorespiratory failure
15	147/81	16-XI	17	F	41	VL; Skin scl.L.; NML NBTE (mural ventricle)	Bronchopneumonia	Sepsis
16	162/81	4-XII	45	F	57	VL; Skin scl.L.; NML	Sepsis	Sepsis
17	164/81	15-XII	17	F	35	VL; Skin scl.L.; NML Fibrosis of thyroid	Perforation of trachea bronchopneumonia	Sepsis cachexia
18	165/81	15-XII	24	F	35	VL; Skin scl.; NML Pancreatitis; Fibrosis of thyroid	Decubital necrosis of trachea; perforation of r. carotid in the trachea	Internal haemorrhage
19	171/81	21-XII	29	M	49	VL; Skin scl.L.; NML NBTE (tricusp. + aortic)	Pulmonary embolia pulmonary infarcts bronchopneumonia	Sepsis cachexia
20	177/81	30-XII	10	F	37	VL; Skin scl.L.; NML NBTE (tricusp.)	Sepsis by candida	Sepsis

A = Autopsy; BW = Body weight; I = Interstitial; NBTE = Non bacterial thrombotic endocarditis; NML = Neuromuscular lesion; Skin scl.L. = Skin sclerodermiform lesions; VL = Vascular lesion. DIC = Disseminated intravascular coagulopathy

Table 2. Biopsies performed in toxic oil syndrome

Organs	1st CPh	2nd CPh	Total
Lung	11	3	14
Peripheral nerves		60	60
Muscle		15	15
Skin	15	73	88
Liver	24		24
Kidneys	4		4
Placenta		6	6

the 1st CPh were made on patients with the so called "atypical pneumonia" and in the 2nd CPh in patients with pulmonary hypertension. The peripheral nerves biopsies were performed in the retromaleolar area obtaining a minimum of 0.8 cm of the sural nerve. The muscle biopsies were made from deltoid, biceps and quadriceps, 11 in the stage of myalgia and 4 in the stage of muscle atrophy.

Fifteen skin biopsies were made in the 1st CPh, when the patients presented generalized exanthemata, and 23 were made in the 2nd CPh, in patients with neuromuscular symptoms. These patients presented more variegated cutaneous symptoms: Disseminated papules in the trunk and extremities lesions similar to pseudoxanthoma elasticum and lesions of scleroderma which began in the face and neck and later on appeared on the extremities. Finally, random biopsies were taken from the retromaleolar region of 50 patients in the 2nd CPh, some of which presented sclerodermiform symptoms. These biopsies were done in order to assess the frequency and dissemination of the primary lesions.

Among 413 patients with the TS, 99 (23.9%) presented with hepatic symptoms or abnormal test of liver function (Solís-Herruzo et al. 1982). Twenty-five of them (13 men and 12 women) were studied prospectively in the months of May and June (1st CPh). The age of the patients ranged from 21–73 years (average age 42 years). Only 6 patients (24%) presented subjective symptoms of hepatobiliary disease (5 icterus and 1 ascitis) and 64% had hepatomegaly. All patients had abnormal liver function. In 24 patients laparoscopy and needle biopsy were done. In one case the biopsy was contraindicated due to thrombosis of the suprahepatic vein (Budd-Chiari Syndrome).

The incidence of clinical renal symptoms has been very low in the TS; only 4 patients, 0.47% of the patients investigated in our hospital, presented with renal symptoms apparently related to the TS (Table 4). All of them developed renal insufficiency. The patients had no previous infections and there was an interval of 29–66 days between the onset of the TS and the appearance of the renal symptoms.

Methods

The following histological stains were performed: HE, Masson's trichrome, Van Gieson, Wilder's silver for reticulin, orcein for elastic fibers, PTAH, PAS and PAS after diastase digestion, Congo red, Sudan, and O.R.O. For the CNS, routine Nissl and Spielmeyer stains, and in some instances silver atains after Rio Hortega Bielschowsky, Polak (for microglia) and Cajal's gold-sublimate were made. The nerves were processed following standard techniques for transversal and longitudinal sections. Also, one fragment of the nerve was fixed in 0.6% glutaraldehyde, embedded in resin and dissected for the study of separated fibers. Skeletal muscle was studied with the following histochemical stains: NADH tetrazolium reductase, succinic deshidrogenase, ATP-ase at pH 9.4 and with preincubation at pH 4.6 and 4.3; phosphofructokinase, phosphorilase and alpha-glycerophosphate dehydrogenase.

Electronmicroscopy. The following organs were studied: Lung (14 transbronchial biopsies), peripheral nerve (60 biopsies), muscle (11 biopsies), skin (50 biopsies), liver (19 biopsies) and kidney (4 biopsies).

For electron microscopy the tissue was minced into 1 mm cubes, fixed in paraformaldehyde (1%) – glutaraldehyde (2.5%) in 0.1 M sodium cacodylate buffer for three hours, and washed twice in 0.1 M sodium cacodylate buffer, followed by postfixation in 1% osmium tetroxide in cacodylate buffer for one hour. The tissue was dehydrated in increasing percentages of ethanol, passed through propilene oxyde and embedded in Epon 812. One micron thick sections were stained with toluidine blue. Thin sections were obtained with a LKB Ultratome III, stained with uranyl acetate and lead citrate and photographed with a Hitachi HU 12 A electron microscope.

Inmunofluorescence. The specimens were deep frozen in liquid nitrogen and cut with a cryotome. The sections were stained with the following antisera conjugated with isothiocyanate of fluorescein: IgG, IgA, IgM, IgE, C_3, C_4, C_{1q}, and fibrinogen. Tissue from the following organs were studied: Lung (14 biopsies), skin (50 biopsies), peripheral nerve (60 biopsies), muscle (11 biopsies) and kidneys (4 biopsies).

Results

I. General Pathology

The most characteristic anatomic-pathological feature of the TS is the vascular lesion. Vessels of almost every tissue and size were affected. In addition, more or less severe interstitial inflammatory infiltrates and/or fibrosis have been observed in most organs. Atrophic changes in the parenchyma of a number of organs were also prominent.

In the 1st CPh, the most affected organ was the lung: The patients died in the early stages due to respiratory failure. Later in the 1st CPh thrombosis of the mesenteric vessels and subsequent intestinal haemorrhagic infarction or pulmonary thromboembolism were the main causes of death. In the 2nd CPh the prevalent lesions were found in the peripheral nerves, muscles and skin. The patients died predominantly of infectious complications and sepsis adquired in the Intensive Care Unit where they had to be admitted because of intense muscular weakness and respiratory insufficiency.

The age, sex, clinical phase, autopsy date, main pathological findings and causes of death are given in Tables 1 a, b.

Vascular Lesions

Four types of lesions have been observed: A) Endothelial lesion; B) Vasculitis; C) Fibrosing sequelae; and C) Thrombo-embolic complications.

A) Endothelial Lesion. This consists of degenerative and proliferative changes (Fig. 1). The degenerative changes were: a) cellular swelling; b) cytoplasmic vacuolization; and, c) cellular necrosis. These changes were observed alone or in combination with proliferative changes of myointimal cells. In order to evaluate the intensity of the lesion we have characterized 3 grades: 1. a non proliferative endothelial lesion; 2. an endothelial lesion with partial luminal obliteration (Fig. 1 a); and, 3. an endothelial proliferation with complete obliteration of the vascular lumen. The endothelial lesion

