THE PATHOGENESIS AND PATHOLOGY OF OCULAR ONCHOCERCIASIS

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FRONTISPIECE

Ocular onchocerciasis; three blind men in the town of Nakong in North Ghana: one is also suffering from leprosy.

CONTENTS

P A R T I.

INTRODUCTION TO THE SUBJECT AND THE THESIS

This thesis will describe the results of the writer's investigations into the pathogenesis and pathology of ocular onchocerciasis in the interior of Africa during the period 1952 to 1956. In British territory this disease has only begun to be recognised since the last war. Now it is appreciated that the problem it presents is of the highest importance to those regions where its ravages occur, an area which embraces the greater part of the African Continent. The northern limit is defined by the Sahara, because of lack of water and the arid atmosphere; the southern limit reaches into Angola in the west and the upper reaches of the Limpopo in the east. The writer has had the opportunity of studying the disease in several selected areas between the Congo rapids and the River Niger, as far north as Timbuctoo, and westwards towards the Gulf of Guinea. For the greater part the work was carried out in Ghana, Nigeria and the Cameroons.

It is an indication of the size of the problem that Stoll, in 1947, estimated that 19 million people in Africa were suffering from onchocerciasis; in 1957 our estimate was double that number. There may be no less than half-a-million Africans blinded by this disease alone. In the territories in which we worked (i.e. in Commonwealth or British Colonial or Mandate

territory) there are approximately 350,000 blind from all causes; of these perhaps 20% have been blinded by onchocerciasis, or, as it has come to be known, 'river blindness'. This figure is high because the areas were for the greater part heavily endemic. The worst area of all was in North Ghana, where in a population of one million, we estimated that 600,000 people suffered from the disease, and 18,000 had been blinded by it. This should make the magnitude of the problem quite clear.

Onchocerciasis is a disease which results from the infestation of man by a filarial worm, Onchocerca volvulus Leuckart. In 1893 Leuckhart was sent samples extracted from subcutaneous nodules excised in the Gold Coast (as Ghana was then called) by an unnamed German missionary. The worm was called Filaria volvulus. It is interesting to note, nevertheless, that nearly twenty years before, in 1874, John O'Neill, an Irish surgeon on board H.M.S. Decoy, a frigate stationed off Cape Coast in Ghana, examined a piece of skin taken from a patient sent from the Addah Fort Hospital at the mouth of the Volta, and recognised the presence of microfilariae (mf.) in the dermis under the microscope. There is little difficulty in identifying them as mf. volvulus from the drawings in the Lancet of 1875.

Following Leuckart's description, however, it was the nodules with their contained adult worms that attracted attention.

Brumpt (1904) suggested Glossines and Simuliidae might be the cause of the nodules, and thus hit the target first time. In 1910, Railliet and Henry classified all the Onchocerca filariae, describing the species' characteristics; among them appeared Leuckart's Filaria Volvulus, given its full name for the first time. It was not until 1915 (publishing being delayed until 1919 because of the Great War) that the worm was discovered in the Americas by Robles. Brumpt (1919) believed this to be a new species which he called O. caecutiens (literally the 'blinding filaria'), an excellent choice of name based on the fact that Robles had been the first to associate blindness with the disease; he had even suggested two species of Simuliidae as vectors; this species of Onchocerca, however, turned out later to be identical with volvulus. Pacheco-Luna (1920) was the ophthalmic surgeon called in by Robles to investigate the ocular manifestations of the anterior segment in detail - just as he had called in Brumpt as a leading helminthologist to identify the worm. The aged Pacheco-Luna may be seen today sitting in his Paris garden puffing at his pipe, the last survivor of these South American pioneers. It is amazing to think that in Africa, where the worm had first been identified long before and where several million people were suffering from the disease, it was not until 12 years after Robles' pronouncements that Hissette (1931, 1932), a Belgian, showed that O. volvulus was an even greater cause of blindness in Africa than in Central America.

The first British worker on the scene was the Scot, Bryant (1935), who, in describing a posterior segmental lesion to Hissette when they met on furlough, was advised to look for mf. volvulus, for although Hissette at that time had not published any description of the chorioretinal changes, he had observed them, and suspected the diagnosis. Bryant promptly discovered onchocerciasis in the Sudan, and generously acknowledged the tip he had received from Hissette. He will always be known as the first to publish a description of the posterior condition. Meanwhile, in Sierra Leone, another British worker was carrying man's knowledge of this strange disease further. Blacklock (1926a,b, 1927) was the first to demonstrate that S. damnosum Theobald was the vector of the larval stage of O. volvulus in Africa. The descriptive terminology given this species of Simuliidae is a particularly happy one, so irritating is its bite. Hissette (1932) found that S. neavei Roubaud was a carrier in Central and East Africa, and Becquaert (1934) later identified three vectors in Central America, S. metallicum Bellardi, S. ochraceum Walker, and S. mooseri Dampf; the first is the most prolific vector. At the moment no other vector has definitely been recognised, as far as the writer knows, although many have been sought.

Hughes (1949) suggests that whilst European doctors were laboriously piecing together the picture of onchocerciasis,

the bush Africans in the endemic areas had formed a fairly accurate idea. Saunders (1929) mentions that in the Gold Coast of his day nodules on the head were associated with blindness by the Lobi-Dagartis. Dry (1921) found that in Kenya blindness and skin irritation were both claimed to be caused by the bite of black flies. Buckley (1949) learned that the inhabitants of an infected village in the Congo also associated blindness with biting flies. Waddy (1951), in Ghana, speaks about a chief who blamed the nodules for all ills, including sterility. With his usual dry wit, Waddy remarks of this man that despite an ample stock of worms he had managed to procreate twenty children! Maybe it is a bit too ingenuous of Hughes to praise the perspicacity of the bush-dwellers; our own experience was that the mass of people were ignorant of the cause and, indeed, even of the fact that the incidence of blindness among them was at all unusual.

After Pacheco-Luna's observations of 1920 several reports in Spanish on the relation of onchocerciasis to blindness were published in Central American journals. They did little to advance our knowledge of the condition, judging by the English summaries. The latter, of course, are often incomplete or incomprehensible. In Africa, after Hissette's exciting new observations, Strong and his Harvard team, who had been studying in Mexico and Guatemala, joined the Belgian in the Congo; no

ophthalmic surgeon had ever worked with Strong previously. As a result of their collaboration a combined monograph appeared in 1938. In this book, Hissette merely repeated himself.

No further interest by British workers in Africa appears to have been taken until Ridley (1945) spent a fortnight, during his war service in West Africa, in North Ghana studying the ocular manifestations. His highly competent and comprehensive monograph is at present the standard work in our own literature. It is remarkable considering the short time he had at his disposal, but then he was the first highly skilled modern ophthalmic surgeon to study onchocerciasis. This work resulted in a spate of activity among other British workers, the writer included. In this regard, Wilson, Director of the Royal Commonwealth Society for the Blind, and a member of the Royal Commission into Blindness in the Colonies (1948), was the driving force which led to my West African Ophthalmic Survey. A blind man himself, Wilson tramped all over West Africa asking questions. His conclusions have proved basically correct, but caused quite a furore at the time.

