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THE MURID FILARIA *MONANEMA MARTINI* : A MODEL FOR ONCHOCERCIASIS

Part I. — Description of lesions[1]

P. N. VUONG*, S. WANJI**, L. SAKKA**, S. KLAGER***, O. BAIN**
With the technical assistance of : T. Ben Assavi*, R. Tcheprakoff**, S. Plateaux**

SUMMARY

A study of the anatomo-pathological lesions induced by *Monanema martini,* a filaria with skin-dwelling microfilariae, was performed using 65 *Lemniscomys striatus* fixed from 30 minutes to 36 months after inoculation of the infective larvae, 5 *Arvicanthis niloticus* and 3 *Meriones unguiculatus* fixed during the patent phase, and controls. Attempts at quantification of lesions in *L. striatus* was made.

Approximately 20 % of *L. striatus* had microfilariae in the eyeballs, and many more presented ocular lesions. The delay of the patent period seems to have more effects on the gravity of lesions than repeated inoculations. The location of the lesions and parasites presuppose that microfilariae enter the eyeball through the lymphatic capillaries of the irido-corneal angles. Cutaneous lesions were often severe: there is a parallel between the importance of lesions and the abundance of microfilariae. Larvae are responsible for damage to various structures of the lymphatic system (thrombo-lymphangitis, acute or granulomatous lymphadenitis...) into which they migrate, explaining the mechanism of elephantiasis. These rodent lesions appear similar to those observed in human onchocerciasis and lymphatic filariasis.

Whatever the *M. martini* stage and the organ examined, major lesions belonged to the inflammatory process. Various types of inflammatory reaction (acute, subacute, or chronic inflammation, scarring sclerosis etc.) can co-exist within a single tissue area. The accidental escape of a microfilaria from a lymphatic capillary into the connective tissue (including the corneal stroma) induces an inflammatory reaction. Thus *M. martini,* as human *Onchocerca* species, causes a chronic disease, associating recent lesions to old ones.

RÉSUMÉ : **La filaire de muridé à microfilaires dermiques, *Monanema martini,* un modèle pour l'onchocercose. Première partie : Description des lésions.**

Une étude anatomo-pathologique des lésions induites par la filaire à microfilaires dermiques *Monanema martini* est effectuée avec 65 *Lemniscomys striatus* fixés 30 minutes à 36 mois après l'inoculation des larves infectantes, 5 *Arvicanthis niloticus* et 3 *Meriones unguiculatus* fixés pendant la phase patente, et des rongeurs témoins. Un essai de quantification des lésions chez *L. striatus* est effectué.

Presque 20 % des *L. striatus* ont des microfilaires dans les globes oculaires, et beaucoup plus ont des lésions. La durée de la phase patente semble influer davantage sur la gravité des lésions que les inoculations répétées. La localisation des lésions et des microfilaires suggère que celles-ci pénètrent dans les globes oculaires par les capillaires lymphatiques de l'angle irido-cornéen. Les lésions dermiques sont souvent sévères ; il y a une relation entre la gravité des lésions et l'abondance des microfilaires. Les larves provoquent des lésions des différentes structures du système lymphatique dans lequel elles migrent (thrombo-lymphangite, lymphadenite aiguë ou granulomateuse...), expliquant le mécanisme de l'éléphantiasis. Les lésions des rongeurs apparaissent semblables à celles observées dans l'onchocercose ou les filarioses lymphatiques.

Quel que soit le stade de *M. martini* ou l'organe du rongeur, les lésions s'intègrent dans un processus inflammatoire. Plusieurs types de réaction (inflammation aiguë, subaiguë, chronique, sclérose cicatricielle etc.) co-existent dans un même secteur tissulaire. Le passage accidentel d'une microfilaire lymphatique dans le tissu conjonctif avoisinant, y compris le stroma cornéen, induit une réaction inflammatoire. Ainsi *M. martini,* comme l'onchocerque humaine, détermine une maladie chronique, associant des lésions anciennes et récentes.

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* Département d'Anatomie et de Cytologie Pathologiques, Hôpital Saint-Michel, 33, rue Olivier de Serres, F 75015 Paris.

** Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, 61, rue Buffon, F 75231 Paris Cedex 05.

*** Tropenmedizinisches Institut, Wilhelmstrasse, 31, 7400 Tübingen 1, Germany.

INTRODUCTION

Since practical models cannot be realized with *Onchocerca* species, different systems have been explored. These systems consist of inoculating laboratory animals — rodents or monkeys — with onchocercal microfilariae using subcutaneous, sub-conjonctival or intra-ocular routes (cf. Lagraulet, 1957; Duke and Anderson, 1972; the review of Donnelly *et al.*, 1985; James *et al.*, 1986; Semba *et al.*, 1988). Another approach is to use rodents infected by non-*Onchocerca* filariae which also have skin-dwelling microfilariae (Bianco *et al.*, 1983; Bain *et al.*, 1985; Spratt and Haycock, 1988). It has been demonstrated that these parasites induce in their hosts cutaneous and ocular lesions similar to those observed in patients with onchocerciasis (Vuong *et al.*, 1985 and 1986; Sakka, 1989). One of these filariae, *Monanema martini* Bain, Bartlett, Petit, 1986, can be maintained in the laboratory, and has allowed a systematic study of visceral, cutaneous and ocular lesions developed during its parasitic cycle.

MATERIAL AND METHODS

A — Filarial strain and parasitological techniques

Monanema martini is maintained in two natural host murids, *Lemniscomys striatus* and *Arvicanthis niloticus,* as well as in *Meriones unguiculatus.* Male or female rodents, 1 to 2 months of age, were inoculated with infective larvae (L3) by injection into the sub-cutaneous tissue of the right dorso-lumbar area. The larvae migrate inside the lymphatic system towards the lymphatic vessels of the large intestine wall (colon and cecum); the third and fourth moults take place respectively at day 10 and day 21 post-inoculation (Wanji *et al.*, 1990).

The microfiladermia appears two months p.i. and persists for more than one year. It was measured every three months and generally just before necropsy, and was expressed by microfilariae/mm². A fragment of ear pinna is taken and teased in RPMI (1640, with glutamate, and sodium bicarbonate buffer) +20 % calf serum; the microfilariae were counted 2h later. Experiments commenced in 1986, and the technique had been improved during the course of the work: in previous experiments, snips were carried out in either of the two ears and the surface area estimated approximately 2 mm² by the calibrated punch; since 1988, we collect snips from the left ear and measure their surface area using a camera lucida attachment on the microscope. In recent experiments a blood sample was taken from the retro-orbital sinus for immunological analyses.

Oxyurosis due to *Aspiculuris tetraptera* was present in one third of the rodents.

B — Anatomo-pathological material

Three series were used.

1 — Control animals: 10 *Lemniscomys striatus* *, 1 *Arvicanthis niloticus,* and 2 *Meriones unguiculatus (Table I)*;

2 — Inoculated animals used for the analysis of lesions related to larval migrations: 21 *Lemniscomys striatus* fixed 30 minutes to 20 days after inoculation. Each rodent received a single dose of 300-400 infective larvae (exceptionally 30 or 80 L3);

3 — Inoculated animals used for the analysis of lesions related to the patent phase of filariasis: 44 *Lemniscomys striatus,* 5 *Arvicanthis niloticus,* and 3 *Meriones unguiculatus* fixed from 5 to 36 months after the first inoculation of the larvae *(Table I).* The *L. striatus* were mono-inoculated (a single dose of 30 L3, rarely a dose of 80 to 200 L3) or multi-inoculated (one dose of 30 L3 and 6-8 subsequent doses of 15 to 30 L3 during the year), or bi-inoculated (one dose of 30 or 80 L3 and a second dose of 80 L3 one year after the first). The *A. niloticus* and *M. unguiculatus* received a single dose each, respectively 30-50 L3 and 50-100 L3.