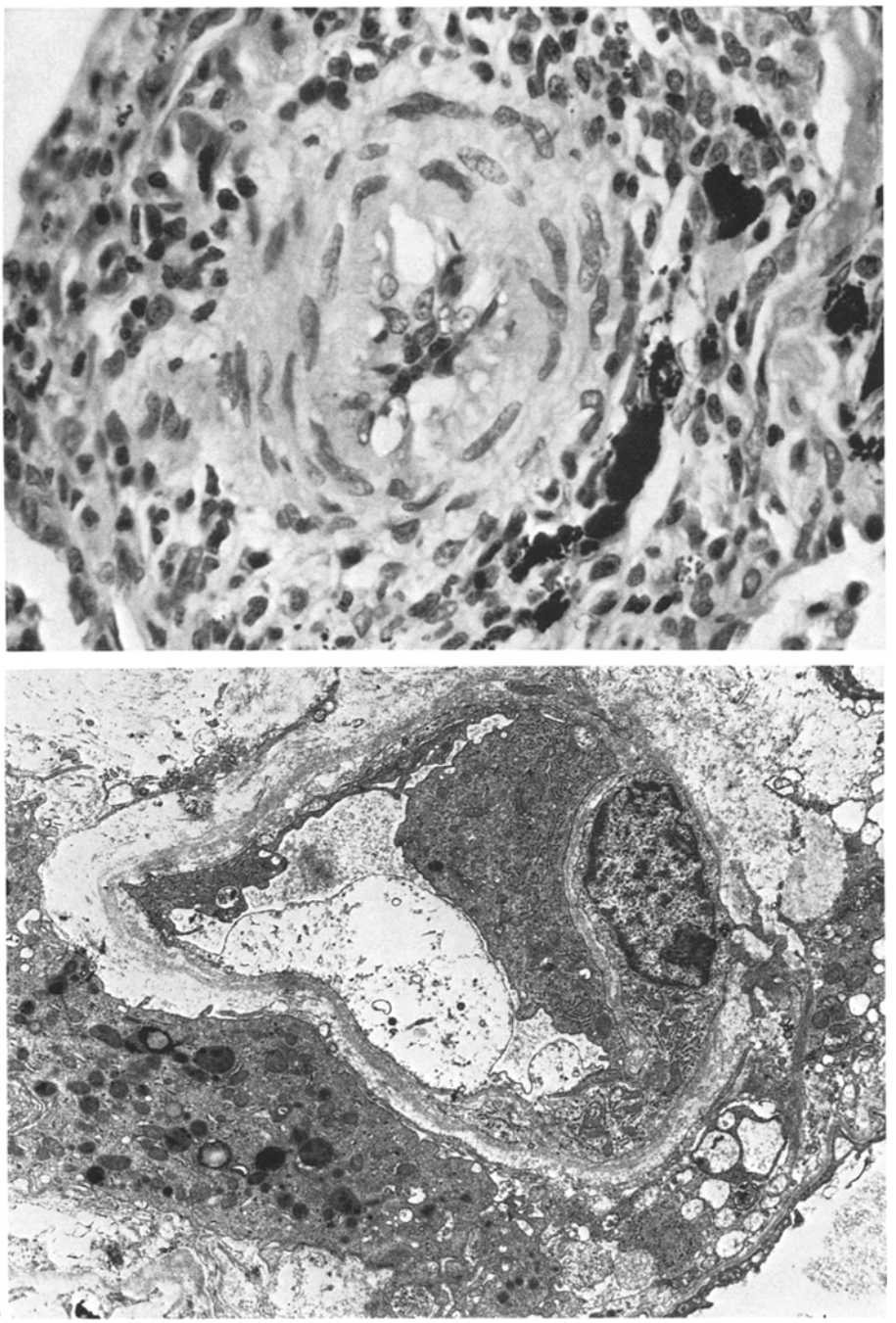

Fig. 1. a Lung: Endothelial lesion characterized by cytoplasmic swelling and vacuolization, accompanied of proliferation with partial obliteration of the lumen (HE × 250). **b** Lung: Electron micrograph showing the hydropic degeneration of an endothelial cell (EM × 4,800)

has been observed in both clinical phases although it has been of a lower grade in the later autopsies. In the early phases of the disease, the pulmonary arteries were most affected but later on we observed that the endothelial lesion was also systemic. Ultrastructural study of the injured endothelial cells showed marked cytoplasmic tumefaction due to hydropic degeneration with destruction of the membranous organelles (Fig. 1b).

B) Vasculitis. Three main types of infiltration of the vascular wall by inflamatory cells have been observed: a) Perivascular; b) Intimal; and, c) Medial. Perivascular infiltration of lymphocytes, histiocytes and eosinophils has been found in vessels of every size and in every organ in a systemic fashion and in both clinical phases of the disease. However, in chronic cases the perivascular infiltrate can be slight or absent. This type of infiltrate should not be considered as a vasculitis.

The intimal infiltration by lymphocytes, histiocytes, eosinophils and occasional neutrophils appeared as an endarteritis or endophlebitis (Fig. 2a). We have observed it since the early stages of the disease. The inflammatory cells were located between the endothelium and the media. In some instances foamy macrophages and later on subintimal fibroblastic proliferation were observed (Fig. 2b).

The infiltration of the media by lymphocytes, histiocytes, eosinophils and, in some cases, by a few neutrophils has been observed less frequently than the perivascular and intimal inflammatory infiltration. Also, it appeared in a systemic fashion. We have never observed fibrinoid necrosis nor the presence of parietal granulomas and have never found leucocytoclasia. Investigations for antibodies with the immunofluorescence technique were consistently negative. We consider the intimal and the medial inflammatory infiltration as a vasculitis "sensu stricto".

C) Fibrosing Sequelae. Intimal fibrosis appeared in the advanced stages of the disease. It can lead to a partial or total obliteration of the vascular lumen (Fig. 3).

In many instances a transitional picture has been observed between the inflammatory and the proliferative changes on the one side and the fibrosing sequelae on the other. Thus, we have interpreted the intimal fibrosis as a consequence of the endothelial lesions, endoarteritis and endophlebitis. We want to emphasize that the lesions described above had a segmental distribution. Fibroblasts and myofibroblasts proliferated in a concentric disposition, immersed in a basophilic myxoid matrix (Fig. 4a) demonstrated by electron microscopy (Fig. 4b).

D) Thromboembolic Complications. Vascular thrombosis was observed in both clinical phases of the TS. In the early stages aggregates of fibrin and platelets were found in the pulmonary capillaries. Later, thrombosis occurred in veins (mesenteric and portal tree) and arteries (femoral, mesenteric, carotid and pulmonary).

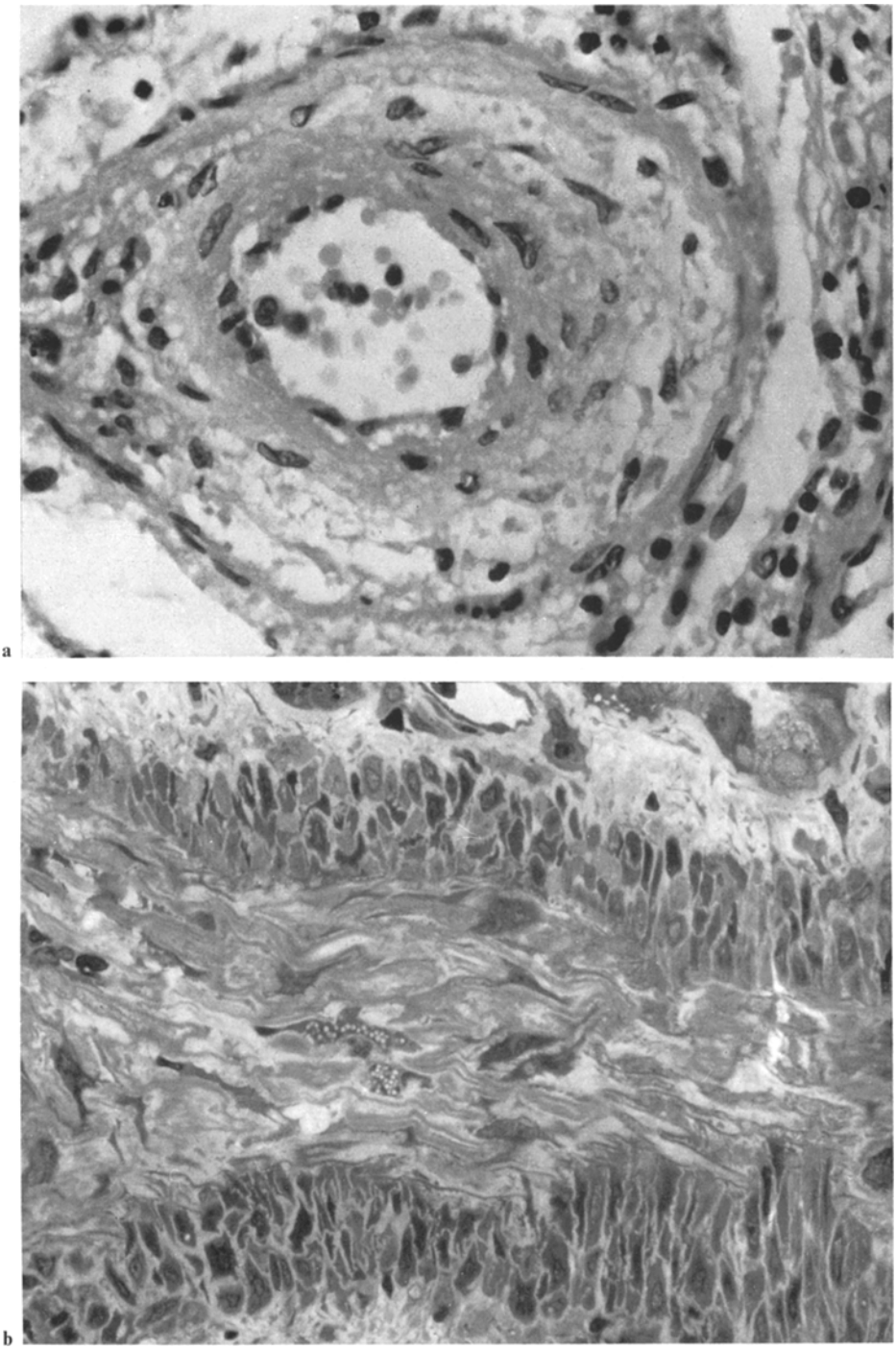

Fig. 2. a Lung: Inflammatory infiltrate (lymphocytes and histiocytes) and edema of the subendothelial space producing a picture of endovasculitis (HE × 250). **b** Kidney: Obliterated small artery showing spindle cell proliferation and occasional foamy macrophages surrounded by well preserved inner elastic membrane and media (toluidine blue, 1 micron section × 250)