Despite the long start in Central America, up to 1930 nobody had reported on the pathology of the condition. Ochoterena (1927), in a paper purporting to cover the pathology of onchocerciasis, does not mention the eyes (1927). It was another three

years before he obtained his first eyeball, quickly followed by two others (1930). These eyes were excised after death. Although in the light of modern pathology they appear somewhat superficial, these papers were a start. Mf. were found in all tissues except the retina and optic nerve. It was Strong, working in Central America, who produced the first really sound paper, describing the association of the parasites in the eye with lesions of the conjunctiva, cornea and iris. This was published in 1934, just before Bryant described the posterior changes. Strong's observations were drawn from two eyeballs and eleven pieces of biopsy tissue. Hissette, in his early papers, discussed the pathology including the presence of mf., although no mention is made of them in the text. It is left to the reader to draw his own conclusions from the excellent photo-micrographs, which showed the parasites in the conjunctiva, cornea, iris and choroid. Hissette does not mention from how many eyes or biopsies he drew his conclusions. It seems to have been one only. The Belgian gives the impression of being a man of great imagination and talent, who unfortunately expresses himself badly in words. In the same year Giaquinto (Mira) reported finding mf. volvulus in the optic nerve. Up to 1934, therefore, apparently there had only been six or seven eyeballs examined, along with an unknown number of biopsies.

Bryant of the British workers first presented pathological observations, somewhat briefly in his paper and

rather more fully in a thesis (1935a,b). He told the writer that two eyes were sectioned, eight others being stored in a building subsequently blown up in the blitz. He purchased his material. Hughes obtained one eye at autopsy, which was the basis of his Oxford thesis. He remarked on the difficulty of persuading the African to undergo a surgical operation of this nature. Ridley also remarked on the difficulty of getting eyes; he was unlucky and got none. As we cannot find any other reference in the literature, it would seem that at a reasonable estimate the pathology of the ocular lesions of onchocerciasis is based on studies from about 10 or 12 eyes (3 by British workers) and a large number of biopsies. The writer can count himself fortunate to have obtained by persuasion 20 selected eyeballs and 80 pieces of biopsy tissue; all together every stage of every lesion is covered. Although a little of the material was subsequently discarded for various reasons which will be given later, there was still enough to provide ample scope for a comprehensive report. The eyes were excised under general anaesthesia, in the bush; biopsies involving keratoplasty, sclerectomy and iridectomy were carried out under local anaesthesia. The background to this surgery has been written up elsewhere (Rodger, 1958).

The pathogenesis of the ocular manifestations has generally been considered dependent upon the death of the mf. in

the affected structure. Early workers, like Robles (1919) and Calderon (1920), were inclined to believe that the eye symptoms were due to a toxin secreted by the adult worms. It was Strong (1934) who first declared emphatically that it was the mf. which led to the tissue reactions. The fact is this was only a hypothesis, based on circumstantial evidence, which is frequently a misleading premise in medicine and surgery. What is remarkable is the lack of interest in the mf. in the early Central American papers; O'Neill's observations in the Lancet went unnoticed for 50 years. Strong's views, therefore, were in a way revolutionary at the time. He was equally emphatic that the ocular manifestations were chronic in nature, citing his pathological material as evidence of this fact. As Hissette held the same views, and as these two men stood above most of their contemporaries, it is not surprising this opinion has held sway ever since. It may be that workers in the '30s' were handicapped by the lack of pathological material, none of which afforded evidence that an acute lesion existed. It is not easy to explain, nevertheless, why nobody other than the writer has ever reported seeing the acute phase, short though the duration of the attack may be. We have carried out iridectomies in several acute anterior uveitis cases, and found the iris tissue filled with the motile parasites. We have viewed mf. in the cornea with the slit lamp in eyes that were

so photophobic the lids had to be held forcibly apart. In the course of investigating treatment we have handled many dozens of acute onchocercal eyes, thereby refuting part of Strong's opinions. Fortune no doubt played a part in first sending the team which the writer led to what is perhaps the most heavily infested area in the whole world. Later, in other parts of the African interior, we went many months without seeing an acutely-infected eye. The importance of all this lies in stressing the vacuum which existed before the war in our knowledge of the disease, not only in regard to the pathogenesis and pathology, but even the symptomatology.

Although the exotoxin theory was generally abandoned in favour of Strong's final arguments that it was the dead bodies of the mf. which caused the inflammation, it did not necessarily mean that he was right*. There may be other factors. Rodhain (1949) is convinced that allergy plays a part, and Toulant (1953) supports this view, believing that a hyper-sensitive state is responsible for some of the ocular lesions, if not all. This, he claims, is why only some subjects are affected. These three possibilities exist, undoubtedly: a direct toxic effect resulting from the products of disintegration of the mf. bodies,

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* Strong made several other suggestions before fixing finally on the theory that it was the disintegrating bodies of the mf. which caused the trouble.

a circulating exotoxin emanating from adults or mf., or the induction of a hypersensitive state with which is bound the question of antibody formation. None of these a priori hypotheses can be ruled out, for they have neither been demonstrated experimentally, nor disproved. It is believed that this thesis advances our knowledge in this direction.

In the dissertation which follows a series of investigations into the problems posed above will be described. Three approaches to the subject have been taken: in Parts II and III the pathogenesis is discussed, largely in the light of animal experiments, based on a technique we evolved to isolate the mf. when alive; in Part IV the microscopic appearances of the various ocular lesions are given, each description being prefaced by the salient clinical and macroscopic features; finally, in Part V the threads are briefly drawn together. Numerical references are given at the end of each Part, except in the case of Part I, this introduction, where references are given by dates, a system that seems to be better suited to a chronological review of this kind. Photomicrographs are not referred to in the text, for we find this much less instructive than the method of placing them at the end in the form of an Appendix, where the story may be followed in pictures. Finally, there is a short index for the benefit of those who may use the University Library, in which it is hoped this thesis will rest.

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Repetition of his earlier work in English.

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Based on many clinical papers, reviewed disease and its possible pathogenesis, suggesting a hypersensitive state determined the onset in certain subjects only.

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P A R T II.

THE PATHOGENESIS

INTRODUCTION

Apart from a preliminary paper by the writer (Rodger[1]), there has been no attempt to investigate the pathogenesis of the ocular lesions experimentally. On the other hand early workers were interested in the hypersensitivity of the skin which occurs as a result of the death of the mf. in man. Rodhain and Dubois[2] described intradermal reactions which they induced but pointed out that it was a group reaction involving F. loa and W. bancrofti as well as O. volvulus. Toulant[3] and Rodhain[4] believed that hypersensitivity also caused the ocular lesions or at least some of them. That the skin of man becomes hypersensitive to the parasites seems at first sight likely. The immediate effect of killing massive numbers of mf. with diethylcarbamazine is, according to these workers, full evidence of this: pruritis, headache, arthralgia, oedema and a transitory eosinophilia, the fact that antihistamines ameliorate the symptoms, all support the contention. Nevertheless, the early experiments we carried out failed to demonstrate either actively or passively the presence of antibodies in infested human subjects in guinea pig and rabbit skin. A capillary response might have been observed if an intravenous dye had been used; this was not done so one cannot

conclude with confidence that an antigen-antibody reaction did not occur. On the other hand the findings might well be correct; there may be a species difference, so that man alone becomes hypersensitive. Whatever we conclude these early experiments were rather disappointing because they proved negative.