Table I. — *List of the rodents analyzed during the patent phase of filariasis and of the control rodents.*

Months	5	7-13	15-17	20-36
L.s. control	n=3	n=5		n=2
L.s.mono-inoc.	n=2	n=11	n=14	n=6
_ mf/mm2	280 - 380	mf - 240	10 - 390	60 - 125
_ mf/mm2	120 - 120	**10 - 10**	15 - 30	1 - 300
_ mf/mm2		260 - 260	90 - 140	3 - 20
_ mf/mm2		1 - 1	0 - 210	? - 30
_ mf/mm2		145 - 145	2 - 200	? - 150
_ mf/mm2		35 -110	50 -75	10 - 100
_ mf/mm2		0 -1	**30 - 180**	
_ mf/mm2		210 - 210	10 - 120	
_ mf/mm2		**40 -60**	**40 -110**	
_ mf/mm2		0 -0	0 - 0	
_ mf/mm2		mf - 90	**80 -100**	
_ mf/mm2			6 - 240	
_ mf/mm2			mf - 250	
			95 - 135	
L.s. multi-inoc.		n=5	n=1	
_ mf/mm2		**40 - 40**	mf - 35	
_ mf/mm2		60 - 60		
_ mf/mm2		250 - 250		
_ mf/mm2		**115 - 115**		
_ mf/mm2		**35 - 35**		
L.s. bi-inoc.			n=4	n=1
_ mf/mm2			mf - 105	mf - 90*
_ mf/mm2			**mf - 130**	
_ mf/mm2			mf - 145	
_ mf/mm2			mf - 90	
A. n. control	n=1			
A. n. mono-inoc.		n=4		
_ mf/mm2		0 - 1		
_ mf/mm2		0 - 3		
_ mf/mm2		**1 - 2**		
_ mf/mm2		0 - 2		
M. u. control		n=2		
M. u. mono-inoc.		n=1	n=2	
_ mf/mm2		0 - 1	0 - 1	
_ mf/mm2			0 - 15	

L.s. : *Lemniscomys striatus,* A.n.: *Arvicanthis niloticus;* M.u. : *Meriones unguiculatus;* mono-inoc.: inoculation of a single dose of L3; multi-inoc: several inoculations during the year; bi-inoc: a second dose one year after the first one; Months: time-interval between inoculation and necropsy, or age of control rodents plus two months; in the column « 20-36 », rodents were necropsied between 20 and 27 months except one at 36 months (*); mf/mm²: density of microfilariae with two figures for each rodent: figure at necropsy (left), maximal figure (right), these figures are replaced by « mf » when presence of parasites is indicated only on histological sections; microfiladermiae expressed in heavy letters correspond to rodents having at least 1 mf in the eyeball.

C — Pathological methods

Control and parasitized rodents were killed under chloroform and fixed in 10 % formaldehyde after incision of the abdominal

* An eleventh *Lemniscomys striatus* was removed because of adenocarcinoma in the colon.

and the chest walls. Tissue samples were taken from the eyes, dorsal skin (right lombar area), ear pinna and viscera. *In A. niloticus, M. unguiculatus,* and nine of the *L. striatus,* eyeballs only were collected.

After blocking the tissues in paraffin, a few serial sections 5 μm thick were cut and stained with hematoxylin-eosin-safran, and if necessary with other special stains such as Masson's trichrome with light green to analyse sclerosis, slow Giemsa to identify inflammatory cells and mast cells, periodic acid-Schiff to identify the basal membrane of the capillaries, and orcein to identify the elastic structures of the vessels.

Both the parasites and the lesions were located and identified. The intensity of lesions was expressed by one, two, or three + and the absence of lesions by −. An attempt at quantification was assumed for the dermal and ocular lesions of *L. striatus* by assigning the score 1, 2 or 3 to increasing intensities +, + +, + + +. The scored results were analyzed using the Man-Whitney U test.

A table corresponding to each animal contains results on experimental parameters, parasitological observations and tissue lesions. The file is retained in the Laboratoire de Biologie parasitaire, Protistologie, Helminthologie under the number N 16,856 for future reference.