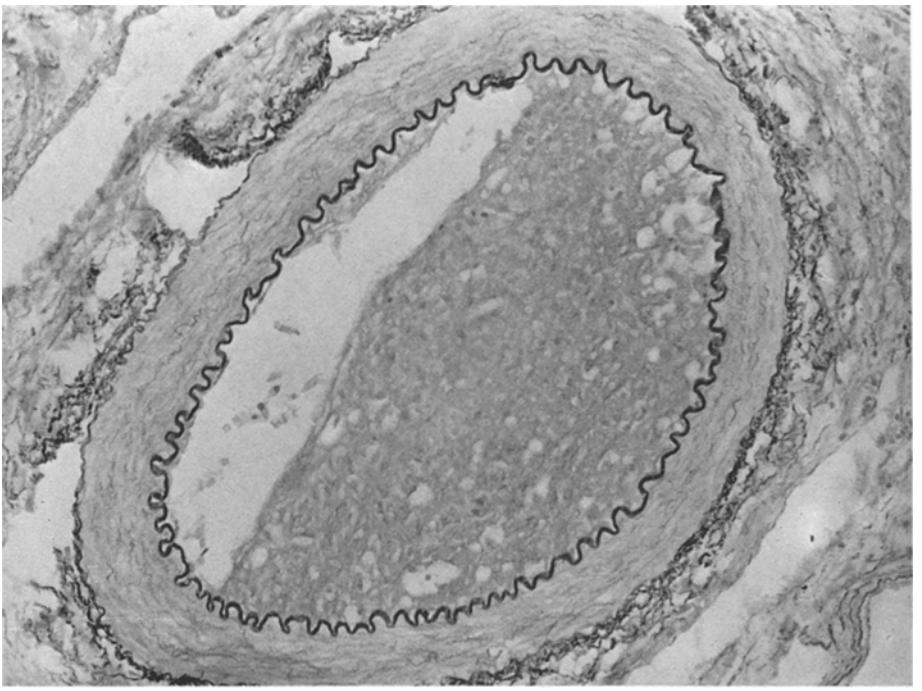

Fig. 3. Heart: Severe fibrous narrowing of the lumen of a coronary artery from a 12 year old girl (orcein stain for elastic fibers × 200)

II. Pathology of the Organs

Lungs

1st CPh. In this phase of the disease the main pathological changes were observed in the lungs, correlating with the clinical picture of so called "atypical pneumonia". The histological study showed: 1) Septal oedema; 2) Slight interstitial infiltrate of mononuclear cells (lymphocytes and monocytes) and scanty eosinophils; 3) Cuboidal metaplasia of type II pneumocytes and decreased number of type I pneumocytes; 4) Alveolar oedema with desquamated type II pneumocytes and macrophages; 5) Lymphangiectasia in the interlobular septae (Figs. 5a). The vascular lesions. The ultrastructural study of the biopsies exhibited: 1) Hydropic degeneration of pneumocytes types I and II and necrosis and desquamation of type I pneumocytes (Fig. 5b); 2) Destruction of cytoplasmic organelles; 3) Separation from the alveolar basement membrane; 4) Presence of hyaline membranes; and, 5) Infiltration by eosinophils with intravascular and interstitial degranulation.

2nd CPh. The histological study of the lung showed more prominent vascular lesions, consisting of vasculitis and/or intimal fibrosis with partial obliteration of the vascular lumina, as well as interstitial oedema and interstitial

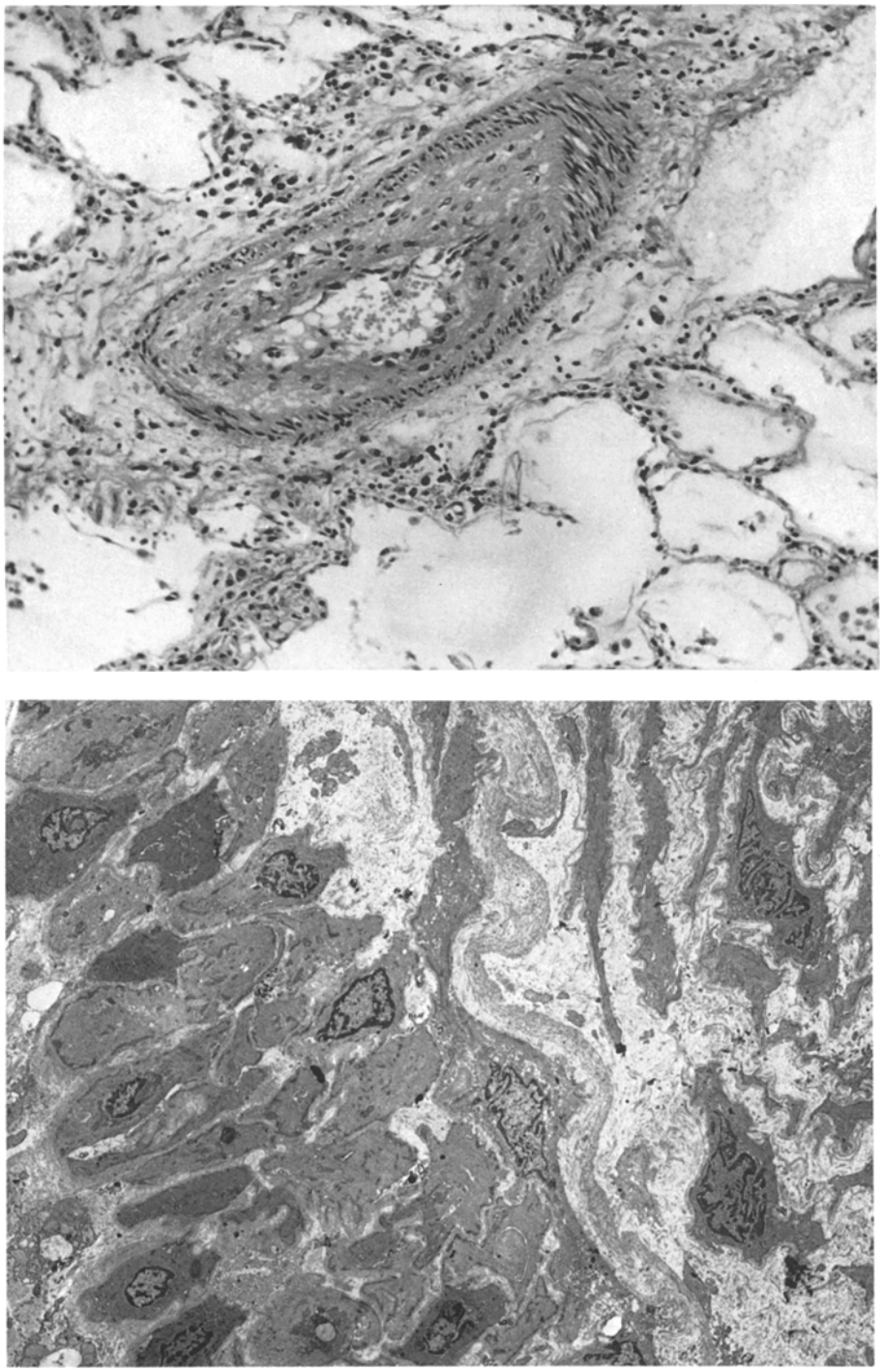

Fig. 4. **a** Lung: Medium size artery with concentric intimal thickenning due to fibroblastic myxoid proliferation and lymphocytic infiltration (HE × 100). **b** Kidney: Electron micrograph of a medium size artery displaying good preservation of the inner elastic membrane and media, and fibroblastic and myofibroblastic concentric proliferation of the intimal layer (× 1,200)