Every structure in the eye has revealed inflammation in association with the presence of the parasites in microscopic sections, as have all the ocular lesions except one. The latter, representing only about 5% of all cases of ocular onchocerciasis, is never associated with mf. in the posterior segment, and appears to have a different aetiology; its pathogenesis will be considered in the next Part of this thesis, separately. If mf. volvulus is present in 95% of the ocular manifestations of onchocerciasis, they are on the other hand frequently also present where no lesion exists. At one time or another various workers have remarked on this finding. Out of 600,000 people suffering from the disease in North Ghana, of whom roughly one-third had parasites within the eyes, only 18,000 had been blinded, and perhaps the same number affected to a lesser degree. High though this figure is, it is surprising it is not much higher. It is difficult to explain why only about one in twenty with mf. in the eyes is affected except in terms of a specifically acquired decreased sensitivity, or immunity. Inherent in the subject of immunity, of course, is the prospect of there also being in some patients

an increased sensitivity, as suspected by Rodhain and Toulant. Sulzberger[5] has defined the dual process of an increase and decrease in sensitivity as one whose component parts are mixed up together, accompanying or following one another in the same tissue or animal, and maybe even resulting from one and the same exposure to the same agent. It would not, therefore, be surprising if the results of any experimental investigation into the pathogenesis of ocular onchocerciasis were to prove contradictory and complex.

The theories around which we built the experiments now to be described can be briefly outlined here. Tests on lower animals, we know, do not necessarily prove the aetiological significance of a suspected human antigen. In passive transfer experiments, nevertheless, to demonstrate the presence of antibodies it is better to use animals rather than humans; the opposite is true in the case of active transfer. At a late stage in the work, we carried out on man some of the procedures earlier performed on animals. This was possible only when we had learned what dosages were safe, and had gained confidence in handling material about which nothing was known but a lot suspected. The problem was approached from three angles:

1. Local tissue hypersensitivity

Although onchocerciasis seems to induce a hypersensitive state in the skin of man, it need not effect the eye in the same way. Using foreign or exogenous antigens an ocular hypersensitivity

can be induced, as by egg albumen; if a local tissue hypersensitivity to volvulus protein were to develop in the eye, therefore, it would not be surprising. Clinically, apart from the fact that the low incidence of ocular crises suggests that the mechanisms of biological adaptation are at work, there is little with which to support or refute the hypothesis that a local tissue hypersensitivity occurs. The type of ocular lesion found is clinically, on the whole, nonspecific. Only the superficial punctate keratitis is suggestive of allergy (Rodger[6]). The histopathological evidence of allergic inflammation, as described in earlier papers, is just as unsatisfactory. It rests entirely on the presence of eosinophil leucocytes, which, as is commonly understood, are to be expected anyway wherever the body is invaded by parasites. Necrotising arteritis and focal necrosis, characteristic of a severe tissue allergy, were never seen. The pathology on the whole does nothing to encourage us to believe that the ocular lesions are those of an allergic inflammation.

2. Primary exogenous inflammation

This is the popular theory as to the pathogenesis. Such an inflammation might result from exotoxins secreted by the living adults or mf., or be liberated during the disintegration of their dead bodies. With a well-adapted parasite like volvulus one would not expect the living organisms to cause inflammation, or their pleasant way of life would be upset. There is enough

clinical evidence to suggest strongly that the living parasites, both adult and mf., do not induce an inflammatory response. The pathology on the other hand, as first claimed by Strong[7], indicates it is the dead mf. which cause the damage inasmuch as their bodies are seen in the midst of inflammatory cells; there is contradiction here, however, for in the skin, mf. have frequently been found unassociated with any inflammatory reaction. The explanation of this probably lies in the fact that Strong assumed the mf. he observed were dead; in fact no clear-cut picture has been presented which differentiates the living from the dead parasite microscopically, a deficiency which we have made good, as will be shown in Part IV.

3. Primary toxicity inducing either a state of sensitisation or of tolerance

Finally, the experiments were planned to allow of the possibility of a substance with a primary toxicity leading either to sensitisation or immunity on repeated application. Dinitrochlorbenzene is one such substance, which induces sensitisation in guinea pig or man only when applied to the skin in sufficient quantities to produce a toxic reaction. Examples of the opposite state of affairs (inducing tolerance) are every day occurrences. These are possibilities which can only be solved experimentally.

METHODS

1. Preparation of a microfilarial suspension

An area of skin found by repeated pinch biopsies to have a large mf. population is excised, portion by portion, drawing as little blood as possible, and placed in 25 ccs. of Ringer-Locke solution at room temperature for half an hour. Nearly all the mf. emerge from the skin during this time, and the pieces of skin and any red blood corpuscles present settle to the bottom. The clear fluid which contains most of the parasites is then decanted and centrifuged at 5,000 revolutions per minute for about 10 minutes. The supernatant contains most of the mf. still; up to 4,000 have been estimated in 25 ccs.; they can be kept alive for as long as 48 hours provided the temperature is not higher than body temperature. Before use 25,000 units of streptomycin are added to the mf. suspension and 25,000 units to the control solution, which consists of Ringer-Locke by itself. Streptomycin does not kill the parasites, nor does it affect the ocular tissues in the amounts injected, while keeping secondary infection under full control.

Counting chambers are now filled with the suspension and the number of parasites in each chamber up to a volume of 1 cc. are estimated. We made up counting chambers ourselves with perspex rings. Fluid is taken with a syringe from the middle of the suspension after inverting the bottle. If the parasites are

required dead the suspension is placed in ice for 48 hours. Although it has been reported that the mf. within onchocercomata flown to a laboratory in ice were alive several days later, ex nodule mf. collected in the manner described above do not survive even 24 hours in a refrigerator. We gave them 48 hours only for good measure.

2. Preparation of antigens

Four antigens were prepared from the mf. suspension:-

(a) the suspension was subjected to supersonic bombardment at a 1 megacycle frequency, 6 watt power output, with a 4 cm. diameter cell for a period of 15 minutes,

(b) the suspension was heated at 90°C for one hour,

(c) two volumes of acetone were added to one volume of suspension and left for 30 minutes at 4°C, after which the acetone was evaporated off in a vacuum,

(d) trichloracetic acid 2% was added to the suspension in equal volume, left for 30 minutes at 4°C, and then neutralised with sodium hydroxide at pH 7.

Streptomycin was added in the same proportion to each of the four antigens, which were then kept in ice until required.

3. Plan of experiments

Experiment 1: The effect of ocular injections of living and dead mf. and of the four antigens in rabbit eye.