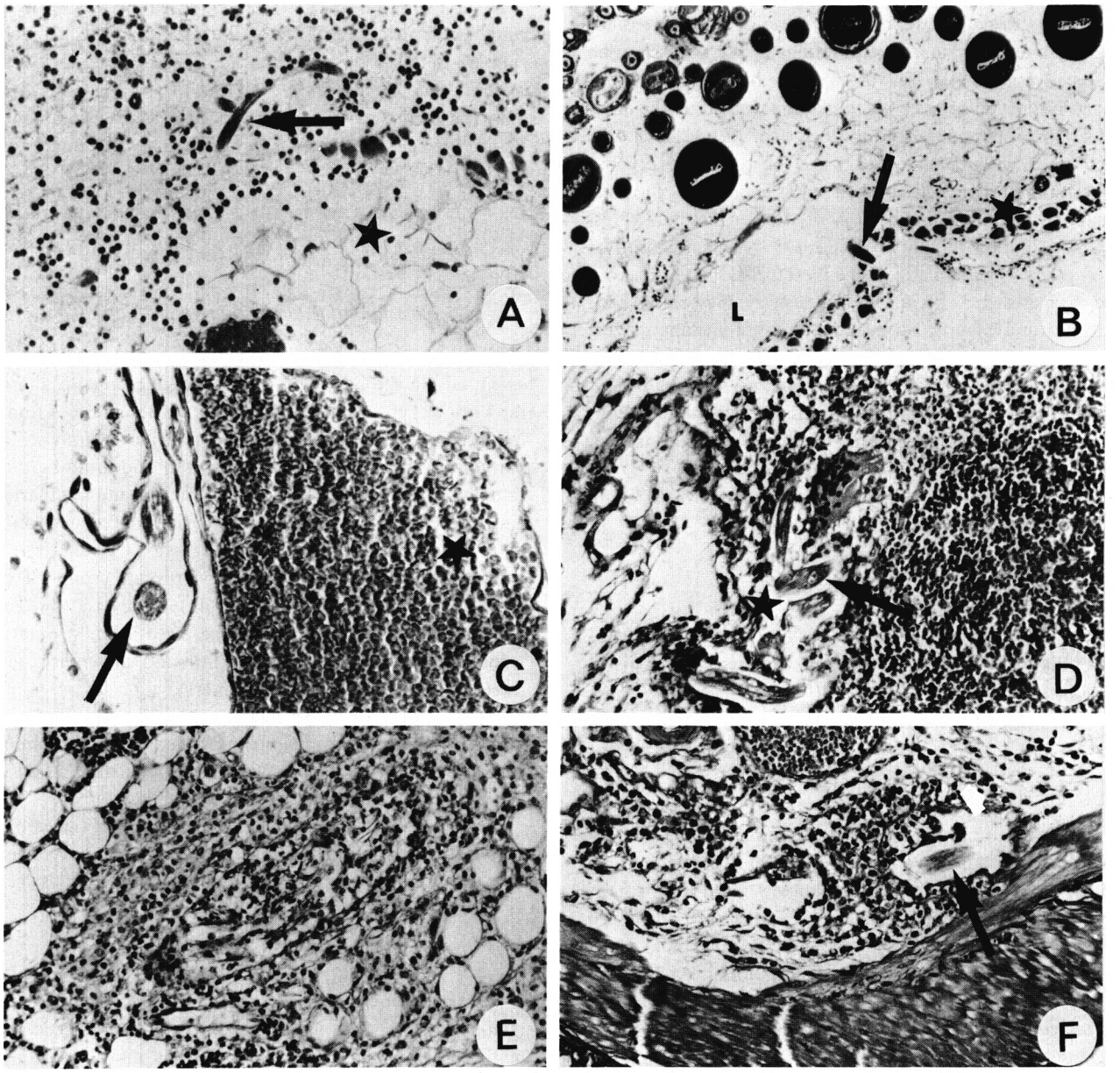

FIG. 1. — Larvae (→), and cutaneous or visceral lesions in *L. striatus* inoculated with 400 L3. A: larva in the dermo-hypodermic tissue (★) surrounded by an acute inflammatory reaction type 2, 6 h. p.i.; B: larva in a sub-cutaneous lymphatic vessel (L) next to the musculus cutaneous (★), 6 h. p.i.; C: larva in a deep lymphatic vessel running alongside a vein (★), at d. 7 p.i.; D: larva inside the marginal sinus (★) of a lymph node with foreign body granuloma type 4, at d. 12 p.i.; E, F: mesenteric lymphatic vessels demonstrating acute type 2 (E) or subacute type 3 (F) thrombo-lymphangitis, at d. 12 p.i.

RESULTS

A — Description of elementary lesions

The lesions belonged either to the inflammatory process or the so-called reactive lesions. The inflammatory lesions included the 5 types which have been previously described (Vuong *et al.*, 1986) and are summarized here: type 1: chronic non-specific inflammation with infiltration of mononucleated lympho-histiocytic cells; type 2: acute inflammation with essentially vasculo-exudative components; type 3: sub-acute inflammation; type 4: granulomatous inflammation with formation of histiocytic granulomas or eosinophilic granulomas; and type 5: lesions of scarring sclerosis.

Reactive lesions were variable: — hyperplasia of the mono-histiocytic system, which is characterized by the accumulation of mononucleated histiocytes or of multinucleated giant cells within connective tissue or lymphoid organs; — lymphoid hyperplasia with enlargement of germinal center of lymphoid nodules; — dilation of lymphatic vessels; — dilation of blood capillaries; — accumulation of mast cells; — hyperplasia of the mesothelial lining of serous membranes such as peritoneum, mesentery, mesocolon, pericardium and pleura; — accumulation of melanophages; — exocytosis; — lesions linked to trauma (hemorrhage linked to blood sampling); — necrosis; — vascular thrombosis; — tumor-like hyperplasia of tissue and miscellaneous lesions (squamous metaplasia of the bronchial mucosa, osseous metaplasia, visceral atrophy).

A hepatic steatosis caused by nutrition was observed in each rodent.

B — Lesions of control rodents

Both inflammatory and reactive lesions existed in control rodents. Their number, degree of severity, and distribution are recorded in *Tables II to V.*

C — lesions related to larval migrations *(fig. 1)*

1 — Localization of larvae

Until 48 h p.i., the larvae were either in the dermo-hypodermal connective tissue or inside the superficial lymphatic vessels of the sub-cutaneous tissue and of the musculus cutaneus. Between days 3-20 they were identified in the lumen of deep lymphatic vessels and sometimes inside the marginal sinus of mesenteric lymph nodes. At 20 d.p.i. larvae were observed in the lymphatic channels of the wall of the colon-cecum. Some erratic larvae were disclosed in the lumen of pulmonary arteries of two rodents at d.p.i. 7 and 12 respectively.

2 — Lesions

At the site of inoculation, severe acute inflammatory lesions and granulomas frequently developed near or around larvae, some of which were lysed. These lesions consisted of inflammatory bands running alongside the muscular cutaneous aponevrosis, sometimes partly necrotic.

In the mesentery, some lymphatic vessels containing larvae developed acute or subacute lymphangitis. These vessels were surrounded by an inflammatory ring containing neutrophils mixed with lympho-histiocytic mononucleated cells; sometimes this ring infiltrated the lymphatic vessel wall, lifting the endothelial lining and eroding it. The vascular lumen may be clogged by a fibrinous thrombus; the whole constitutes a thrombo-lymphangitis.

In the lymph nodes, the presence of larvae may be associated with foreign body granulomas, while the neighboring lymph node parenchyma presented a monohistiocytic hyperplasia.

In the lungs, the presence of larvae were accompanied by histiocytic or eosinophilic granulomas, with congestion.

D — Lesions related to the patent phase of filariasis

1 — Viscera *(Table II, fig. 2)*

a — *Localization of filariae and microfilariae*

Filariae were identified in 21 *L. striatus*: they resided essentially in the lymphatic channel of the mucosa and of the serosa of the colon-cecum. They were sometimes found inside the mesenteric lymphatic vessels, more rarely in the marginal sinus of the mesenteric lymph nodes, and exceptionally in the pulmonary vessels (arteries and capillaries) and in the right heart atrium; an adult filaria was found in a mesenteric vein.

Microfilariae were found alone or in small groups. They were in the lymphatic vessels, and specially the thoracic duct, the lymphatic channel of the mesothelial stroma of serous cavities (pleura, peritoneum) and mesentery, and rarely the marginal sinus of mesenteric lymph nodes. Sometimes they were observed in the connective tissue of skeletal muscle, serous lining stroma (pleura, mediastinum), gastric wall, or inside the mesentery and in the lumen of the pulmonary alveoli.

The blood also harbored some microfilariae (in cardiac cavities, myocardial capillaries, aorta...), but they were very rare. No microfilariae were found in the brain, liver, spleen, kidneys, adrenals of the rodents.

b — *Lesions*

In the colon-cecum, the presence of filaria inside the lymphatic vessels was associated with inflammatory lesions in the surrounding tissue: these lesions may be chronic, acute, subacute or granulomatous; Peyer's patches demonstrated lymphoid hyperplasia; the cecal wall presented infiltration of mast cells; some lymphatic vessels were dilated; the mesentery showed hyperplasia of mesothelial lining.

