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Mémoire.

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ONCHOCERCA-LIKE LESIONS INDUCED BY THE FILARIOID NEMATODE *CERCOPITHIFILARIA JOHNSTONI,* IN ITS NATURAL HOSTS AND IN THE LABORATORY RAT[1]

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SUMMARY

Histo-pathological analysis of the eyes, ears, skin and associated skeletal muscles of the back of 14 animals infected with a filarioid worm with dermal microfilariae, *Cercopithifilaria johnstoni:* seven *Rattus fuscipes,* of which four were naturally infected, two marsupials, *Perameles nasuta* and *Isoodon macrourus,* and five *R. norvegicus.*

This filarioid nematode induces skin and eye lesions in all the infected animals. These lesions are similar to those described earlier with other filarioid species with dermal microfilariae, such as *Monanema martini* and *Onchocerca volvulus;* the pathogeny is similar: microfilariae live inside the lymphatic vessels and their accidental exit gives rise to a localized inflammatory reaction leading to fibrosis.

C. johnstoni is particularly interesting because it may be adapted to the laboratory rat, and because the ocular lesions are severe.

RÉSUMÉ : **Lésions de type onchocerquien induites par la filaire *Cercopithifilaria johnstoni* chez ses hôtes naturels et chez le rat blanc.**

Analyse anatomo-pathologique des yeux, des lobes auriculaires et de l'aponévrose dorsale de 14 animaux parasités par la filaire à microfilaires dermiques *Cercopithifilaria johnstoni :* 7 *Rattus fuscipes,* dont quatre naturellement infestés, 5 *R. norvegicus,* et deux marsupiaux, *Perameles nasuta* et *Isoodon macrourus.*

Comme pour toutes les filaires à microfilaires dermiques déjà étudiées et comme pour *O. volvulus,* l'espèce détermine chez tous les animaux parasités des lésions dermiques et oculaires. Ici encore, la pathogénie réside essentiellement dans le fait que les microfilaires, situées normalement dans les lymphatiques, déterminent lorsqu'elles en sortent un processus inflammatoire local puis une sclérose cicatricielle.

C. johnstoni est particulièrement intéressant parce qu'il est adaptable au rat blanc, et parce que les lésions oculaires sont intenses.

INTRODUCTION

Cercopithifilaria johnstoni (Mackerras, 1954), a filarioid nematode with dermal microfilariae, is a parasite of *Rattus fuscipes* and of marsupials in Australia. The life cycle was elucidated by Spratt and Haycock (1988) in ticks belonging to the genus *Ixodes.* These authors proposed this filarioid as a study model for onchocerciasis, as its microfilariae induce dermal lesions in its hosts and it successfully completes its cycle in the laboratory rat.

C. johnstoni was maintained in the laboratory in naturally and experimentally infected native hosts and in laboratory rats. Histo-pathological findings are described and compared with the lesions observed in onchocerciasis and in murids which have been infected experimentally with a filarioid *Monanema martini* Bain, Bartlett, Petit, 1986, with dermal microfilariae.

MATERIALS ET METHODS

Naturally infected *R. fuscipes* from the population described by Spratt and Haycock (1988) were used. Proce-

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dures for harvesting ticks from wild rats and their maintenance in culture in the laboratory were as described by those authors. Captive-bred bush rats *(R. fuscipes)* and a short-nosed bandicoot, *Isoodon macrourus,* and laboratory rats were used in experimental studies. Infection of mammalian hosts was effected by subcutaneous inoculation of third stage larvae dissected from *Ixodes trichosuri* or by permitting ticks to feed directly on the host. Patency was detected by taking a small skin snip (approximately 1 mm²) from the ears of rats or the dorsal midline of bandicoots, generally at weekly intervals, dissecting this in saline and examining it for emerging microfilariae of *C. johnstoni.* Infected animals (n = 14) were killed by intramuscular inoculation of pentobarbitone sodium (350 mg/ml). At post mortem examination, samples of skin and associated skeletal muscles, eyes and associated dermal tissues and ears were taken from infected animals — seven *R. fuscipes,* of which four were naturally infected, five *R. norvegicus,* one *P. nasuta* and one *I. macrourus* — and fixed in 10 % neutral buffered formalin. Parasitological data for these animals and the samples taken are given in *Table I.*