Experiment 2: The active and passive transfer of hypothetical antibodies to guinea pig and rabbit eye.

Experiment 3: The effect of subconjunctival injections of dead mf. in blind human subjects not suffering from onchocerciasis,

or suffering from onchocerciasis without ocular invasion, or suffering from onchocerciasis with ocular invasion but without an ocular lesion.

No experiment was carried out on onchocerciasis subjects with ocular invasion and ocular lesions, as this was considered too hazardous.

RESULTS

Experiment 1

a. The effect of living mf. on the eye

No. mf. injected	Subsequent history of mf.	Structure involved	No. of animals	Onset in hours	Effects	Sequelae
10	None seen after first day in 2 animals; doubtfully once or twice owing to flare in third.	Ant.chamb. and iris	3	48	Slight aqueous flare, cotton-wool exudates at pupil, lasting 3 days.	Total absorption in 2 animals and persistent tag of exudate in 1. Killed at 1, 2 & 3 weeks.
12 (repeated once)	One living and one dying mf. seen in same conj. biopsy.	Subconj.	3	24-36	Local hyperaemia, slight grey opacity of adjacent corneal margin.	Complete resolution 2-3 days later. No sequelae. All killed 6 days after repeat.
20	3 motile mf. seen daily for 5 days; a single motile mf. on 12th day; after this doubtful.	Anterior chamber	1	120	Transient flare lasting less than 2 days.	No sequelae. Killed after 3 weeks.

b. The effect of dead mf. on the eye

6	Nil	Subconj.	6	2	Severe bulbar hyperaemia, then neovasc. of adjacent corneal sector with grey opacity. Chemosis limbus.	Resolution started 7th day and took another week.
6	Nil	Subconj.	Same group of 6, 20 days later.	2	As above	As above, then killed, i.e. 7 days after 2nd injection.

No. mf. injected	Subsequent history of mf.	Structure involved	No. of animals	Onset in hours	Effects	Sequelae
6 (repeated at 7-day intervals 8 times)	Dead mf. in conj. biopsy on two occasions.	Subconj.	2	2 (each time)	As above, only after 4th inj. less reaction; lid oedema marked; last inj. did not cause reaction.	Chronic bulbar hyperaemia and chemosis of lids after 2 months, when killed.
12-20 (varied in each animal)	Fragments in sections.	Iris	5	1-2	Aqueous flare & fiery hyperaemia of bulb. Cotton wool exudates covering pupil; in 4 out of 5 part tumbled into Ant.chamb. Iris blood vessels engorged; slight chemosis of lids. All above at height in 7 days; healing took another 3-4 wks, when 4 killed. One rabbit killed at height of reaction.	Resolution began after 9 days. Exudates organised, pupil membranes shrank & split. At 21st day pupil cleared in 3 but with residual exudate at margin(rolled border) or depigmented fringe; in 2 ant.syn. & in 1 post.synechia; 1 eye had persistent occluding membrane. Where pupil clear ant. lens capsule showed colour pattern, lent. opacities & free pigment granules. All eyes(4) now quiet. Control eyes very slight exudate lasted 3-4 days & then became absorbed.
2-3 (repeated in 2 rabbits after 5 days)	Fragments in sections.	Corneal stroma	4	immediate	White opacity & neovascularisation from limbus. Bulbar hyperaemia. One killed on 3rd day.	Part resolution in other rabbits after 2-3 weeks, leaving corneal macula. Control corneae opaque for 2-3 days only, & left no residuum. Killed about 21st day.

No. mf. injected	Subsequent History of mf.	Structure involved	No. of animals	Onset in hours	Effects	Sequelae
50	Fragments in sections	Retro-bulbar	2	Nil	No clinical change after 1 week when rabbit killed.	See 'Microscopic appearances' in this Part of the thesis.
250 (Approx.) Weekly for two months.	Nil	Sub-cutaneous	4	Nil	Attempt here to produce chorio-retinal changes was unsuccessful.	No conclusions reached. Inserted for record.

c. The effect of the four antigens on the eye

The experiments just described were carried out using a simple suspension of living or dead mf. mixed with streptomycin. The experiments with dead mf. were now repeated using the four antigens. Where the bodies of the parasites had been subjected to bombardment, the results were similar to those described above. In the case of the second antigen the reaction was slight; in the third and fourth the results were inconclusive by comparison with the control eyes. Heat, acetone and trichloracetic acid seem to destroy the toxin produced as a result of the disintegration of the mf. bodies, which leads to inflammation when not so treated.

Experiment 2

a. Active transfer of hypothetical antibodies

Each of the antigens in turn was tested on a pair of animals in a guinea pig and rabbit series, that is 16 animals in all were used. Subcutaneous injections of the antigen being tested were given from 15-25 days before the exciting dose was administered. The latter was given subconjunctivally in the guinea pig eye and subconjunctivally and into the iris in the rabbit eye. A state of hypersensitivity could not be elicited. In addition, the qualitative response to an equal number of dead mf. in the sensitised animal was identical to that in the non-sensitised. The recovery periods were also roughly the same. When the sensitising and exciting doses were both administered locally into the ocular tissues the same negative results were obtained. There can be no question that neither with the repeated 'sensitising' injections, as described in Experiment 1, nor with the single 'sensitising' subcutaneous or intraocular injections described here, did an 'exciting' dose in the eye produce an allergic reaction. There was, in other words, no suggestion at any time that a hypersensitive state existed.

b. Passive transfer of hypothetical antibodies

The purpose of this experiment, as the last, was to demonstrate the presence of an antigen-antibody system in human onchocerciasis. On the assumption that heavily-infested patients

had a high antibody content in the blood, it was hoped to inject into the eye 0.05 ml. of serum from such patients mixed with equal volumes of the four antigens. Subconjunctival injections of the serum alone, however, resulted in a violent inflammatory reaction in the rabbit. It was obvious that this procedure could not be used, human serum itself acting as a foreign protein. Rabbits we sensitised to human serum died of anaphylactic shock. Guinea fowl egg albumen produced an exudative iritis similar to that following injection of human serum into the iris. In an earlier paper[1] passive transfer of human antibodies using this technique could not be demonstrated in guinea pig or rabbit skin.

Experiment 3

a. Effect of dead mf. on the eye of man not suffering from onchocerciasis

For this experiment fresh suspensions of dead mf. were used. About 45 dead parasites were injected under the conjunctiva at 6 o'clock in the case of 2 volunteers, one blinded by cataract, the other by optic atrophy (positive Ide test). Neither suffered from onchocerciasis or the bloodborne filarial diseases. Within two hours a very violent bulbar hyperaemia had developed, and the palpebral conjunctiva was like red velvet. This reaction reached its peak at the end of 24 hours, and then began to resolve. A crescent of white corneal opacification developed at the site of the injection. It took six days for the inflammation to subside.