Pulmonary arteries or capillaries containing filariae did

TABLE II. — *Visceral lesions in L. striatus.*

	Intensity	Mf ev	Mf il	Mf is	F il	F is	1	2	3	4	5	HMono	HLym	E vas L	E cap S	Masto	HMeso
AP	+	- -	- -	- -	- -	- -	- -	- -	- 0.5	- 0.2	- 0.2	- -	- -	- -	- -	- -	- -
MU	+	- 0.4	- -	- -	- -	- -	- -	- 0.5	- 0.2	- -	- -	- -	- -	- -	- -	- 0.2	- -
	++	- 0.2	- -	- -	- -	- -	- 0.5	- 0.2	- -	- -	- -	- -	- -	- -	- -	- -	- -
	+++	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 0.2	- -
CO	+	- -	- -	- 0.2	- -	- -	1 0.8	- 0.5	1 0.2	- 0.2	1 -	- -	- -	- -	1 0.2	- 0.2	- -
	++	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 0.5	- -	- -
	+++	- -	- 0.2	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 0.2	- 0.2	- -	- -
VS	+	- -	- -	- 0.2	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
	+++	- -	- -	- 0.2	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
VL	+	- -	- 0.8	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
	++	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 0.2	- -	- -	- -	- -	- -
	+++	- -	- 0.2	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
PO*	+	- -*	- 0.2	- -	- 0.2	- 0.5	- 0.8	- 0.8	1 3.1	- 0.2	- 0.5	- -	- -	- -	7 2.8	- 0.2	- -
	++	- -	- -	- -	- -	- 0.2	- -	- -	- 0.5	- -	- -	- -	- -	- -	- 0.2	- 0.2	- -
	+++	- -	- -	- -	- -	- -	- 0.2	- -	- 0.2	- -	- -	- -	- -	- -	- 0.2	- 0.2	- -
PM	+	- 0.8	- -	- -	- -	- -	- 1.1	- -	- 1.4	- 0.2	- -	- -	- -	- 0.2	- -	2 1.7	- 3.7
	++	- -	- -	- -	- -	- -	- -	- -	- 0.2	- -	- -	- -	- 0.2	- 0.5	- -	- -	- 0.5
	+++	- 0.2	- -	- -	- -	- -	- -	- -	- 0.2	- 0.8	- -	- -	- -	- -	- -	- 0.8	- -
OE	+	- -	- -	- -	- -	- -	1 -	- -	- -	- -	1 -	- -	- -	- -	- -	- -	- 0.5
ES	+	- 0.2	- -	- -	- -	- -	- 1.1	- 0.2	1 3.1	- -	1 2.2	- -	1 0.8	- 0.2	- 0.5	- -	- -
	++	- -	- -	- -	- -	- -	- -	- 0.2	- 0.2	- -	- 0.2	- -	- -	- 0.2	- -	- 0.5	- -
	+++	- -	- -	- -	- -	- -	- -	- -	- 0.5	- 0.2	- 0.5	- -	- -	- -	- -	- 0.2	- -
GR	+	- -	- -	- -	- -	- -	- 0.8	- 1.1	- 1.1	- -	- 0.8	- -	2 5.1	- 0.2	- -	- -	- 0.2
	++	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 0.2	- -	- -	- -	- -
	+++	- -	- -	- -	- -	- -	- -	- 0.2	- 0.2	- -	- -	- -	- -	- -	- -	- -	- -
CA	+	- -	- -	- -	- 2.8	- -	- 0.5	1 0.2	1 2	- 0.5	- 2.	- -	2 3.7	2 1.7	1 -	- -	- -
	++	- -	- -	- -	- 0.5	- -	- -	- 0.5	- 0.8	- 0.2	- 0.2	- -	- 0.2	- -	- -	- 0.2	- -
	+++	- -	- -	- -	- 2.	- -	- 0.2	- 0.5	- 0.2	- 0.5	- 0.5	- -	- 0.2	- -	- -	- -	- -
ME	+	- 0.2	- 0.2	- -	- 0.5	- -	2 0.5	- 1.4	- 2.8	- 0.2	- 0.5	1 0.2	- 0.5	- 0.8	- 0.8	1 0.5	- 0.2
	++	- -	- -	- -	- -	- 0.2	- -	- 0.5	- 2.5	- 0.5	- -	- -	- -	- -	- 0.2	- 0.5	- 0.2
	+++	- -	- -	- -	- -	- -	- 0.2	- -	- 1.7	- -	- 0.2	- -	- -	- 0.5	- -	1 2.8	- -
GG	+	- -	- -	- -	- -	- -	- -	- 0.2	- 0.5	- -	- -	3 4.	- 1.1	- -	- 0.2	- 0.2	- -
	++	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 0.2	- 0.5	- -	- -	- 0.2	- -
	+++	- -	- 0.2	- -	- 0.2	- -	- -	- -	- -	- -	- -	- 0.8	- -	- -	- -	- 0.2	- -
FO	+	- -	- -	- -	- -	- -	5 1.4	- 0.8	- 2.	- 0.2	- -	- 0.2	- -	- -	- 2.5	- -	- -
	++	- -	- -	- -	- -	- -	- -	- -	- 0.2	- -	- -	- -	- -	- -	- 0.2	- -	- -
	+++	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 0.2	- -	- -
RA	+	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 0.5	1 2.2	- -	1 0.2	- -	- -
	+++	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 0.2	- -	- -	- -	- -	- -
NE	+	- -	- -	- -	- -	- -	- 1.1	- -	4 1.7	- -	- -	- -	- -	- -	1 0.8	- 0.2	- -
	++	- -	- -	- -	- -	- -	- -	- -	- 0.5	- -	- -	- -	- -	- -	- -	- -	- -
EN	+	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	2 1.1	- -	- -

Intensity of parasitism and of lesions are indicated by +, ++, or +++. In each column, the left figure corresponds to the sum of elementary lesions observed in the 10 control *L. striatus;* the right figure corresponds to the sum of lesions observed in 35 *L. striatus* (divided by 3,5 to permit comparison); small lines represent value O; when all the values are nil in a line, this one is suppressed.

Mf ev, Mf il, Mf is: respectively extra-vascular, intra-lymphatic, and intra-vascular microfilariae; *: microfilariae in the pulmonary alveoli; F il and F is: respectively intra-lymphatic and intravascular filariae; 1, 2, 3, 4, 5: the five types of the inflammatory reaction: HMono = hyperplasia of the mono-histiocytic system; HLym = lymphoid hyperplasia; E vas L = dilation of lymphatic vessels; E cap S = dilation of blood capillaries, Masto = infiltration of mast cells; HMeso = hyperplasia of the mesothelial lining of serous membranes.

AP = aponevrosis; MU = skeletal muscles; CO = heart; VS = aorta, pulmonary arteries and other great blood vessels, VL = thoracic duct and other lymphatic vessels; PO = lungs; PM = pleura and mediastinum; OE = esophagus; ES = gastric wall; GR = small intestine wall; CA = cecal wall; ME = mesentery and pancreas; GG = lymph nodes; FO = liver; RA = spleen; NE = kidneys; EN = brain.

not present inflammatory vasculitis; a thrombosis without additional infarction was observed in one *L. striatus.* The pulmonary parenchyma, in close proximity to infected vessels, disclosed non-specific subacute or granulomatous alveolitis with numerous eosinophils.

Inflammatory lesions associated with microfilariae were observed in the mediastinum, the mesenteric lymph nodes and digestive wall.

2 — Skin *(Tables III and V, fig. 3)*

a — *Localization of microfilariae*

Microfilariae were concentrated in the dermis of the ear pinna but parasite density was variable from one area to another in a given ear pinna. Generally, microfilariae were disclosed in the lumen of lymphatic capillaries but they