TABLE I. — *Hosts infected with* C. johnstoni, *parasitological data and fixed organs.*

N°	Host	L3	D		Mf		eye	ear	APO
1	I. macrourus	6	195	51	1	2	+	+	+
2	* P. nasuta	8	793	696	0	323	+	+	
3	R. fuscipes	nat.	?	?	70	70	+		
4	-	nat.	?	?	2	2	+		
5	* -	nat.	?	?	198	198	+	+	+
6	-	nat.	?	?	260	260	+	+	
7	* -	?	262	184	6	21	+	+	+
8	-	13	728	648	0	1	+	+	+
9	-	18	219	133	0	678	+	+	
10	R. norvegicus	45	223	18	0	2	+	+	
11	-	Ix.	379	210	0	4	+		
12	-	Ix.	459	328	0	17	+		+
13	-	15	182	82	3	9	+	+	
14	-	8	182	82	1	16	+	+	

No: number of animals. Host: species of host parasitized; *I: Isoodon; P : Perameles, R: Rattus.* L3: mode of infection; nat: natural; *Ix: Ixodes trichosuri*-transmitted; numbers: sub-cutaneous inoculation of a precise number of infective larvae. D: days between infection to fixation (left) and between infection to end of microfiladermia (right). Mf: skin microfilarial count per mm², at fixation (left) and maximum level (right). Eye: eye samples; Ear: pinna samples; APO: skin of dorsum and associated muscles.

The histo-pathological methods used were those described for *M. martini* (Vuong *et al.,* 1986 and 1991). For each tissue sample, one or two sections 5 μm thick were studied. The presence of parasites (adult nematodes and microfilariae) was noted and their densities per section expressed by the symbol (+): one + for 1-3 parasites, + + for 4-6 parasites, + + + for higher values. The lesions were identified and their intensities ranked on a scale of one + to three +. Each animal is represented by a file in which all the results have been documented; the files are held at the MNHN, Paris, number N 17066.

RESULTS

The lesions observed in the mammalian hosts belonged either to an inflammatory process or to the so-called « reactive » changes (Vuong *et al.,* 1991). The inflammatory lesions included 5 types: chronic non-specific (type 1), acute (type 2), subacute (type 3), granulomatous inflammation (type 4) and fibrosis (type 5). Reactive changes were various in type: dilation of lymphatic vessels, dilation of blood capillaries, mastcells infiltration, accumulation of melanophages, eosinophilic necrosis etc.

1. — APONEUROSE AND MUSCLES OF THE BACK

A — Adult nematodes and lesions

Adult nematodes were identified in the aponeurose of the back of two animals. In *R. fuscipes* No 7, two filarioid worms were observed, a live male residing in a cavity (the nature of which was difficult to identify) with mild sub-acute inflammatory lesion in the neighbouring tissue *(fig. 1 and 2);* a moribund or dead female, coated by fibrinous material, surrounded by fibrosis with inflammatory granulomas in areas in contact with the parasite *(fig. 3).*

In *R. norvegicus* No 13, two live filarioid nematodes were also observed: a single male located in a well-delimited space, possibly lymphatic *(fig. 4),* and a female with microfilariae, surrounded by acute and sub-acute inflammatory lesions.

B — Microfilariae and lesions

Small numbers of extra-lymphatic (el) or intra-lymphatic (il) microfilariae were identified in the dorsal aponeurose of the back and in the striated muscular tissue of *R. fuscipes* which had natural infections (No 3, 4, 5 and 6).

2 — SKIN

— *Microfilariae*

Microfilariae were observed in the intra-lymphatic or extra-lymphatic sites in the pinna of *R. fuscipes* No 5.