Prepared as we were for what was likely to happen, by having previously completed our animal experiments and graded the dose carefully, nevertheless, the dramatic onset of this violent reaction in the first patient was rather frightening. No harm was done, however, and the patients were subsequently handed over to hospital for treatment of their diseases and benefited in the long run. This was done in the case of all the human volunteers.

b. Effect of dead mf. on the eye of man suffering from onchocerciasis but without ocular invasion

Only one subject, blinded by glaucoma, was selected. Following the subconjunctival injection of about 45 dead mf. from the same suspension, a less violent reaction of the bulbar conjunctiva occurred compared with the two previous cases. The hyperaemia took two days to develop and disappeared in a week.

c. Effect of dead mf. on the eye of man suffering from onchocerciasis and with ocular invasion but without an ocular lesion

There were three subjects, all blinded by senile cataract. The same procedure was carried out as before, using the same mf. suspension. There was absolutely no reaction in any of the cases. This was repeated in one case immediately, but the eye still remained white. The dead mf. which led to such a dramatic inflammatory reaction in the other subjects had no effect on this type of case.

d. Effect of dead mf. on the skin of man

This was carried out on two subjects: one with an I.D.F. of 31 and the other who did not suffer from onchocerciasis or any other filarial infestation. In both, about 50 dead mf. were injected subcutaneously into the thigh, and the control solution placed in the opposite leg. There was a moderate inflammatory reaction in each patient, slightly more severe than that in the control limbs, and approximately the same in both subjects. These results, to be included in another paper, are inserted here for two reasons: first, the immunity or partial immunity of the eye which Experiment 3 appears to demonstrate does not apparently occur in the skin; it is in short probably a local tissue immunity. Second, the reaction of the skin need not necessarily be an allergic one; it too may be the result of a primary toxic effect, as is illustrated in the case of the second of these two subjects.

MICROSCOPIC APPEARANCES OF THE EXPERIMENTAL EYES

The experiments just described gave rise to lesions clinically identical with most of the ocular manifestations of onchocerciasis seen in man; the limbitis, sclerosing keratitis, keratouveitis and anterior uveitis were clearly demonstrated. We were unable to reproduce the punctate keratitis, for where only one or two dead mf. were placed in the cornea the resultant opacity was much bigger than those appearing in a punctate keratitis. We did not attempt to place dead mf. in the choroid, retina or optic nerve, although we did produce by retrobulbar injection what proved on autopsy to be a perineuritis; but there was nothing in the clinical picture to associate this with what we observed in man.

The eyeballs were embedded in celloidin and stained with haematoxylin and eosin. The microscopic appearances in the first paragraphs below are given under the clinical heads of the ocular lesions so they may be compared the more readily with the descriptions of the human pathology reported in Part IV. There is one important difference in the two groups, which must be underlined at the start. The animal eyes for the greater part (but not entirely) represent the acute stages of the disease in the different structures of the eye, while the human represent the subacute or the chronic.

1. Primary exogenous inflammation around dead parasites

a. Limbitis

The tissue in the region of the limbus where subconjunctival injections of dead mf. had been made, was markedly oedematous and showed a diffuse infiltration in which eosinophil leucocytes and lymphocytes were present. The infiltration extended into the superficial half of the corneal stroma for a few mms. and also in a posterior direction into the episcleral tissue and even into the sheaths of the extra-ocular muscles. The advancing edge of the corneal infiltration consisted mainly of eosinophil leucocytes, and the perivascular infiltration of the conjunctival vessels at the limbus was derived from the lymphocyte element. This no doubt explains why the resolution of the corneal opacification in the lightly affected case was complete. The iris was quiet and unaffected. Invasion of the cornea by small capillaries, accompanied by the usual cells, more particularly in repeated infections, occurred, the new vessels passing under the epithelium and above Bowman's zone. There is, of course, no Bowman's membrane in the rabbit.

b. Sclerosing keratitis

The cornea in the neighbourhood of the dead mf. placed in the stroma revealed peripheral vascularisation and an organising subacute keratitis. The dominant cells were eosinophil leucocytes and lymphocytes among which a few plasma cells were present. The eosinophils were present in large numbers at the advancing edge of

the pannus beyond the foremost vessel. The latter lay close under the epithelium; fibroblasts were also seen passing along with the vessels from the limbal area; it was not possible to say whether they were corneal corpuscles or scleral fibroblasts which had invaded the cornea. There was marked congestion and perivascular infiltration of the limbal blood vessels outside the cornea, some of which had become occluded as a result of the endothelial swelling.

c. Anterior uveitis

Involvement of the corneal margin in the neighbourhood of the needle puncture was not altogether due to trauma by comparison with the control eye. It may be that the fluid of the mf. suspension contained some toxic products of the mf. within it, and that that caused the keratitis which developed around the track of the needle. The main effect, however, as was intended, was seen in the anterior uvea, in the iris and ciliary body, especially the former into which mf. had been injected.

The affected part of the cornea reflected to a minor degree the picture seen in sclerosing keratitis, and may be quickly dismissed. The vessels of the limbal circle were very congested and cuffed with inflammatory cells. There was also constant involvement of the ciliary body, although in none of these eyes had mf. been placed in that structure. This was more than vascular congestion; plasma cells and an occasional nodule of lymphocytes in the ciliary body were seen in some eyes. One assumes that toxins had diffused there from the iris.

The iris itself was severely affected. The chromatophores of the stroma migrated in large numbers to the anterior face and there formed a solid band. The posterior epithelium became swollen and darker. There was an infiltration of eosinophil leucocytes with some lymphocytes, plasma cells and an occasional monocyte also present. The endothelium of the vessels had swollen and in places they were occluded. The space normally present in the iris arteries could not readily be distinguished.

Finally, an inflammatory exudate was present not only within the iris but also in the anterior, and sometimes the posterior, chamber; occasionally exudate lay on the surface of the anterior part of the retina. A cyclitic membrane was well-formed in some. The filtration angle of each of the eyes, although to varying degrees, was encircled with organising fibrinous exudate in which many inflammatory cells could be made out. Where the occluding membrane over the pupil persisted it was fully organised; posterior synechiae or ectropion of the pigment fringe were also noted. In two eyes the lenses showed early cortical opacities.

d. Optic perineuritis

The nerve itself was not affected, and appeared healthy. There was suspected congestion of the septal vessels. Inflammatory cells were present in abundance in the subarachnoid space, consisting for the greater part of lymphocytes. Eosinophil leucocytes were present in large numbers in the adjacent posterior ciliary vessels.

2. Possibility of inflammation being induced by exotoxins from living mf.

Where living mf. were placed under the conjunctiva, oedema and infiltration were slight and variable in degree and corneal involvement negligible. The total absence of eosinophil leucocytes was a feature. In these animals where living mf. were placed in the anterior chamber and iris, no inflammatory changes at all were observed in the sections other than a little albuminous exudate, probably post mortem in origin. As the changes, minor though they were, were significantly greater than those seen in the control eyes there must be some explanation for them. Either living mf. do secrete an exotoxin, or one or two died, perhaps, before the suspension was injected, or maybe their movements when up against a barrier, like the inner pupillary ring, led to irritation.

As not every eye gave evidence of these changes (slight aqueous flare, hyperaemia and exudation at the pupil), and as they disappeared within a few days of onset, we are inclined to believe that the last of the three explanations is the most probable.