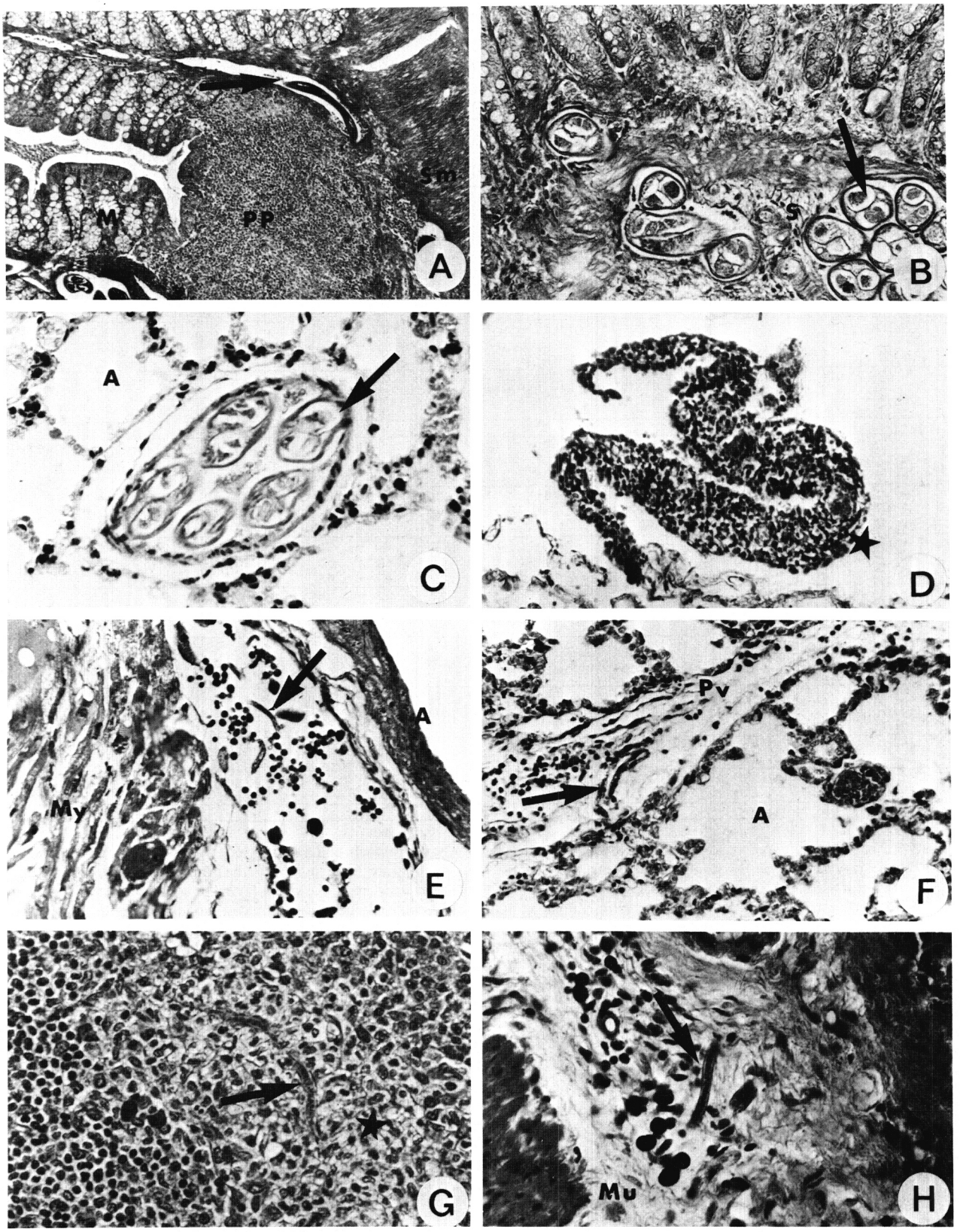

FIG. 2. — Filariae (→), microfilariae (→) and visceral lesions in *L. striatus*. A: filariae in a lymphatic channel of the mucosa (M) next to a Peyer's patch (PP); B: *idem,* in the lymphatic channel of the sub-mucosa and of the serosa (S) of the cecum; C: filaria in a blood capillary of a pulmonary alveolus; D: hyperplasia of mesothelial lining of the mesentery (★); E: microfilariae inside the thoracic channel (My: myocardium; A: aorta); F: extra-lymphatic microfilaria in the mediastinum with sub-acute inflammatory reaction type 3 (A: pulmonary alveoli); G: microfilariae inside the marginal sinus (★) of a lymph node demonstrating acute lymphadenitis with mono-histiocytic hyperplasia; H: microfilaria in the gastric sub-mucosa giving rise to an inflammatory reaction type 3 with infiltration of mast cells.

TABLE III. — *Cutaneous lesions in* L. striatus.

	Intensity	Mf ev		Mf il		1		2		3		4		5		E vas L		E cap S		Masto		Mela		Nec	
Back	+	-	-	-	-	-	1.4	-	0.8	-	1.1	-	0.2	-	1.1	-	-	-	-	-	-	-	-	-	-
	++	-	-	-	-	-	-	-	0.2	-	0.2	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-
	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	0.2	-	-	-	-
Ear	+	-	1.4	-	4.2	10	10	-	4.2	2	5.7	-	3.4	8	5.1	-	0.5	1	2.2	4	0 .8	-	0.2	-	0.2
	++	-	0.8	-	2.8	-	3.1	1	1.7	-	2.8	-	0.8	2	3.1	-	-	-	0.2	1	1.4	-	-	-	-
	+++	-	1.4	-	2.5	-	0.2	-	-	-	0.8	-	1.1	-	1.1	-	-	-	-	-	3.4	-	-	-	-

The sum of the elementary lesions in the ten control rodents is represented by the left figure, and the sum of the elementary lesions of the 35 inoculated rodents (divided by 3,5) by the right figure.

Mela = infiltration of melanophages; Nec = necrotic lesion; Back = dorsal skin, Ear = ear pinna. Definitions of the other abbreviations are given in *Tables I and II.*

may be extravascular, inside the connective tissue of the dermis, or both intra- and extra-lymphatic.

b — *Lesions*

The five types of the inflammatory lesions were disclosed in the ear pinna; they generally co-existed in the same ear. The lesions were multiple, irregularly distributed, and sometimes of very severe intensity; three quarters of these lesions developed around microfilariae. In some rodents, the predominance of foreign body granulomas gave the skin a pseudo-tumoral aspect which finally resulted in an extensive thickening of the ear, contrasting with that of control rodents.

Reactive lesions were variable: dilation of lymphatic vessels, dilation of blood capillaries, infiltration of mast cells in cases of severe inflammatory lesions, necrosis of dermal connective tissue.

The scored results did not vary greatly between the different groups of *L. striatus*; most importantly, no significant difference (U test) was noted between multi-inoculated and mono-inoculated groups with similar patent phase (5-13 months).

3 — Eyeballs and accessory organs *(Tables I, IV and V, fig. 4 and 5)*

a — *Localization of microfilariae*

Microfilariae were seen in the eyelids, extravascular connective tissue or lymphatic vessels, and among the Harder glands. Microfilariae were also found in the eyeballs, as illustrated in *figure 4.* In the anterior segment, they were identified outside or inside the vessels of the irido-corneal angle or corneal periphery. In the retina, a single microfilaria was extra-vascular and partially destroyed, the 5 others were found in the lumen of vessels, the type (blood or lymphatic) of which remains unknown. Two of the nine *L. striatus* harboring intra-ocular microfilariae had a mixed infection, in the irido-corneal angle and the retina. One *A. niloticus* showed a single extra-lymphatic microfilaria in the corneal stroma.

b —. *Lesions*

Regardless of host, extralymphatic microfilariae were associated with acute, subacute, and/or chronic inflammatory reactions with mild sclerotic changes. Inflammatory lesions were common in the irido-corneal where they were associated with an infiltration of mast cells and melanocytes. In the retina, dilation of blood capillaries was common. A retinal hemorrhage was seen in the vicinity of the extravascular microfilaria.

The scored result of mono-inoculated *L. striatus* with a long patent period (20-36 months) was high: 8,16. It is significantly higher than that of a mono-inoculated group with a patent period of 5-13 months (U test).

DISCUSSION

A — NATURE OF LESIONS INDUCED BY *M. martini*

Whatever the stage (larva, adult or microfilaria) and whatever the organ examined, major lesions belong to the inflammatory process, a common phenomenon, here induced by the presence of the parasite.