— *Lesions*

Acute inflammatory lesions, which may or may not be associated with extra-lymphatic microfilariae, and sub-acute lesions, were observed in *R. fuscipes.* In these animals, mast cells were numerous, often degranulated, and formed a coat around the blood and lymphatic vessels. Punctuate

fibrosis was marked, with numerous melanophages, in most *R. fuscipes (fig. 5)* and in the two bandicoots; in the latter, fibrosis was prominent and surrounded the dilated lymphatic vessels. It was less marked in *R. norvegicus.*

3 — EYES AND ASSOCIATED STRUCTURES *(Table II A and B)*

— *Microfilariae*

Microfilariae were found at the limbus: one in each of *Perameles nasuta* and *R. fuscipes* No 7 *(fig. 6)* and six in *R. fuscipes* No 5; in the cornea: 11 in *R. fuscipes* No 5 *(fig. 7);* in the eyelids: numerous in *R. fuscipes* No 5; microfilariae were concentrated in the stroma of the mucosal aspect of the eyelid *(fig. 8);* they were intra- or extra-lymphatic.

— *Lesions*

Inflammatory as well as reactive lesions were of variable types and intensities in the different host animals. They were particularly frequent and significant in the limbus. Acute inflammatory lesions, sometimes near an extra-lymphatic microfilariae, were mild to severe in *R. fuscipes (Table II A),* and were mild in *R. norvegicus (Table II B, fig. 9).* Fibrosis was common, particularly in *R. fuscipes* and in the two species of bandicoots, and moderately severe to mild in *R. norvegicus.* The principal reactive lesions were dilation of lymphatic and blood vessels, vascularization (especially in the marsupials) and infiltrations of melano-phages and mast cells *(R. fuscipes).*

Acute inflammatory lesions of the limbus frequently spread onto the periphery of the cornea. A sub-acute