3. Onset of tolerance with repeated infections

The subconjunctival tissues into which dead mf. were repeatedly injected, when finally examined, showed marked oedema and a diffuse chronic-inflammatory-cell infiltration, in which plasma cells dominated; eosinophil leucocytes and lymphocytes

were present but in decreased quantity. This series of animals was the first where the condition might be classified as chronic; as it turned out the microscopic appearances exactly corresponded with those we found later in man. The sector of cornea adjacent to the area of limbitis was vascularised and some chronic inflammatory cells and fibroblasts had invaded the superficial stroma. Some patches of interstitial keratitis were present, although mf. had not been placed in these areas and no sign of mf. fragments were visible. One assumes these patches arose from the diffusion of toxins from the primary focus. The anterior chamber contained a small amount of exudate, but that was all; the exudate was albuminous and there were no cells. Considering the great reaction and severe structural changes which result from a first injection of parasite bodies, the microscopic changes after repeated injections confirm the clinical evidence that the ocular tissues become locally immune, or partially so at least. The appearance of pannus in the interstitial layers of the cornea, where no mf. had been placed, was a new and interesting feature, and probably reflects what happens in the human eye. Several mf. in the process of dissolution were found in the subconjunctival tissue at the site of the last injection. The two rabbits were sacrificed 3-4 days after the last injection, at which time there was no change in the clinical appearance existing before that injection, namely a chronic mild irritation. The pathology supports this observation.

4. Possibility of allergy after sensitisation

With the first three or four injections into the eye we observed the same degree of inflammation. Being able to grade the dosage it was possible to assess the probable degree of the reactions, and to forecast them. Sensitisation by repeatedly injecting the parasites into the eye, as we have shown above, did not produce allergy (hypersensitivity). Hyposensitivity resulted instead. Sensitisation by administering the dead mf. as an antigen and then about 15 days later giving a shocking dose provided no evidence that a hypersensitive state could be created in this way any more than by repeated injections. The clinical and microscopic appearances were similar no matter whether it was the first, second, third or fourth injection divided in point of time by one, two or three weeks. The so-called shocking dose, given after the period of time usually prescribed for antibody formation, did not produce a reaction any more violent than did a second injection given within this period.

5. Changes in the control eyes

The effect of the Ringer-Locke-Streptomycin solution on the eye was slight and inconstant and usually disappeared in a couple of days. It consisted of a slight granular exudate in the anterior chamber, sometimes in the posterior also, and around the perforating wound, site of the original injection, a slight inflammatory infiltrate. Eosinophil leucocytes were noticeably

absent, and the infiltrate was strictly localised. Where the needle had penetrated the iris there might be a few inflammatory cells, or nothing at all. Slight vascular congestion at the limbus at the site of the limbal puncture was general, but quickly settled. The eyes excised several days after the initial operation (5 to 60 approximately) revealed no abnormality. As a result of these findings we have every confidence that the structural changes in the eye treated with mf. suspensions depended entirely on the presence of the parasite bodies.

DISCUSSION

There is no doubt that the clinical lesions produced in the animals in the experiments just described exactly represent those seen in man. We reproduced the limbitis, sclerosing keratitis, interstitial keratitis, and anterior uveitis in full detail. The acute phase is brief, and it seems that within a matter of one or at the most two weeks the picture of an established chronic ocular lesion presents itself. Thus the animal experiments afforded evidence of the most important kind of what we alone had previously concluded on clinical grounds, namely that an acute phase does occur in man, contrary to past opinion (Rodger[8]). To be able to observe the acute attack from its onset to its resolution by slit lamp and to note how complications arise, all fitting into the clinical pattern seen in man where they are revealed at different stages like a jig-saw puzzle, was most enlightening. With the knowledge that ocular onchocerciasis does lead to occlusion of the pupil, does cause exudate to drag the pupillary margin down, that the exudate does tend to resolve in whole or in part, and frequently leaves a 'rolled border' of organised tissue at the pupil, or if it completely resolves uncovers a depigmented pigment fringe with or without synechiae, with or without lenticular opacities, and so on, greatly strengthens one's confidence in making a diagnosis.

It is an experience to be recommended. For this reason alone, therefore, the experiments were worth while, although that was not their main purpose. We wanted to try and formulate the pathogenesis.

There seems little doubt that the essential factor is death of the mf. in the tissues concerned. The reaction is immediate and violent in an eye not previously infected. With repeated infections, it was clearly demonstrated the tissues become less and less sensitive. This state of tolerance appears to be a property best defined as a local immunity, and does not appear to occur in the skin of man. The opposite state of affairs, hypersensitivity of the eye to repeated or sensitising injections, we failed to demonstrate. Perhaps this claim should not be unduly pressed, because these latter experiments were not altogether comprehensive. More work must be done in the case of the human eye.

One thing that did emerge clearly was that it is the dead and not the living parasites which have a primary toxicity. It is not easy to explain the mild inflammatory reaction which occurred in some of the eyes into which living mf. had been injected. It may be, as we have suggested earlier, that the original suspension had been slightly contaminated with mf. toxin by one of them having died before the injection was administered, but that would not explain the delayed reaction which occurred in some of the animals. This reaction admittedly was slight and

transient, but it none the less requires an explanation. We do not accept it as evidence that the living mf. secrete an exotoxin. It is more probably due to mechanical irritation, such as might happen if a parasite became entangled in the stroma of the iris, or kept pushing away at the pupillary margin. One only needs to observe the activity of the parasites under the microscope in iris or corneal biopsies to accept this theory more readily. The fact that we did not always observe the mf. we had placed in the anterior chamber was at times disconcerting, but is fully in accord with our experiences in man, where eyes revealing no signs of parasites in the aqueous humour when subsequently examined microscopically revealed their presence. They appear to be photophobic, and hide behind the iris. The fact remains that they were observed at times. So, the evidence is quite strong that living mf. do not secrete an exotoxin. The technique holds out fascinating prospects for future work.

Probably the most important of all the experiments were those on man. The total absence of any reaction whatever when dead mf. were placed under the conjunctiva of patients suffering from onchocerciasis with positive conjunctival biopsies <u>but without ocular lesions</u> could not have been more striking, especially as we had previously demonstrated with similar dosages a particularly violent reaction in patients who were not suffering from onchocerciasis. There is no question here that the dead mf. have a primary toxicity in man, and that in some infected subjects an

immunity exists, however we may explain its formation. It is a pity we did not carry out any experiment in patients exhibiting some of the manifestations of ocular onchocerciasis, but the reader no doubt understands our reluctance to do so. It is impossible to be sure that a state of tolerance has arisen; it need not necessarily have been reached even in a blind onchocercal eye, so we argued; to produce an endophthalmitis leading to great pain and total destruction of the eye was a prospect unpleasant to contemplate and ethically indefensible; eyes with previous lesions might even be hypersensitive; we have no human evidence precluding this, although it seems unlikely.