The control rodents also show inflammatory reactions, but these reactions are rare, of weak intensity, and of few types: lesions of type 1 and 5 dominate, while acute (type 2) and granulomatous lesions (type 4) are exceptional. The relationship between the lesions and the filariasis is based upon the higher number, type diversity, and intensity of lesions in parasitized rodents compared with controls; this is clearly confirmed by the scoring results of these two series of *L. striatus (Table V).*

In parasitized rodents, various lesions can co-exist within the same tissue area regardless of the organ. This feature demonstrates that filariasis is a chronic disease, developing over a length of time in a relatively long time-lapse, associating recent lesions to old ones.

B — VISCERAL LESIONS

The mesentery, mesocolon, mediastinum, and lungs are the most frequently damaged organs. In the mesentery and

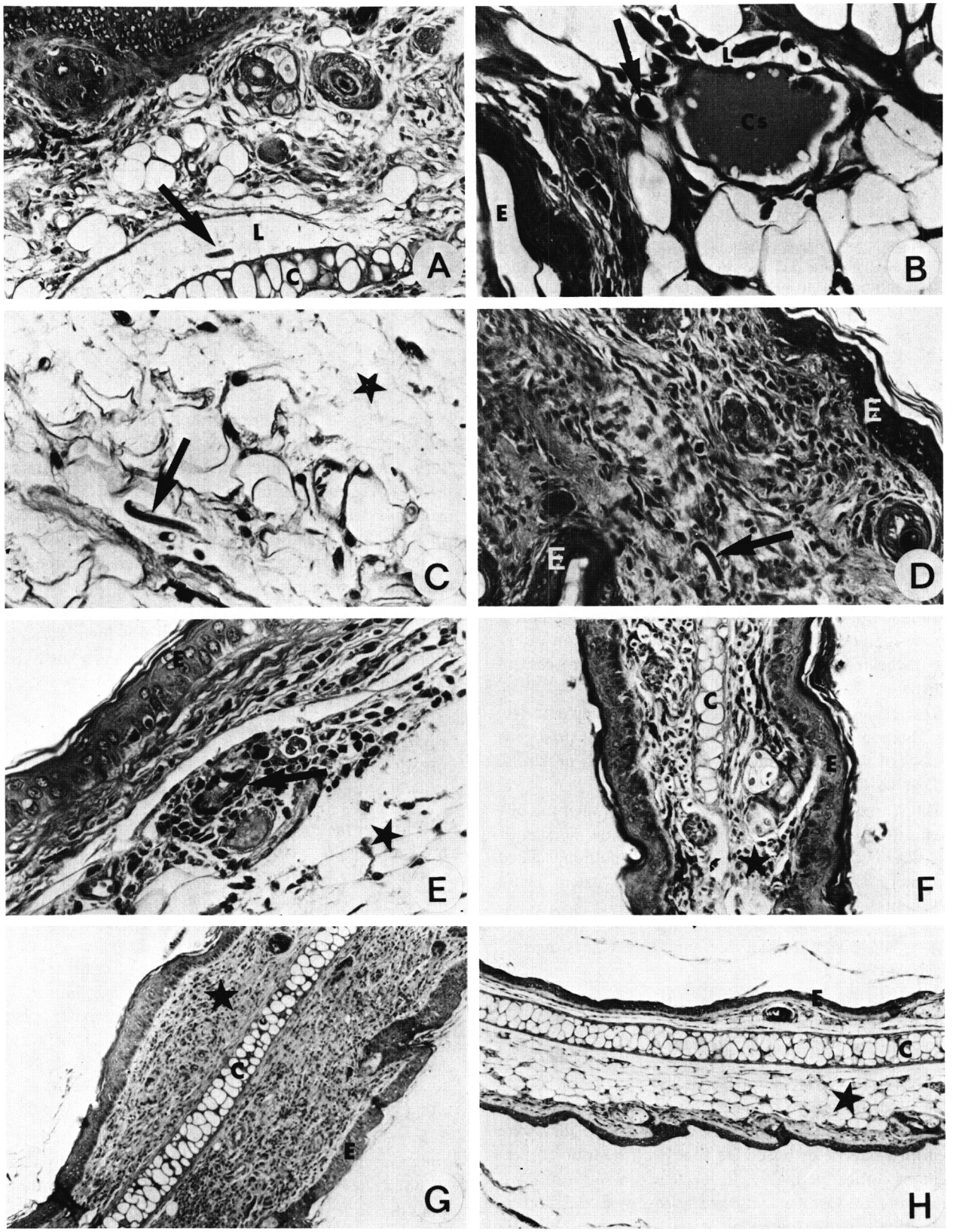

FIG. 3. — Microfilariae (→) and cutaneous lesions of the ear pinna in *L. striatus;* A: microfilaria in a lymphatic vessel (L) running alongside the auricular cartilage; B: *idem,* in a lymphatic vessel (L) alongside a blood capillary (Cs); C: extra-lymphatic microfilaria with a mild acute inflammatory reaction type 2; D: sub-acute inflammatory reaction type 3; E: microfilaria phagocytized by a giant multinucleated cell; F: coexistence of acute type 2 and sub-acute type 3 lesions; G: pseudo-tumoral aspect induced by profusion of foreign body granulomas type 4; H: ear pinna of a control rodent.

C: cartilage; E: epidermis; ★: dermis.

TABLE IV. — *Ocular lesions in* L. striatus.

L.s.	Intensity	1	2	3	4	5	HMono	HLym	E vas L	E cap S	Masto	Mela	Trau
PA	+	4 3.8	- 1.8	- 2.	- 0.2	2 3.4	- 0.2	- 0.2	- 0.2	1 0.9	- 0.6	- 1.5	- 2.2
	++	- 1.1	1 2.5	- 1.1	- -	- 2.9	- -	- 0.4	- 0.2	- 0.4	4 1.1	- 1.5	- 0.6
	+++	- -	- 0.2	- 0.2	- -	- -	- -	- -	- -	- -	- 1.1	- 0.2	- -
AI	+	3 6.8	- 1.8	- 1.3	- -	1 4.7	- -	- -	- 0.2	- 0.4	- -	- 0.6	- 0.6
	++	- -	- 1.1	- 0.6	- -	- 3.1	- -	- -	- -	- 0.2	- -	- 0.6	- -
	+++	- -	- -	- 0.2	- -	- 0.2	- -	- -	- -	- -	- 0.4	- -	- -
CR	+	- 0.4	- 0.9	- 0.4	- -	- 1.3	- -	- -	- -	- 0.6	- 0.2	- 0.9	- 0.2
	++	- -	- 0.9	- -	- -	- 1.5	- -	- -	- -	- -	- 0.2	- 0.2	- -
	+++	- -	- -	- -	- -	- 0.2	- -	- -	- -	- -	- -	- -	- -
RE	+	- -	- -	- -	- -	- -	- -	- -	- -	1 7	- -	- -	- -
SC	+	- -	- -	- -	- -	- -	- -	- -	- -	1 -	- -	- -	- -
	+++	- -	- -	- 0.2	- -	- -	- -	- -	- -	- -	- -	- -	- -

The sum of the elementary lesions in the ten control rodents is represented by the left figure, and the sum of the elementary lesions of the 35 inoculated rodents (divided by 3,5) by the right figure.
Abbreviations: PA : eyelid; SC : sclerotic; others : see *Tables I, II* and *III.*