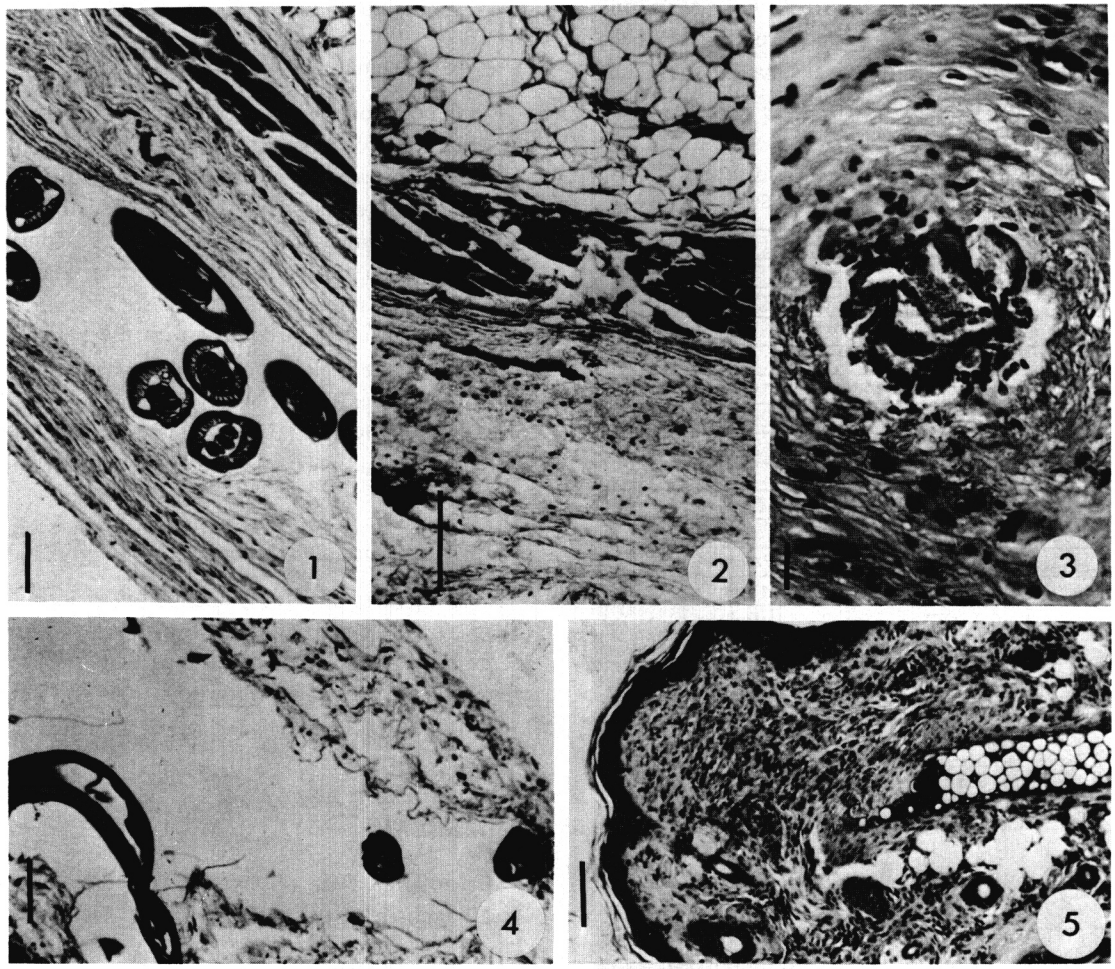

FIG. 1-4. — *Cercopithifilaria johnstoni* in the subcutaneous aponeurose of dorsum of rats. 1 to 3, in *Rattus fuscipes;* 1 — male in cavity of uncertain nature; 2 — tissue adjacent to that Fig. 1 showing sub-acute inflammatory lesions; 3 — dying female surrounded by a fibrinous coat with surrouding inflammatory and fibrotic tissue; 4 — in *Rattus norvegicus:* male in lymphatic cavity surrounded by acute and sub-acute inflammatory reactions (Hematein-Eosin-Saffron; bar = 100 μm).

FIG. 5. — *Cercopithifilaria johnstoni* in *Rattus fuscipes:* acute and sub-acute inflammatory lesions, and chronic lesions with fibrosis in the pinna (HES; bar = 100 μm).