The local ocular immunity demonstrated in man could not be explained by any of the animal experiments; in the latter we only demonstrated the development of such an immunity with repeated infections, each in itself potentially a destructive one. One assumes that it all depends upon the weight of the initial infections. The death of a single mf. in the eye might lead to little or no visible evidence of an inflammatory reaction ever having occurred, yet might lead to partial immunity; on the other hand, if, as in our experiments, a number of parasites happened to die in the eye simultaneously on the first occasion, then an ocular crisis would almost certainly result. This is given indirect support by the fact that we frequently noted in the course of routine examinations with the slit lamp the relation of living mf.

in conjunctival biopsies, or in the anterior chamber, with only very minor signs of disease; a common one was the presence of pigment granules on the anterior lens capsule in young persons, or of a small quiescent tongue of pannus onchocercosus. These are interesting conjectures, which cannot be proved until the toxic product of the dead mf., evidently destroyed by heat, acetone, and trichloracetic acid, is identified, isolated and tested further. That tolerance of the type we demonstrated in rabbits occurs in man, we have no doubts; we believe it to be widespread and common. Where intraocular mf. in a subject with an ocular lesion are killed by diethylcarbamazine, there is not always a recrudescence of symptoms. The literature is full of such apparent contradictions; here is the explanation - these patients have acquired a partial immunity.

The microscopic appearances of the experimental eyes supply a few other facts worth emphasizing. One is that the acute phase is characterised by the presence of eosinophil leucocytes. Their exact relationship to the dead mf. was better understood in the human material we studied, and will be given later in Part IV. Another point of importance is that the dissolution of the parasites is not associated with the presence of macrophages or giant cells, which is surprising, and warned us to look for other evidence of mf. dissolution when we came to examine the human eyes.

Another point is that the nature of the infiltrate changes rapidly from one in which eosinophil leucocytes dominate to one where plasma cells are present in great numbers. This confirms the belief that the acute phase is short. The development of pannus, hugging the undersurface of the epithelium, comparable to its characteristic position in the human eye, is rather difficult to understand. Why this preference for Bowman's zone? We had always supposed that the situation of pannus onchocercosus in man's eye between Bowman's membrane and the epithelium depended on the fact that the mf. preferentially passed in that direction as being the locus minimae resistentiae, and on dying there led to pannus formation. But here we placed dead mf. in the eye of the rabbit either subconjunctivally or into the corneal stroma, not necessarily just underneath the epithelium - the technique could not be as accurate as that; yet the new capillaries always passed between Bowman's zone and the epithelium. There may be some simple explanation of this phenomenon which has escaped our attention.

Another interesting feature which emerged from these studies of the histo-pathology was the presence of interstitial keratitis in areas deep to the site of the injection. As the site could be localised accurately by the corneal wound there can be no doubt of this. Our assumption that toxins emanating from the dead parasites diffuse through the cornea and lead to these localised patches of inflammation seems a reasonable

one. The same argument would explain the onset of a sclerosing keratitis following repeated attacks of experimental limbitis.

In all respects the microscopic appearances of the experimental eyes dovetail well with those found in the human, provided it is not forgotten that on the whole the animal eyes were excised during or at the end of the acute phase and the human at the beginning or during the chronic phase. Where we did obtain an acute eye in the human the pictures corresponded, the opposite also being true.

CONCLUSIONS

1. The inflammatory reaction in ocular onchocerciasis is a primary intoxication depending on the presence of dead mf. The unsensitised eye is highly susceptible.

2. Living mf. may cause slight irritation with a correspondingly slight and transient inflammation, probably when the local cytoarchitecture hampers them in their movements.

3. It is almost certain that the toxins diffuse from the primary focus to neighbouring tissues. Involvement of the interstitial corneal stroma and the ciliary body can only be explained in this way in the experimental eyes.

4. Some human eyes were found to be immune to the mf. toxin or toxins. Other eyes appeared to have a partial tolerance. It is suggested that local immunity can arise in the eye if the number of mf. which dies in the eye on the first few occasions is low. In eyes apparently immune there can usually be found evidence of a slight inflammatory reaction having occurred in the past, which lends support to this hypothesis. In support of the claim that an acquired partial tolerance occurs the usual clinical history of the disease - a few acute attacks becoming less frequent and less acute until inflammation is mild and chronic even in eyes still heavily invaded - fully supports this view.

5. Hypersensitivity to the mf. does not appear to occur. We were unable to demonstrate in experimental animals the presence of antibodies by active transfer. Intraocular passive transfer was impossible because human serum acts as a foreign protein.

6. It is generally believed that the skin becomes hypersensitive to mf. volvulus. There is no evidence, however, other than equivocal, to show that the reaction in this structure, supposedly sensitised, is any greater than it is in a primary infection. Signs attributed to allergy when a microfilaricide is given in dermal onchocerciasis might equally well occur as the result of a primary intoxication, especially when so many parasites are being simultaneously killed. Among the signs to which we refer are headache, arthralgia, raised temperature, reaction to antihistamines and even oedema. The transient eosinophilia which results cannot be used as evidence of allergy as we are dealing with a filarial infestation anyway.

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PART III.

THE POSTERIOR SEGMENTAL LESION*

*This is the only part of the thesis which has previously been published (Rodger, F.C., 1958, Brit. J. Ophthal., 42, 21). It is included so that all aspects of the pathogenesis may be considered together. It is a revised version of the original.

GENERAL INDICATIONS

The posterior lesion is the most interesting manifestation of onchocerciasis because it is the least understood. Bryant[1], after a conversation with the Belgian worker, Hissette, reached the conclusion that a progressive chorio-retinitis which he had seen in the Sudan was caused by onchocerciasis. This opinion, later supported by Hissette[2,3] and by Richet[4], has since been confirmed by the observations of many other workers. The current view when the writer started was that the cause of the condition is invasion of the choroid by the parasites and their death. Bryant[1] produced histological evidence of this. His belief was given some support by the finding of microfilariae in the choroid by Ochoterena[5] and Hissette[2]; one autopsy case with several mf. in or adjacent to this tissue was also reported by Hughes[6].

When we started our investigations into onchocerciasis in West Africa we visited first a very heavily endemic area in the Northern Territories of Ghana. There many examples of the posterior segmental lesion were found, and nearly all of them (as it was a heavily endemic area) in densely infested subjects. Subsequently, however, we surveyed Northern Nigeria, and here, in areas of light endemicity, the incidence was paradoxically higher. Having evolved by this time an index of the density of infection in the individual (Rodger and Brown[7]), the index figures were

compared in cases of anterior and posterior lesions in Nigeria. The results were extremely interesting. Whereas the mean Individual Density Figure for the anterior lesion was about 26, it was 11 for the type of posterior lesion under discussion; four cases had sterile skins and conjunctiva, and of these two had no nodules although the lesions were characteristic. This raised a problem of pathogenesis, for it no longer appeared so certain that the cause of the condition was an inflammation arising around the dead bodies of mf. in the tissues concerned. In the heavily endemic areas of Ghana we seldom saw the posterior lesion except in association with an anterior lesion, but in Nigeria this was far from being true; 9% of 165 recorded cases were associated with a sclerosing keratitis, 2% with a kerato-uveitis, and 9% with an anterior uveitis; 80%, in short, were not associated with anterior lesions. Another factor which suggested that there might be more to the pathogenesis than the accepted view concerned the average number of nodule sites in the different types of lesion. Where the posterior lesion occurred alone, the average number (nodule mean) was 0.9; when the posterior lesion was combined with the anterior segmental lesion in the same geographical areas the average was 2.0; with anterior segmental lesions alone, it was as great as 2.6. The possibility that another factor might be involved thus gained strength. This view was lent further support when it was observed that in high-forest areas (where onchocerciasis is common although not severe)

no posterior lesions were discovered, whereas in the Northern scrub, even in areas with equal density quotients, the posterior lesion was extremely common. For these reasons, therefore, it was concluded that although onchocerciasis plays some part in producing this lesion, as it has not been reported from places outside the endemic onchocerciasis areas, another factor or factors must be sought.