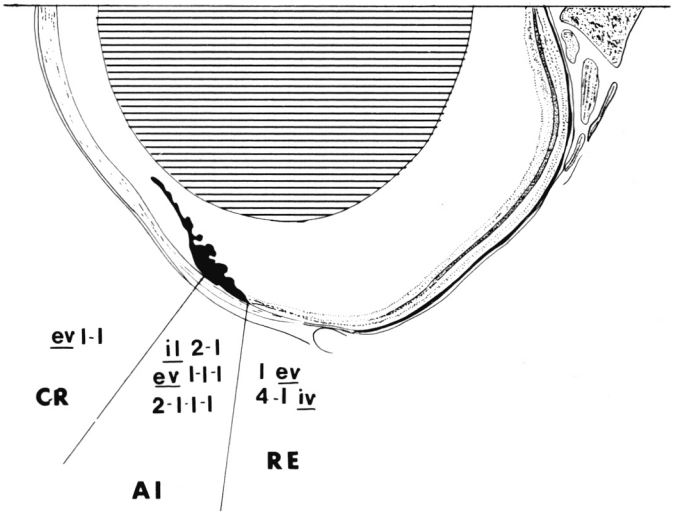

FIG. 4. — Diagram of antero-posterior section of an eyeball of *L. striatus* indicating location of intra-ocular microfilariae. For each location, each figure represents one rodent; its value expresses the number of microfilariae.
Al: irido-corneal angle; CR: cornea; RE: retina; *il*: intra-lymphatic microfilaria; *iv*: intra-vascular microfilaria; *ev*: extra-vascular microfilaria.

the mesocolon, migration of larval stages 3 and 4 provokes severe inflammatory lesions; their presence within lymphatic vessels of the mesentery induces thrombolymphangitis. These data are similar to those observed by Schacher and Sahyoun (1967) with *Brugia pahangi* in the dog, although *B. pahangi* lesions appear to be linked with the third molt, while in *M. martini,* they begin earlier, at day 7.

No reaction of similar intensity has been observed with adult filariae, whichever the method of inoculation (mono-inoculation with 30 to 200 L3, bi- or multi-inoculation). This phenomenon seems to illustrate notions currently admitted by immunologists: filarial camouflage, decrease of the host's immunological response.

TABLE V. — *Scoring results of the cutaneous and ocular lesions in* L. striatus.

		Contr 2-27	Inoc 5-36	mono 5-13	mono 15-17	mono 20-27	multi 7-17	bi* 15-36
cut.	n L.s.	10	35	13	7	5	5	5
	mf	0	+	100	40	+	100	++
	S.R.	**3,4**	**8**	**7,8**	**8,3**	**9**	**7,2**	**8**
	S	3,3	4,9	3,8	5,1	5	6,8	7,2
ocu.	n L.s.	10	44	13	14	6	6	5
	mf	0	+	100	35	+	90	++
	S.R.	**0,5**	**5,1**	**3,7**	**5,8**	**8,16**	**3,6**	**5,6**
	S	0,7	4,9	4,3	5,1	5,9	2,8	6,5

S.R.: mean scoring result; S: standard deviation; other abbreviations: see previous tables.

The mild intensity of lesions associated with adult *M. martini* is contradictory with that described with *Brugia* in dog. Inflammatory lesions of lymphatic vessels could be at least partially secondary to the irritation of vascular wall by endless movements of *Brugia* filariae within the lumen of the lymphatic vessel, while the filariae of *M. martini* are not as motile but coiled and completely clog the vascular lumen.

In some rodents, larvae and microfilariae are found in the marginal or intermediate sinus of lymph nodes; their presence induces a lymphadenitis which may be acute or granulomatous with foreign body reaction. Damage of various structures of the lymphatic system, the starting point of an ailment of lymph circulation, may explain the mechanism of deep visceral lesions in elephantiasis.

In the lungs, the presence of larvae as well as of adults in the collateral of pulmonary arteries is not associated with villous endarteritis, thrombosis, or vasculitis. These changes are reported with *Dirofilaria immitis* in dogs (*cf.* Ducos de La Hitte, 1990). Extra-lymphatic microfilariae can induce inflammatory lesions. Penetration of microfilariae inside the lumen of the alveoli is associated with eosinophil alveolitis, resembling eosinophilic pulmonary syndrome.