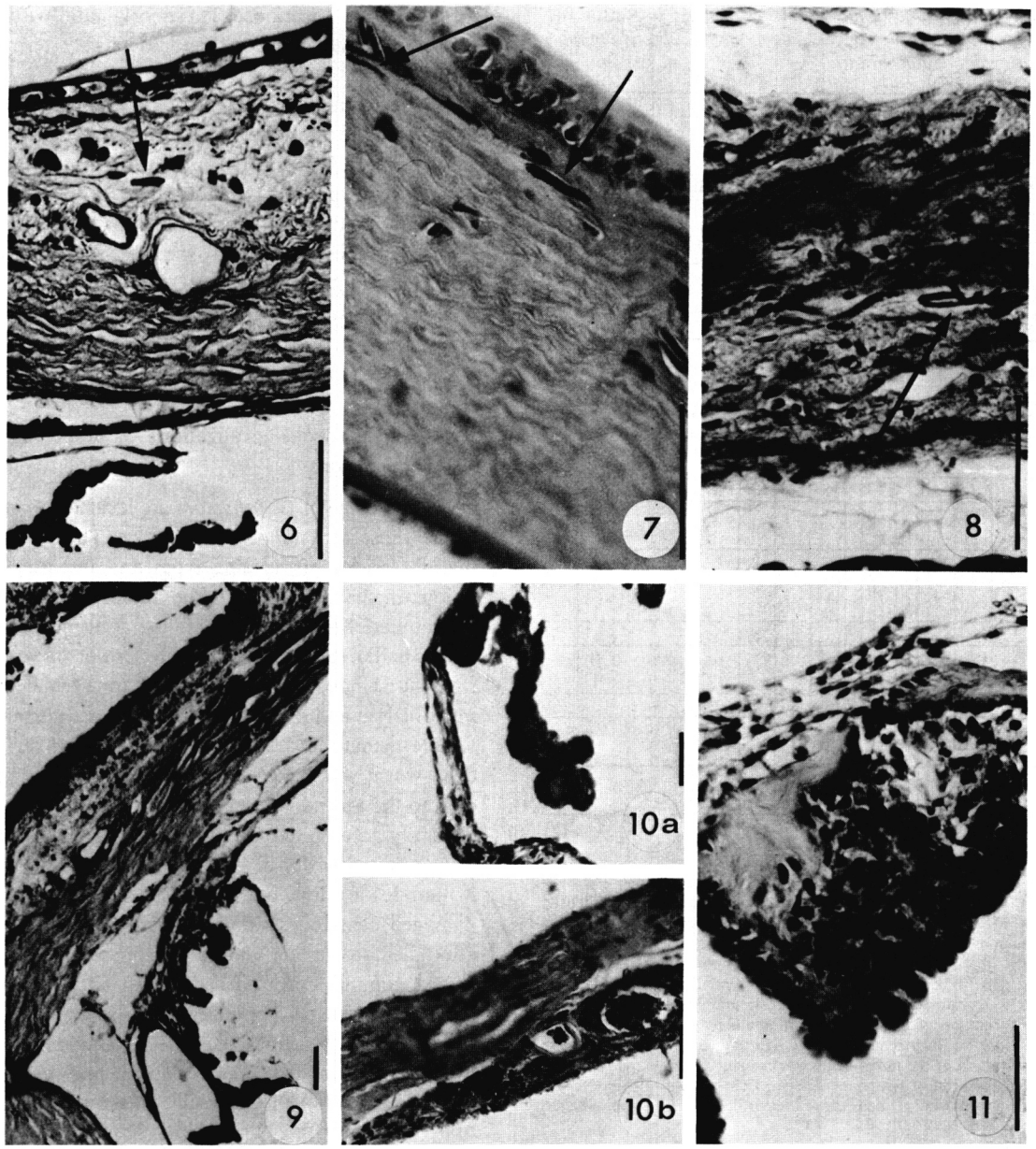

FIG. 6-8. — *Cercopithifilaria johnstoni* in *Rattus fuscipes;* 6: limbus containing an extra-lymphatic microfilaria with inflammatory reaction at contact points; 7: cornea showing microfilariae without inflammatory reactions at contact points; 8: microfilariae at mucosal aspect of eyelid with inflammation in the periphery (HES; bar = 50 μm).

FIG. 9-11. — *Cercopithifilaria johnstoni* in *Rattus norvegicus;* 9: sub-acute inflammatory lesions are predominant in the limbus; 10: absence of melanin pigment permits identification of inflammatory lesions of iris (a) and choroid (b); 11: nodular fibrosis at base of insertion of iris (HES; bar = 50 μm).

inflammatory lesion was observed in this tissue in *R. fuscipes* No 4. Corneal fibrosis was mild to severe in *R. fuscipes* and the bandicoots, and mild in *R. norvegicus.*

The absence of melanin pigment in laboratory rats facilitated identification of the sub-acute and acute inflammatory lesions, and of fibrosis in the iris and choroid *(fig. 10 a, b, and 11).* In the retina, dilation of the blood capillaries was common.

DISCUSSION AND CONCLUSION

Cercopithifilaria johnstoni induces dermal and ocular lesions in both its natural hosts and in the laboratory rat.

TABLE II. — *Ocular microfilariae and lesions. A: in* Rattus fuscipes *and B: in* Rattus norvegicus. *The numerals represent number of rats sustaining microfilariae or lesions in an organ.*

Org	Inten	Mfe	Mfi	1	2	3	4	5	HMo	HLy	EvL	EcS	Ma	Me
PA	+			1								1		
	++			2	2			1	1		1			1
	+++	1	1		1			1					2	1
AI	+			1	1			1			2			
	++	1	1	1	2			4		1	2		1	
	+++				1									
CR	+			1	1	1		1				2		
	++				1			1			1			
	+++		1		1			1						
RE	+											3		
SC	+							1				1		
	++							1						

A

Org	Inten	Mfe	Mfi	1	2	3	4	5	HMo	HLy	EvL	EcS	Ma
PA	+				1	1		1					2
	++			2	1								
AI	+			1	2			2			1	2	1
	++							3		1		2	
	+++											1	
CR	+							5					
RE	+											3	
	++											1	
SC	+							1					
CH	+					1							
	++					1							
IR	+				1	1							
	+++							1				1	

B

Org: organ; PA: eyelid; Al: limbus; CR: cornea;RE: retina; SC: sclerotic; Ch: choroid; IR: iris. Inten: density of microfilariae or intensity of lesions. Mfe: extra-vascular microfilaria; Mfi: intra-lymphatic microfilaria. Columns 1 to 5: the five types of inflammatory reaction; 1: chronic non-specific inflammation with infiltration of mononuclear lymphohistiocytic cells; 2: acute inflammation with essentially vasculo-exsudative components; 3: sub-acute inflammation; 4: granulomatous inflammation with formation of histiocytic granulomata or eosinophilic granulomata; 5: fibrosis. Further columns on right: types of reactive lesions; HMo: hyperplasia of the mono-histiocytic system; HLy: lymphoid hyperplasia; EvL: dilation of lymphatic vessels; EcS: dilation of blood capillaries; Ma: infiltration of mast cells; Me: infiltration of melanophages.