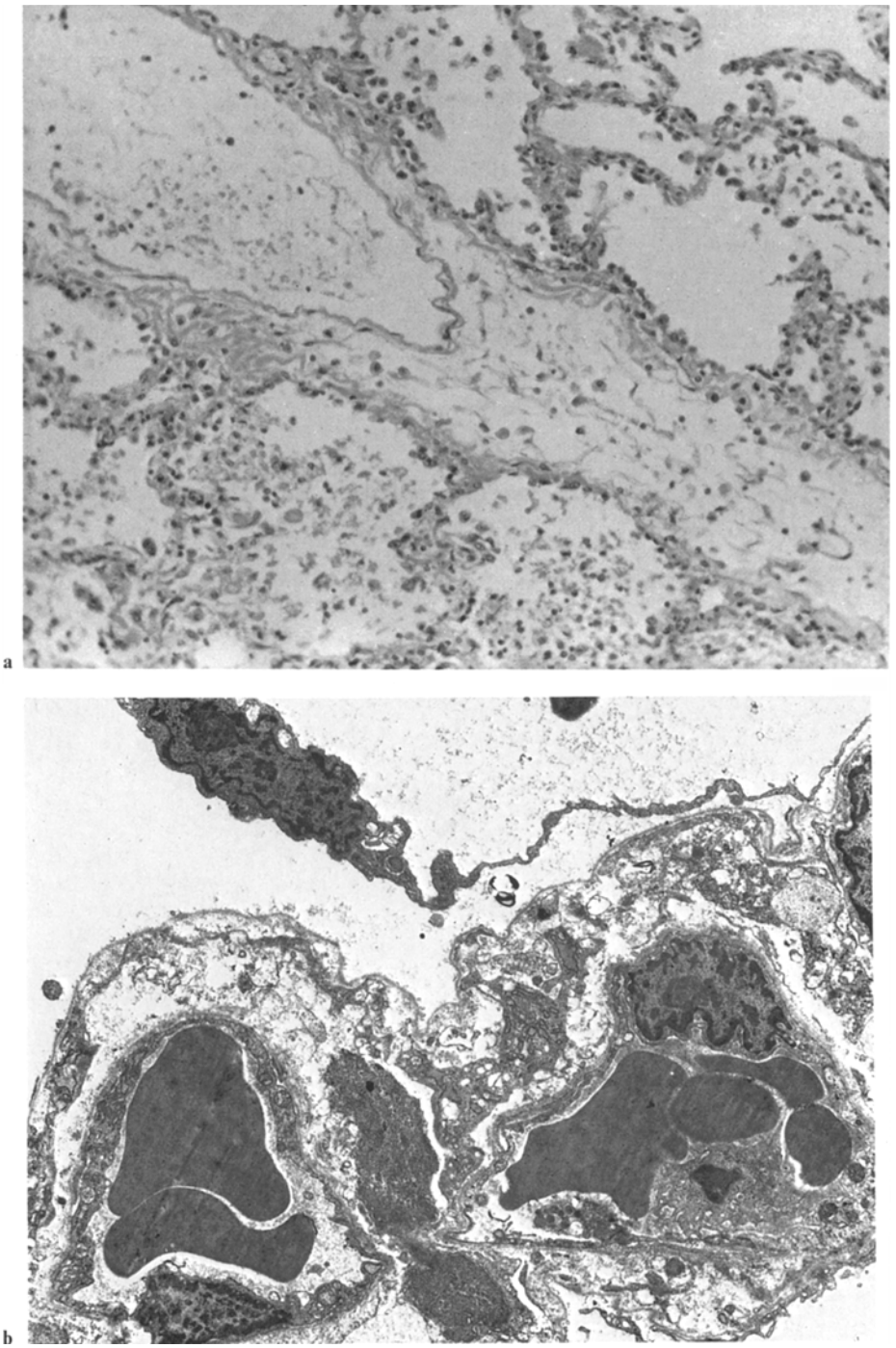

Fig. 5. Lung: Marked interstitial oedema, lymphangiectasia and moderate desquamation of pneumocytes (HE × 140). **b** Electron micrograph of the alveolar lining; note the necrosis and detachment of type I pneumocyte (EM × 2,900)

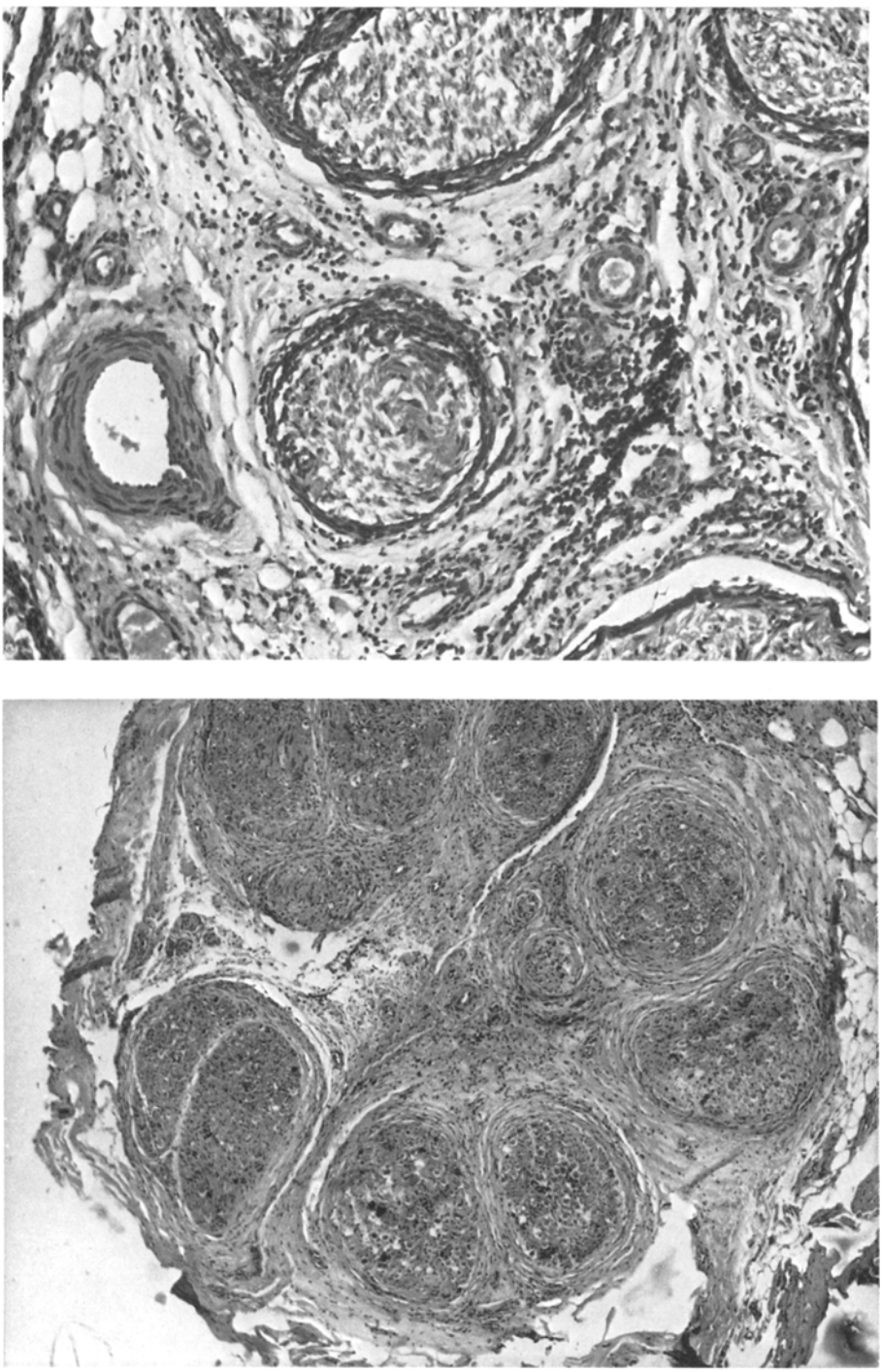

Fig. 6. Transverse section of sural nerve with inflammatory cell infiltration of the perineurium and around an epineural vein (HE × 250). **b** Marked fibrous thickenning of the perineurium around nerve fascicles (HE × 100)

Table 3. Pathology of the peripheral nerves

Neuromuscular	No.	Slight	Moderate	Severe
Age	7–36 (years)	17–71 (years)	29–53 (years)	12–6 (years)
Sex	6F–2M	14F–2M	2F–1M	30F–3M
Evolution	15–25	17–30W	24–29W	18–31W
Perivascular				
Epineuritis	8 (75%)	16 (68.7%)	3	33 (100%)
Epineuritis diffuse	2 (25%)	9 (56.2%)	2	26 (78.7%)
Perineuritis	7 (87%)	7 (43.7%)	2	23 (69.6%)
Perineural fibrosis	2 (25%)	1 (6.2%)	2	21 (63.6%)
Arterial stenosis	0	2 (12.5%)	0	7 (21.2%)

inflammatory infiltrates of variable proportions by lymphocytes, monocytes and eosinophils. However pulmonary fibrosis was not observed in any case, even in the autopsy cases of the 2nd CPh, which clinically presented with pulmonary hypertension.

Peripheral Nervous-System

All biopsies were performed in the advanced stages of the 2nd CPh. The age, sex and main clinicopathological features of the 60 cases studied by biopsy are given in Table 3. The most prevalent lesion consisted of perivascular, venulocapillary infiltrates of lymphocytes and histiocytes in the epineurium of variable intensity (Fig. 6a); occasionally those infiltrates were also present around endoneural vessels. In some cases the inflammatory infiltration had a diffuse character. Inflammatory infiltration of the vascular walls or infiltration around arteries or arterioles was not observed. The epineural artery showed subintimal fibrosis in some cases with partial obliteration of the lumen.