Among so-called reactive lesions, mesothelial hyperplasia

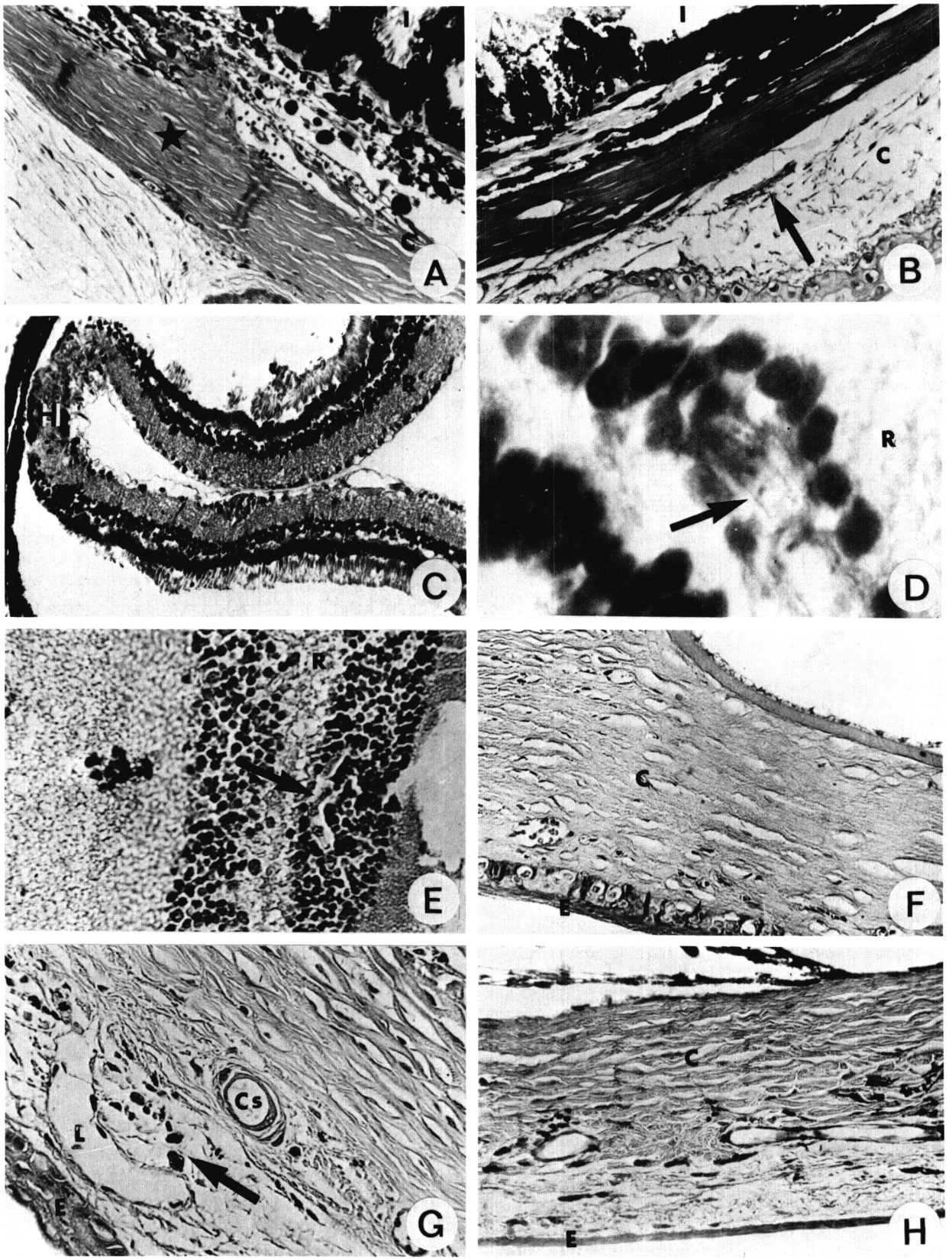

FIG. 5. — Microfilariae (→) and ocular lesions in three species of inoculated rodents; A to E, *L. striatus;* A: sub-acute inflammatory reaction type 3 associated with infiltration of mast cells and of melanophages in the irido-corneal angle (★); B: extra-vascular microfilaria, in the peripheral rim of the cornea, associated with a vasculo-exsudative reaction type 2; C and D: retina demonstrating hemorrhage (H) next to a destroyed extra-vascular microfilaria (low and high power magnification, respectively in C and D); E: microfilaria inside a retinal vessel; F and G, *A. niloticus;* F: extra-vascular microfilaria in the corneal stroma and vasculo-exsudative reaction type 2; presence of an amorphous deposit thickening the blood capillary wall (Cs); G: cornea showing sclerotic changes; H: *M. unguiculatus,* cornea with chronic inflammatory reaction and sclerotic changes.

A: irido-corneal angle; C: cornea; E: corneal epithelium; I: iris; L: lymphatic vessel; R: retina.

is relatively common. It is due to circulation of parasites within the lymphatic channel, which criss-crosses the stroma of the serous linings, specially the mediastinum. In any case, this lesion is of a mild intensity as is mast cells infiltration, noted only in one third of parasitized rodents.

Other viscera and deep tissues (*e.g.* aponevrosis, brain, oesophagus, liver, spleen, adrenals, kidneys) do not present any appreciable lesions, in contrary to data with *Loa loa* for example (Duke, 1960) and several other filariae with blood microfilariae as cited, or described by Gantier *et al.* (1987).

C — Skin

Just after introduction of larvae, the inoculation site developed an acute inflammatory reaction, essentially vasculo-exudative with infiltration of neutrophils and eosinophils. This explains the destruction of 50 % of the larvae *(fig. 1A)* during the 48 hours following inoculation (Wanji *et al.*, 1990). The surviving larvae are those which can penetrate inside the superficial lymphatic channel; hence, they can escape the inflammatory process produced by the host *(fig. 1B)*.

Beyond this short period, most of the cutaneous lesions are induced by microfilariae. There is a parallel between the scarcity of microfilariae and the scarcity of lesions whatever the lesion type: lesions are few and of mild intensity in the dorsal skin, where the microfilariae are rare. In the ear pinna, where microfilaria are concentrated, lesions are so numerous and intense that the dermis is profoundly damaged *(fig. 3G)*. These lesions observed with *M. martini* are most similar to those described in human onchocerciasis (*cf.* Connor *et al.*, 1969, Buck, 1974, Bonucci *et al.*, 1979). Generally, moderate (+ +) or severe (+ + +) lesions of types 1, 3 and 4, with infiltration of mast cells, are characteristic of parasitized rodents.

Changes observed in non-inoculated control rodents are caused by scratching lesions (type 1) which, in the short or long term, give rise to sclerosis (type 5). Sometimes, sub-acute inflammatory lesions are induced by additional bacterial infection.

D — Ocular lesions

Data on the frequency of intra-ocular microfilariae, based upon the observation of one to four serial sections per eyeball, show:

— 19 % of *L. striatus* with one to four microfilariae in the anterior segment or in the retina. These rodents are mono-, bi- or multi-inoculated. Their microfiladermia ranges between 10 and 180/mm^2;

— one of the four *A. niloticus* with a microfilaria in the cornea, although microfiladermia is very low in this host species.

Lesions developing in the eyeballs are more frequent, and relatively important when compared to those observed in control rodents *(Table V)*. These lesions lead to scarring sclerosis which is localized principally in the irido-corneal angles. Sclerotic changes overflow onto the periphery of cornea and develop into sclerosing keratitis. In one rodent, exocytosis of neutrophils into the intermediate and superficial layers of corneal epithelium may be responsible for punctuate keratitis. No lesion of typical uveitis is demonstrated; but, the presence of melanophages inside the irido-corneal angle demonstrates that this structure has been the site of chronic irritation with a scattering of melanin pigment into the neighboring tissue. No lesion of vasculitis was observed in our material.

E — Tentative quantification of pathological data in *L. striatus*

Cutaneous lesions do not vary significantly between the various groups *(Table V)*, while ocular lesions of the mono-inoculated group with a long patent period is highest: 8,16. Our data are not sufficient to state that there is a difference between multi- and bi-inoculated groups (respectively 5,6 and 3,6). At present time, the data seem to indicate that the length of patent period has more effect on the gravity of lesions than do repeated inoculations.

F — Circulation of *M. martini* and pathogenesis

The combined analytic data demonstrate that inoculated larvae, filariae, and microfilariae do have a preference for the lymphatic system. Some parasites, however, present an erratic location in the bloodstream; this is probably due to the connection of the thoracic duct with the superior vena cava.

The present observations confirm our previous results (Vuong *et al.*, 1985 and 1986): *M. martini* microfilariae circulate from deep lymphatic channel (mesentery, thoracic duct, serous linings, irido-corneal angles...) into superficial lymphatic vessels (skin, ear pinna, muscle...). They concentrate in the lymphatic capillaries of the ear pinna where they are most frequently intravascular in position than extravascular. This feature is also confirmed with *Cercopithifilaria johnstoni* (Spratt and Haycock, 1988). The principal localization of *Onchocerca* microfilariae do not differ from these species, as suggested by Hissette (1932) and stated by Semadeni (1943), Bonucci *et al.*, (1979), Grundzig (1984), Vuong *et al.*, (1988).

Observed lesions in rodent eyeballs allow us to suppose that microfilariae enter this organ by the lymphatic capillaries of the irido-corneal angles. These lesions are similar to those described in human eyeballs (Hissette, 1932; Semadeni, 1944). Some microfilariae accidentally escape these capillaries and pass through either the corneal stroma, or

into the anterior or posterior segments of the eye, where they eventually die.

CONCLUSION

The difficulty of studying ocular lesions in humans and the cost of primate models make it necessary to resort to murid models. The advantage of *M. martini* filaria is that it achieves its complete cycle in a natural host, and that the pathology linked to various biological phases can be easily analyzed. The adult filariae only cause mild changes of the deep lymphatic vessels where they live, but larvae and microfilariae are responsible for lesions of the deep lymphatic system during their migrations. These lesions explain the pathogenesis of lymphadenitis observed in onchocerciasis.

In the ear-pinna where microfilariae concentrate, lesions of the dermis are common, and often severe. Nineteen per cent of the inoculated *L. striatus* show intra-ocular *M. martini* microfilariae — *i.e.* a prevalence similar to that found in human onchocerciasis, in the 5-9 year old groups (Anderson *et al.*, 1974) — and the ocular pathology of the inoculated rodents is at least three times higher than that of the control rodents.

In the skin and eyeball, lymphatic microfilariae trigger inflammatory processes which are localized and asynchronous, when they accidentally pass into the connective tissue. Cumulative lesions produce a chronic disease with scarring sclerosis, even in the corneal stroma, so that, as in onchocerciasis, the disease becomes more severe with time.

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