1 — PATHOLOGY INDUCED BY THE « DERMAL » MICROFILARIAE

The observed lesions, similar in four host species, are of the same nature as those described for other filarioid nematodes with dermal microfilariae: *M. martini* and *Cercopithifilaria roussilhoni* Bain, Petit and Chabaud, 1986 in the African porcupine, and the human parasite *Onchocerca volvulus* (cf. Vuong *et al.*, 1985).

The fundamental mechanism of onchocercal pathology previously proposed by Vuong *et al.* (1985) is subtantiated by these new observations. The dermal microfilariae live in the lymphatic vessels but they may, by accident, arrive in the perivascular connective tissue, and there they induce inflammatory reactions. These originate with an acute vasculo-exudative reaction and inevitably terminate with fibrosis.

The frequency and intensity of lesions in the limbus, as well as the presence of microfilariae at this site, confirm that this vascularized region of the eye is the fundamental pathway for the microfilariae to reach the eye, regardless of the location of the adult nematode. It is therefore not necessary to imply the presence of filarial nodules in proximity of the eye, as Duke did (1976) for human onchocerciasis.

2 — INTEREST OF *Cercopithifilaria johnstoni*

A — Sub-cutaneous localizations of adult filariae and lesions

Adult filariae of *C. johnstoni* are located sub-cutaneously, as are those of *Onchocerca volvulus*. They induce inflammatory lesions of variable types and intensity: sub-acute lesions in the connective tissue adjacent to a well-defined (? lymphatic) cavity containing the adult nematode (in this case a male), acute and sub-acute lesions of varying intensities surrounded by diffuse fibrosis (one female filaria with microfilariae and one male nematode) or dense fibrosis (one female nematode dead or moribund). The surface of the dead worm was covered by fibrinous material, corresponding to the Splendor-Hoeppli phenomenon (Johnson, 1976), generally interpreted as the parasite's camouflage.

The low intensity of the inflammatory lesions when the nematodes are live, and the absence of giant cells around damaged nematodes can be explained by the fact that the adult nematodes are motile to some extent.

The granular reaction around an adult nematode with fibrosis replicated to some degree the structure of an onchocercal nodule (Martinez-Baez, 1935; Buchard *et al.*, 1979; Büttner & Racz, 1983). *C. johnstoni* may help to understand the genesis of these nodules and to determine the eventual links with the lymphatic system.

B — Vasculitis and lymphangitis in the dermis

In the skin, the coatings of mastocytes which surround the blood and lymphatic vessels are similar to the vascular lesions (vasculitis) observed in patient with onchocerciasis (cf. Buck's review, 1974). We suggest that, with *C. johnstoni,* these lesions are in fact lymphangitis.

C — Ocular lesions

C. johnstoni is particulary interesting in view of the ocular lesions. The important acute inflammatory reactions in the mucosal aspect of the eyelid, linked to the presence of microfilariae, are the source of oozing and encrusting lesions leading to synechiae of the eyelids, reported by Spratt & Haycock (1988) in wild and laboratory-bred *R. fuscipes* infected with *C. johnstoni.*

The microfilariae lodged in the eyelids are in fact particularly well situated to gain access into the eyeball. The number and frequency of microfilariae in the eyeball (limbus and cornea) appear higher than those in the *M. martini* model, especially if one notes that six in 14 animals studied here had a negative microfiladermia at the time of post mortem examination and fixation of tissues *(Table I)*.

Finally, the opportunity of using the laboratory rat as an experimental host of *C. johnstoni* reveals the existence of lesions such as iriditis and choroiditis, which are probably frequent as in onchocerciasis (Hissette, 1932), but which are usually hidden by the presence of melanin pigments.

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