In many cases, focal or diffuse perineural lymphohistiocytic infiltrates were observed, affecting the whole perimeter of a thickened perineurium. In the more chronic cases, the perineural cells had disappeared and proliferating fibroblasts were seen. Finally, in the most advanced cases the perineurium showed fibrosis (Fig. 6b). This feature was observed in 43.3% of the biopsies and in all autopsy cases of the 2nd CPh. In the autopsy cases in which the proximal and the distal zones of the sural and sciatic nerves were separately studied, the perivascular infiltrates were slightly more intense in the distal zone than in the proximal one. The perineural fibrosis had a focal character and affected, with a variable intensity, each one of the fascicles. In these fascicles a patchy or total axonal loss was observed. Total axonal loss occurred in relation to a concentric fibrosed and thickened perineurium; partial loss was detected in cases of perineuritis with partial perineural fibrosis.

In teased fibers axonal degeneration and, occasionally, focal myelin irregularities were observed. The lesions were variable from fascicle to fascicle,

so much so that some undamaged fascicles lay side by side with others which were severely damaged. Furthermore, the incidence of these lesions did not maintain any apparent relationship to the clinical neuromuscular picture; these lesions were observed in some patients that did not present neuromuscular symptoms (see Table 3).

Skeletal Muscle

All biopsies were performed in the 2nd CPh. In the earlier clinical stages of myalgia, the study of the biopsies revealed a picture of inflammatory myopathy. The prevalent lesion in this phase was an intense inflammatory infiltration by lymphocytes, histiocytes, eosinophils and neutrophils. These infiltrates were predominantly superficial, affecting the fascia, epimysium and perimysial septae. A less intense endomysial infiltration was also observed. The inflammatory infiltration was diffuse with a tendency to concentrate around vessels, muscle spindles (Fig. 7a) and intramuscular nerve fibers. Furthermore, initial signs of atrophy of isolated type II fibers were observed. These type II fibers frequently presented an inversion of the reaction for oxidative enzymes. No increase of the fibrous tissue nor endomysial fibrosis was observed in this stage. Some muscle fibers presented superficial areas of necrosis. Ultrastructurally, these areas presented disorganization of the myofibrils which were substituted by irregulary shaped, dense subsarcolemmal aggregates.

In advanced clinical stages of weakness and atrophy of the muscles, the lesion was characterized by severe and diffuse denervation atrophy, especially in the last autopsies which presented also severe endomysial fibrosis. Groups of atrophic fibers, with inversion of the reaction for oxidative enzymes were observed in many muscle fascicles. Different degrees of atrophy were present in the same muscle. The most severe cases had atrophy of whole fascicles with intense endomysial fibrosis (Fig. 7b). At this stage the inflammatory cell infiltration was slight, predominantly localized in the perimysium around veins and capillaries, and composed mainly by mononuclear cells. The inflammatory infiltrates diminished in intensity as the denervation atrophy progressed. In two autopsy cases subintimal arterial fibrosis with thickening of the arterial wall was observed.

Central Nervous System

The lesions were non specific and some pathological features were common to both CPh such as. Small perivascular mononuclear infiltrates in the leptomeninges and in the nervous parenchyma, fresh ischaemic lesions of variable extension, and multiple microinfarcts. In 9 cases of the 2nd CPh central chromatolysis, of variable intensity, frequently associated with cytoplasmic vacuolization, was present in the motor neurons of the anterior horns of the spinal cord. Similar lesions, though less marked, were observed in the motor nuclei of the VI and VII craneal nerves, nuclei of the griseum pontis and nuclei of the reticular substance of the medulla. In the two last locations they were surrounded by hypertrophic astrogliosis and microglial mobiliza-

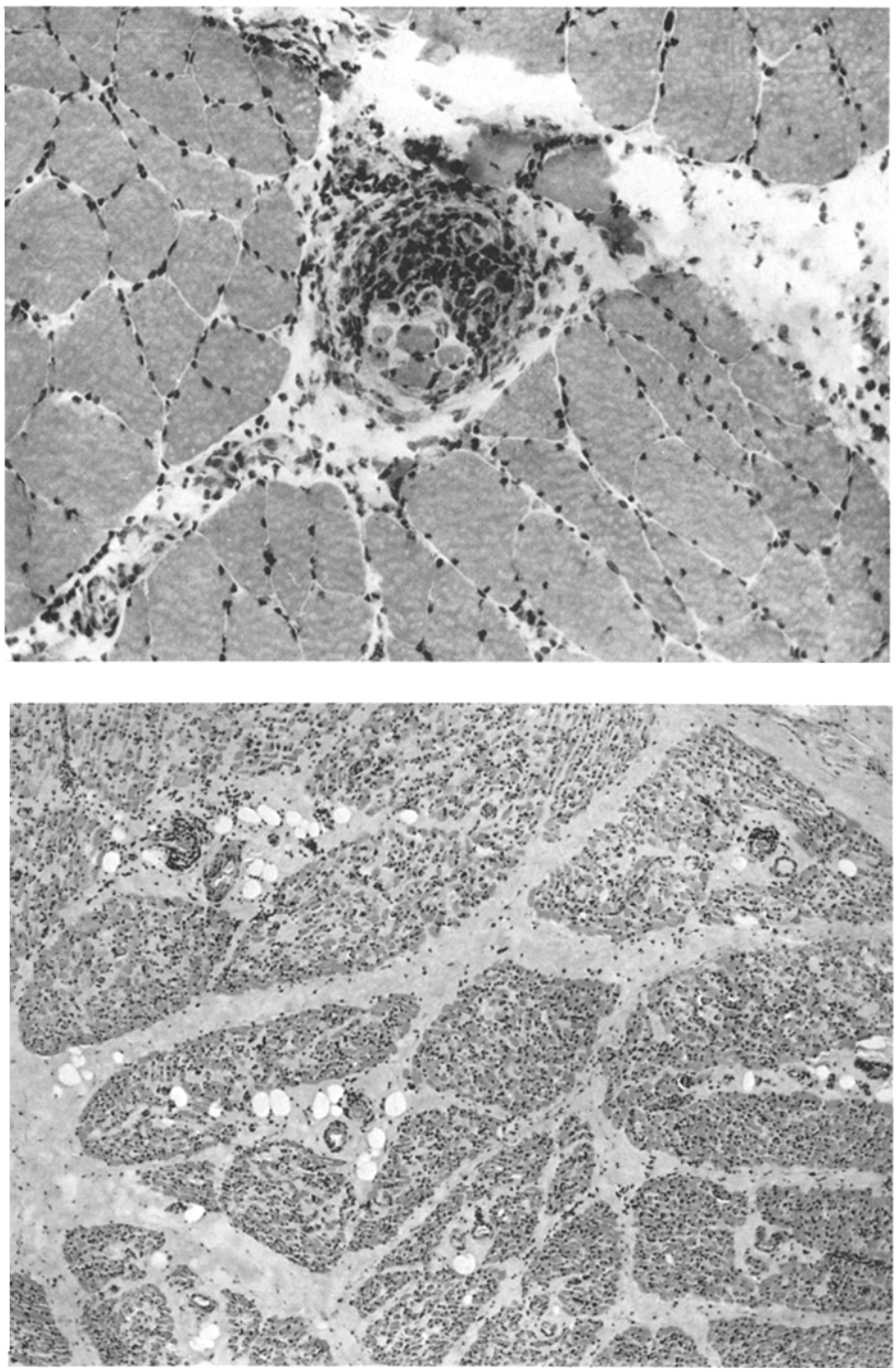

Fig. 7. a Cross section of muscle showing inflammatory cell infiltration of the perimysium and of a muscle spindle (HE × 250). **b** Low power view of a transverse muscle section displaying marked atrophy of muscle with endomysial and perimysial fibrosis (HE × 100)

tion with occasional formation of small nodules around the neurons. The cerebral cortex showed focal anoxic lesions.

Skin

The pathology observed in each CPh was characterized by a complex of several histological changes which allows to differentiate one from the other. However, other microscopical features were found, some of them being common to both CPh.

1st CPh. Slight to moderate lympho-histiocytic infiltrates were present around the superficial or superficial and deep dermal vascular plexuses. The vessels showed dilated lumina and swollen endothelial cells, with occasional mitotic figures; the vascular lumina were sometimes occupied by agglutinated erythrocytes. Frequently, foci of extravasated erythrocytes were observed. Fibrinoid necrosis or leucocytoclasia were not observed. The epidermis was normal or showed only minimal and scattered foci of spongiosis and exocytosis. The dermal papillae were oedematous.

2nd CPh. The histological picture is more complex. Basically it is made up of 3 components: 1) Interstitial inflammatory infiltrates; 2) Mucinosis; and, 3) Sclerosis. Inflammatory infiltrates predominated in the lesions observed in the early stages (Fig. 8a) and the sclerosis in the lesions studied in advanced stages. In certain cases there was a prominent diffuse inflammatory infiltrate made up of lymphocytes and histiocytes, that dissected the collagen fibers which showed blurred outlines. In some cases mucinosis was observed. In the biopsies in which sclerosis was prevalent, thick bundles of collagen occupied the reticular dermis and compressed the atrophic skin adnexa (Fig. 8b).

Other Lesions. Other morphological findings were: a) Vasculitis of small arteries in 61% of the biopsies of the 2nd CPh; b) Eosinophils present in variable amounts in 73% of the cases of the 1st CPh and 33% of the 2nd CPh; c) Fusiform cells with wide cytoplasm and large vesicular nuclei were observed; they occasionally showed round eosinophilic cytoplasmic inclusions, 8–10 microns in diameter; ultrastructurally the inclusions were electron-dense and presented a fibrillar aspect; d) The elastic fibers were irregularly arranged in other cases; with the orcein stain dense globular and refringent particles were seen; e) Perineural inflammatory infiltrates and perineural fibrosis were present in a few cases of both CPh.

Random Skin Biopsies. The histological study of the 50 biopsies of the external retromaleolar region revealed consistently, including those biopsies of patients without clinical esclerodermiform symptoms, the presence of intense fibrosis as described above. The fibrosis extended to the hypodermis. In 42% of cases the fibrosis was light and in 58% moderate. Furthermore in these biopsies endothelial lesions (85%), vasculitis (42%) and perivascular infiltrates (62%) have been found. The immunofluorescence did not discover the presence of Ag-Ab complexes.

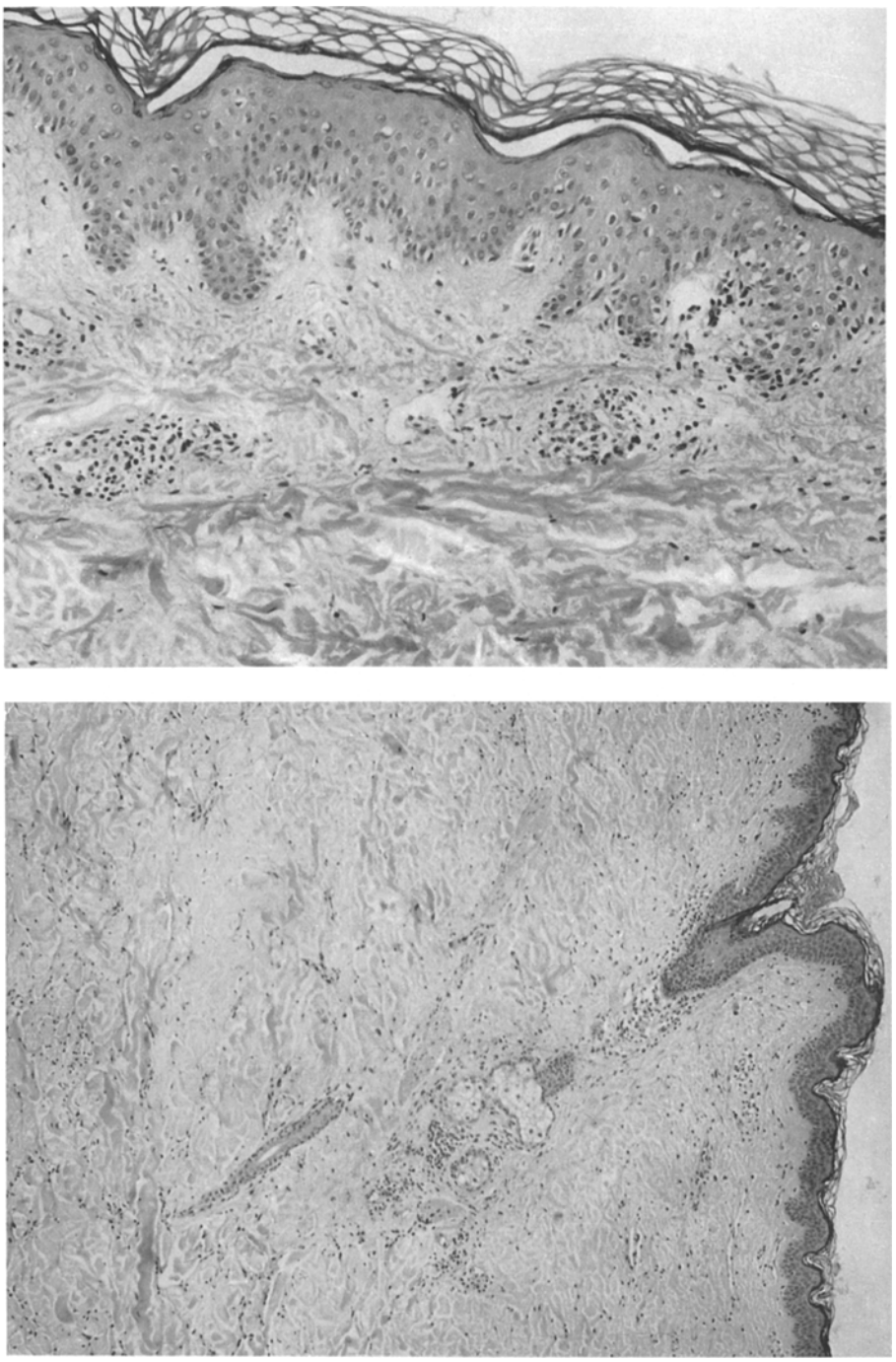

Fig. 8. **a** Perivascular lymphocytic infiltration of the upper dermis (HE × 100). **b** Panoramic view showing marked dermo-hypodermal fibrosis with trapping of the skin adnexa (HE × 40)

Liver

The liver presented: a) portal; and, b) lobular lesions. a) The portal areas showed inflammatory infiltrates of variable intensity made up by lymphocytes, histiocytes, polymorphonuclear leukocytes and eosinophils. Lesions of the biliary ducts were also present. Lesions of the epithelial cells of the ductal epithelium were observed in 11 cases, consisting in nuclear stratification, cytoplasmic vacuolization and inflammatory exocitosis. In 9 cases vasculitis of the portal vein and in 4 cases endothelial lesion of the portal arteries were detected. The portal reticulin framework was slightly reinforced in 14 cases in a similar way to that which occurs in mild chronic active hepatitis. b) The lobular lesion consisted mainly of eosinophilic degeneration of scattered hepatocytes (17 cases) and diffuse mixed inflammatory infiltrates of variable intensity (14 cases). In 22 patients scattered eosinophils were observed. 13 cases had cholestasis, 7 slight and 6 more intense. In 14 cases mitotic figures were conspicuous.

These histological changes were combined in variable proportions resulting in an heterogeneous histological picture. However on the whole the changes can be considered to be an histological spectrum whose extremes are: 1) Microscopic cholestasis with little inflammatory and degenerative changes, on the one side; and 2) Predominance of degenerative and inflammatory changes with minimal presence of biliary pigment on the other.

The ultrastructural study was performed on periportal and centrolobular hepatocytes. In addition to the changes described with light microscopy (eosinophilic bodies, cholestasis, etc.), the following changes were observed: a) Lamellar bodies in the hepatocytes and Kupffer cells, with no preferential cytoplasmic localization (17 cases); b) Giant mitochondria (9 cases) with paracrystalline inclusions (8 cases); c) Hyperplasia of the SER; d) Lesions of the billiary pole of the hepatocytes (15 cases): alteration of the microvilli and condensation of the pericanalicular ectoplasm.

Kidneys

In the 20 autopsies studied only interstitial inflammatory infiltrates and vascular lesions were found.

The morphological findings in the four biopsies were as follows: Light microscopy. The glomerular lesion consisted of diffuse proliferative endocapillary glomerulonephritis (Cases 1 and 3), diffuse membranoproliferative glomerulonephritis (Case 2) and diffuse extracapillary glomerulonephritis (80% of crescentic forms) (Case 4). Immunofluorescence microscopy: Cases 1, 2 and 3 showed immunoglobulins (IgG and IgM) an different components of the complement (C_3 and C_{1q}) associated with the capillary walls. Case number 4 displayed fibrin in the crescents with uninvolved glomerular tufts. The electron microscopic study revealed subepithelial, endothelial, and mesangial deposits in cases 1, 2 and 3. No deposits were present in case 4 (see Table 4).

Table 4. Renal pathology

P	Age	Sex	Haema-turia	Proteinuria	Hyper-tension	Renal insuf.	Oede-ma	Oli-guria	Need of dy-alisis	Clinical symptoms	Pathology
1	41	M	+m	+2.54 g/D	+	+	+	−	−	A.A.N.S.	P.D.End.GN
2	5	M	+m	+1.24 g/D	+	+	+	−	−	A.A.N.S.	P.D.M.-P.GN
3	53	M	+n	+14.5 g/D	−	+	++	−	−	N.S.+R.I.	P.D.End.GN
4	72	F	+Ma	+1.72 g/D	+	+	+	+	+	A.N.S.+R.I.	P.D.Ext.GN

Patient 2 was the son of patient 1; m=microscopic; Ma=macroscopic; A.A.N.S.=Atypical acute nephrotic syndrome; N.S.=Nephrotic syndrome; R.I.=Renal insufficiency; A.N.S.=Acute nephritic syndrome; P=Patients; P.D.End.GN=Proliferative diffuse endocapilar glomerulonephritis; P.D.M.-P.GN=Proliferative diffuse membrano glomerulonephritis; P.D.Ext.NG=Proliferative diffuse extracapilar glomerulonephritis

Other Organs

Non bacterial thrombotic endocarditis (NBTE) was observed in 6 cases (Table 1). The lesion of the endocardium presented similar features to the endothelial lesion that has been described in the vessels. It is interesting to point out that NBTE predominantly affected the right chambers of the heart (5 cases). Furthermore, in three cases myocarditis was present; the inflammatory infiltrate showed the cytological features of the TS.

Interstitial inflammatory infiltrates followed by parenchymal atrophy and fibrosis were observed in the parotid gland, pancreas and thyroid. In two cases, the inflammatory infiltrate of the pancreas was the cause of clinical symptoms of acute pancreatitis.

The placenta showed no significant pathological changes.

Discussion

The TS caused by ingestion of adulterated rapeseed oil in Spain is a new disease of multisystemic character whose aetiology and pathogenesis remains unknown. As has been already emphasized, the most prominent pathological feature is the vascular lesion. It has an ubiquitous character and segmental distribution, as in systemic vasculitis; however in general we did not find lesions which fulfilled the criteria of systemic vasculitis (i.e. inflammatory infiltration of the intima and/or media) and we never observed fibrinoid necrosis. Similar endothelial lesions to those observed in the TS have been described in the radiation vasculitis (Reinhold and Buisman 1973; Fajardo and Berthrong 1978) or after ingestion of diets rich in polyunsaturates fatty-acids (Nofstand 1974). The deposition of myxoid basophilic material in the intima and the proliferation of myointimal cells have been described

in several diseases (Corson 1972; Pangalis et al. 1978; Furst et al. 1979; Spargo et al. 1980). The intimal foamy macrophages and the fibrous obliteration of the intima have been described in radiation vasculitis (Reinhold and Buisman 1973; Fajardo and Berthrong 1973) and in the blood vessels of rejected organs (Porter 1974; Fauci et al. 1978; Furst et al. 1979). Several organs showed similar vascular lesions to those described in Progressive Systemic Sclerosis (PSS) (Cannon et al. 1974; Salerni et al. 1977; Rodnam et al. 1980): The perivascular inflammatory infiltrates, found consistants are not specific. However, in a large number of cases eosinophils predominated in these infiltrates, which corresponds to the observed clinical eosinophilia of the patients.

The pulmonary microscopical picture of the TS in the early phases was characterized by vascular endothelial lesions, septal oedema, slight mononuclear infiltrates, cuboidal metaplasia of type II pneumocytes and descreased number of type I pneumocytes. We believe that the vascular lesion might be causing increased capillary permeability and would be responsible for the respiratory illness and X-ray pictures. The pulmonary lesions are comparable with lesions observed in the early stages of interstitial pneumonitis, idiopathic interstitial fibrosis, rheumatoid lung, PSS, and eosinophilic pneumonitis induced by drugs, especially by nitrofurantoin (Spencer 1977; Boyd et al. 1978). The pulmonary symptoms of the 1st CPh usually disappeared in a short time and had a rapid and favorable response to high doses of corticosteroids (Gilsanz 1982). In more advanced stages of the TS, pulmonary hypertension developped in a certain number of patients. The angiomatoid or plexiform changes, that are characteristic of the advanced stages of primary pulmonary hypertension, were not observed in the pulmonary arteries of TS lungs. Pulmonary fibrosis has not been found to date, in neither our cases nor in the reported data from other institutions (Reports of the Pathology Commision, 1981 and 1982). However, the alveolar diffusion of oxygen is diminished in numerous chronic patients; this alteration could be caused by the lesions in the pulmonary vessels.

The neuromuscular lesion is one of the most dramatic and characteristic features of the TS. The histological picture of the initial neuromuscular symptoms (myalgias) has some similarity with the inflammatory myopathies of collagen diseases (Adams 1975). In the more advanced lesions the muscle changes correspond to a denervation atrophy with intense endomysial fibrosis. The study of the peripheral nerves showed essentially a perineuritic lesion. The clinico-pathological picture does not correspond to any known peripheral nerve disease although perineuritic lesions have, exceptionally been described in PSS (Gordon and Silverstein 1970; Conn and Dyck 1975), sarcoidosis (Oh 1980) and in the very rare sensory perineuritis (Asbury et al. 1972).

The central chromatolysis seen mainly in the motor neurons of the spinal cord is interpreted as an axonal reaction secondary to the peripheral nerve lesion. The finding of similar alterations in some motor nuclei of the brainstem without lesions of its pathways may suggest a primary pathological lesion in the neurons.

The skin lesions in the 1st CPh appeared few days after the onset of fever as generalized exanthemata, of a morbiliform or scarlatiniform character, and disappeared after one or two weeks. In the 2nd CPh the skin lesions were more variegated: disseminated papules in the trunk and extremities, pseudoxanthoma elasticum-like lesions and also sclerodermiform lesions. The histological findings suggest an evolution in the lesions since transitional microscopical pictures between both clinical phases have been observed. In the 2nd CPh the microscopical changes of mucinosis showed a certain similarity to the diffuse type of the granuloma annulare (Umbert and Winkelmann 1977). In the most advanced lesions sclerosis of the reticular dermis and hypodermis, with atrophy of the dermal appendages, were observed. This picture is comparable to those of the PSS and scleroderma-like skin lesions (Spiegelvogel et al. 1977). The described activated fibroblast with eosinophilic cytoplasmic inclusions are similar to those that have been seen in the infantile digital fibroma (Bhawan et al. 1979).

The hepatic lesions are nonspecific; nevertheless they are reminiscent of the cholestatic hepatitis induced by drugs, especially liver injury produced by chlorpromazine (Pérez et al. 1972). As happens with this drug (Boyer 1978) a twofold pathogenesis of the lesion may be operative: an hypersensitivity reaction and direct hepatotoxicity.

The mononuclear infiltrates, fibrosis and parenchymal atrophy of the salivary glands are comparable to lesions of those glands caused by immunological mechanisms (Bloch et al. 1965); a certain number of patients admitted to our hospital presented features of the "sicca syndrome".

Neither the placentas studied by our group or by other groups showed any significant pathology; also, to date, no fetal lesions nor sequelae have been reported in babies born to sick mothers (Report of the Pathology Commision, 1981 b).

The aetiology and pathogenesis of the TS are still unknown. It has been found that the oil contained aniline (an additive used to denaturate rapeseed oil imported for industrial use) and acetylanilide, and that these chemicals form strong bonds with fatty acids, yielding oleanilide (Tabuenca 1981). It was suggested that the anilides found in the oil were responsible for the disease (Tabuenca 1981), but the syndrome produced by those toxic substances is different to this syndrome (Starmer et al. 1971; Gordon 1981 a). Also, it has been suggested that free radicals (FR) could produce the lesions described (Gordon 1981 b) since FR have been implicated in lesions that show some similarity to those of the TS, such as those produced by radiation or nitrofurantoin (Fajardo and Berthrong 1978; Reinhold and Buisman 1973; Harley et al. 1969; Del Maestro 1980). Finally, there are certain aspects of the TS that suggest that some immunological mechanisms could be involved in the pathogenesis of certain lesions, i.e. the anatomoclinical features similar to diseases of immunological cause (PSS, "sicca syndrome", CGVD, etc.), the richness in lymphocytes and/or eosinophils of the inflammatory infiltrates. However, there is no evidence for humoral immune mechanisms. Vascular or tisular immunocomplexes have not been found. Tabuenca (1981) and Brostoff et al. (1982) found high IgE levels

in the sera of patients with the TS; but these authors did not discover specific IgE antibodies. Only 4 out of 842 patients developed glomerulonephritis mediated by immunocomplexes (Gutierrez-Millet et al. 1982).

The thromboembolic complications could have been caused by a consumption coagulopathy or disseminated intravascular coagulation, as detected in a certain number of patients. The observed endothelial lesions could also induce thrombosis, as this has been seen in cases of radiation vasculitis, whose lesions have a similarity to those of the TS. Other possible explanations could be the action of FR, by direct activation of thrombocytes (Demopoulos et al. 1980). Moreover, the eosinophilia could be related to the thromboembolic complications (Fauci et al. 1982; Venge et al. 1979; Venge et al. 1980). One of the hallmarks of the 1st CPh was the peripheral blood eosinophilia. It might be thought that some complications of the TS were secondary to an hypereosinophilic syndrome. However, the complications of the 2nd CPh appeared without evidence of eosinophilia and moreover the pathological components of the hypereosinophilic syndrome are different (Chusid et al. 1975; Flaum et al. 1981; Schooley et al. 1981).

The treatment has been symptomatic and mainly directed to influencing the possible immunopathological mechanisms involved in the progression of this condition. Corticosteroids have produced a beneficial influence on the pneumonitis and skin lesions during the 1st CPh, but the results obtained in later phases have been controversial (Gilsanz 1981).

Acknowledgements. The authors want to thank the members of the "Clinical Commision for the Toxic Syndrome", Drs. A. Rodriguez Noriega, J. Gómez Reino, A. López Encuentra, P. Martín Escribano, Solis Herruzo, J. Valle Gutiérrez, for supplying biopsies (lung, skin, peripheral nerve and muscle) for a programmed study of the disease. Also, to Mr. Michael Hall for the usage of the language.

References

Adams RD (1975) Diseases of the muscle, 3rd ed, Harper and Row, New York

Asbury AK, Picard EH, Baringer JR (1972) Sensory perineuritis. Arch Neurol 26:302–312

Bhawan J, Bachetta C, Joris I, Majno G (1979) A myofibroblastic tumor, Infantile digital fibroma (recurrent digital fibrous tumor of childhood). Am J of Pathol 94:19–36

Bloch KJ, Buchannan WW, Whol MJ, Bunim JJ (1965) Sjögren's Syndrome a clinical pathological and sereological study of sixty-two cases. Medicine 44:187–231

Boyd MR, Catignani GL, Sasame HA, Mirchell JR, Stiko AW (1978) Acute pulmonary injury in rats by nitrofurantoin and modification by vitamin E, dietary fat and oxygen. Am Rev Respir Dis 120:93–99

Boyer JL (1978) Mechanisms of chlorpromazine cholestasis: Hypersensivity or toxic metabolite. Gastroenterology 74:1331–1332

Brostoff J, Blanca M, Boulton P, Serrano P (1982) Absence of specific IgE antibodies in toxic oil syndrome. Lancet I:277

Cannon PJ, Hassar M, Case DB, Casarella WJ, Sommers SC, Le Roy EC (1974) The relationship of hypertension and renal failure in scleroderma (progressive systemic sclerosis) to structural and functional abnormalities of the renal cortical circulation. Medicine 53:1–46

Chusied MJ, Dale DC, West BC, Wolff SM (1975) The hypereosinophilic snydrome: Analysis of fourteen cases with review of the literature. Medicine 54:1–27

Conn DL, Dyck PJ (1975) Angiopathic neuropathy in connective tissue diseases. In: Dyck PJ, Thomas PK, Lambert EH (eds) Peripheral neuropathy. WB Saunders Philadelphia, pp 1149–1165

Corson JM (1972) The pathologist and the kidney transplant. In: Sommers C (ed) Pathology annual, Appleton Century Crofts, New York, pp 251–265

Del Maestro RF (1980) An approach to free radicals in medicine and biology. Acta Physiol Scand (Suppl) 492:153–168

Demopoulos HB, Flamm ES, Pietronigro DD, Seligman ML (1980) The free radical pathology and the microcirculation in the major central system disorders. Acta Physiol Scand (Suppl) 492:91–119

Fajardo LF, Berthrong M (1978) Radiation injury in surgical pathology (Part 1) Am J Surg Pathol 2:159–199

Fauci AS, Haynes BF, Katz (1978) The spectrum of vasculitis. Ann Intern Med 89:660–676

Fauci AS, Harley JB, Roberts WC, Ferrans VJ, Gralnick HR, Bjornson BH (1982) The idiopathic hypereosinophilic syndrome: Clinical, pathophysiological and therapeutic considerations. Ann Intern Med (in press)

Flaum MA, Schooley RT, Fauci AS, Gralnick HR (1981) A clinicopathologic correlation of the idiopathic hypereosinophilic syndrome. I. Haematologic manifestations. Blood 58:1012–1020

Furst DE, Clemens PJ, Graze P, Gale R, Roberts N (1979) A syndrome resembling progressive systemic sclerosis after bone marrow transplantation. A model for scleroderma. Arthr Rheum 22:904–910

Gilsanz (1982) Late features of toxic syndrome due to denatured rapeseed oil. Lancet I:335–336

Gordon RM, Silverstein A (1970) Neurological manifestations in progressive systemic sclerosis. Arch Neurol 22:126–134

Gordon RS (1981a) Little headway on toxic oil syndrome. Lancet II:1102

Gordon RS (1981b) Oleanilides and spanish oil poisoning. Lancet II:1171–1172

Gutierrez-Millet V, Navas-Palacios JJ, Gomez-Reino J, Fernandez Epifanio JL (1982) Renal involvement in toxic syndrome. Lancet I:1–20

Harley DA, Goldman AL (1969) Pleuropneumonic reactions to nitrofurantoin. N Engl J Med 281:1087–1090

Nofstad I (1974) Endothelial damage and platelet associated with PUFA-rich vit. E deficient diet fed to pig. Thromb Res 5:251–255

Oh SJ (1980) Sarcoid polyneuropathy: a histologically proved case. Ann Neurol 7:178–181

Pangalis GA, Moran EM, Rappaport H (1978) Blood and bone marrow findings in angioimmunoblastic lymphadenopathy. Blood 51:71–83

Perez V, Schaffner F, Popper H (1972) Hepatic drug reactions. In: Popper H, Schaffner F (eds) Progress in liver disease, vol IV. Grune and Straton, New York, pp 597–625

Porter KA (1974) Renal transplantation. In: Heptinstall RH (ed) Pathology of the kidney. 2nd ed, Little Brown and Co, Boston, pp 977–1041

Reinhold HS, Buisman GH (1973) Radiosensitivity of capillary endothelium. Br J Radiol 46:54–57

Report of the Pathology Commision of the Ministerio de Sanidad (1981a) Brote epidémico de "Neumonía atípica". Anatomía Patológica. Bol Epidemiol Sem 1486:161–162

Report of the Pathology Commision of the Ministerio de Sanidad (1981b) Informe de la Comisión de Anatomía Patológica del síndrome tóxico, sobre el material de biopsias realizadas. Bol Epidemiol Sem 1503:297–299

Report of the Pathology Commision of the Ministerio de Sanidad (1982) Informe de la Comisión de Anatomía Patológica. Bol Epidemiol Sem (In press)

Rodnam GP, Myerowitz RL, Justh GO (1980) Morphologic changes in the digital arteries of patients with progressive systemic sclerosis (scleroderma) and Raynaud phenomenon. Medicine 56:393–408

Salerni R, Rodnan GP, Leon DF, Shaever JA (1977) Pulmonary hypertension in the CREST syndrome variant of progressive systemic sclerosis (scleroderma). Ann Intern Med 86:394–399

Schooley RT, Flaum MA, Grabnick HR, Fauci AS (1981) A clinicopathologic correlation

of the idiopathic hypereosinophilic syndrome. II Clinical manifestations. Blood 58:1021–1026
Solis-Herruzo JA, Castellanos G, Colina F, Morillas JD, Muñoz Yagüe MT, Diaz Rubio C (1982) Hepatopatía en el síndrome tóxico por consumo de aceite adulterado en el área de Madrid. Gastroenterología Hepatología 5:113–124
Spargo BH, Seymour AE, Ordoñez NG (1980) Renal biopsy pathology with diagnostic and therapeutic implication. John Wiley and Sons, New York, pp 254–265
Spencer H (1977) Pathology of the lung. 3rd edn. Pergamon Press, Oxford
Spielvogel RL, Goltz RM, Kersey JH (1977) Scleroderma-like changes in chronic graft versus host disease. Arch Dermatol 113:1424–1428
Starmer GA, McLean S, Thomas J (1971) Analgesic potency and acute toxicity of substituted anilides and benzamides. Toxicol Appl Pharmacol 19:20–28
Tabuenca JM (1981) Toxic-allergic syndrome caused by ingestion of rapeseed oil denatured with aniline. Lancet II:567–568
Toxic Epidemic Syndrome Study Group. Hospital 1° de Octubre Madrid (1982) Toxic epidemic syndrome. Spain 1981. Lancet (in press)
Umbert P, Winkelmann RK (1977) Histologic, ultrastructural and histochemical studies of granuloma annulare. Arch Dermatol 113:1681–1686
Venge P, Dahl R, Hällgren R, Olsson J (1980) Cationic proteins of human eosinophils and their role in the inflammatory reaction. In: Mahmoud AA, Austen KF (eds) The eosinophil in health and disease. Grune and Stratton Inc. New York, pp 141–146
Venge P, Dahl R, Hällgren R (1979) Enhancement of F XII – dependent reactions by eosinophil cationic protein. Thromb Res 14:641–649

Accepted July 21, 1982