DISCUSSION OF CLINICAL FEATURES

The different diseases which bear some resemblance to the posterior degenerative lesion of onchocerciasis suggest that it is not easy to diagnose. Most workers have emphasized the variety of appearances of the lesion itself. With the recognition of an exogenous chorio-retinitis of onchocercal origin (Rodger[8]), distinct from the degenerative lesion, the diagnostic features become somewhat clearer. No author that we are aware of has so far attempted to discuss the different conflicting descriptions, and it would now seem worth while to do so briefly. It will be most useful when describing the pathology.

The description with which we are most in agreement is that of Ridley[9], who described the lesion in its advanced condition, with or without the corpuscular aggregation of retinal pigment at the periphery. Ridley does not emphasize sheathing of the retinal vessels, an observation on which Sarkies[10] and Toulant and Boithias[11] laid great stress. In our view it is not a specific sign of the disease, being more common in luetic conditions. Sarkies described what he considered to be an early case of the lesion in a schoolboy, as a very slight, patchy increase of pigmentation in the macular area associated with intervening lighter areas, suggesting that the sclera was shining through an atrophic retina. This seems rather slender evidence on which to diagnose the posterior segmental lesion. Sarkies went on to say that no

intermediate stage between that picture and the advanced appearance was seen, and added that only one of his advanced cases resembled the description given by Ridley. This is difficult to explain unless on the basis that confusion has existed as a result of the posterior exudative uveitis of onchocerciasis, common where Sarkies worked in Ghana in an area with a high density quotient. As this is a non-specific inflammatory uveitis, it bears only a slight similarity to what we have called the posterior degenerative lesion of onchocerciasis. Ridley's description is based on twelve cases he saw in a village called Funsi in Northern Ghana. This village is isolated, and the nutrition of the people very poor. We visited it seven years later and saw ten cases, eight with advanced degenerative lesions, and two inflammatory. At the advanced stage, the degenerative lesion, is, as Ridley said, typical. The appearance is that of a circumscribed choroidal sclerosis, with peripheral corpusculation occurring in some instances. This is not a hypothetical combination, as Ridley remarks. It was described by Duke-Elder[12], and later by Falls and Cotterman[13], in genetically determined lesions. The latter type of degeneration is extremely rare in the non-Moslem areas of Africa, however, if it occurs at all.

Hissette and Bryant also appear to have confused the inflammatory with the degenerative type. For example, both workers mention that plaques of choroidal atrophy revealing white sclera are often seen, whereas in our view this is rare in the

degenerative type. Hissette describes photophobia and lacrimation as part of the symptomatology, which is something we ourselves never observed. He also says that the lesion is a late symptom of onchocerciasis, whereas in our own cases it appeared to be the opposite, the most common age group being 20 to 30 years. Hissette finds difficulty in deciding whether cupping of the disc, which is frequent, is atrophic or glaucomatous, because in many cases the cupping exactly simulates the glaucomatous type. A secondary glaucoma was common in our cases of onchocercal exudative uveitis, but glaucomatous cupping was never seen in the degenerative lesion, although atrophic cupping was usual in long-standing cases. Hissette also remarks on the frequent incidence of colloid bodies, which we found to be more common in the inflammatory than in the generative type.

Toulant, Robineau, and Puyuelo[14] also appear to have been confused by the multiplicity and diversity of the lesions. We have never seen the retinal haemorrhages nor the colloid bodies which Toulant describes at the posterior pole save in the inflammatory condition. Nor is it clear what he means when he says that he has never noted the tapeto-retinal degeneration, so-called by Ridley, unless there is some confusion here in the nomenclature. This term well describes what is the most characteristic stage of the disease. Toulant and Boithias qualified this statement later by saying that a secondary retinitis pigmentosa is seen but is rare, only three cases having been

recorded in the course of several years' work; this is not our experience. One significant remark of theirs is that a juxtapapillary chorio-retinitis is a frequent finding. This is our own view, the lesion usually first appearing in such a position. Toulant and his colleagues, who have produced much fine work on this subject, disagree with Hissette over the symptomatology, and are in general agreement with other workers that it rests entirely on the presence of hemeralopia.

All these points suggest that the variable appearances of the lesion so emphasized by Hissette, Bryant and the others were due to a lack of understanding that we are dealing with two conditions. In fact, the former worker stated that he found it impossible to suggest a pathogenesis to cover the many aspects of the disease; he was not helped, of course, in that the pathological material obtainable at the time was extremely small. Both Hissette and Bryant, nevertheless, must be given every credit for being the first to describe the changes.

TABLE I

Average daily nutrient intake of adult male Nigerian peasants in four different areas, 1954 - 1956*

Group			A	B	C	D
Occupation			Farming and Fishing	Farming	Farming	Farming
Area			Banks of Lake Chad. Sahel savannah	Bunga-Ningi, Bauchi Prov. Sudan savannah	Langai, nr. Panyam. Plateau prov.	Mbanege, Ogoja. Rain forest
Lesions			No Onchocerciasis & no Posterior Lesion	Onchocerciasis and Posterior Lesion	Onchocerciasis and Posterior Lesion	Onchocerciasis but no Posterior Lesion
Calories			3,000	2,900	2,600	2,400
Protein	Animal	(g.)	29	11	15	3
	Vegetable	(g.)	78	84	81	62
Fat		(g.)	37	40	45	31
Calcium		(mg.)	1,390	880	612	640
Iron		(mg.)	56	37	30	23
Vitamin A		(i.u.)	1,200	4,240	4,100	11,900
Thiamine		(mg.)	3.6	2.9	2.3	2.1
Riboflavin		(mg.)	1.1	1.4	1.3	1.0
Nicotinic Acid		(mg.)	27	24	24	16
Ascorbic Acid	Uncooked	(mg.)	24	133	43	316
	Cooked	(mg.)	12	44	12	54

*These four areas correspond with those in which we worked. The cases quoted in Tables II and III come from Area C. The intakes were measured by methods described by Nicol (1956).

SOME NEW OBSERVATIONS RELEVANT TO THE PATHOGENESIS

The absence of the posterior degenerative lesion of onchocerciasis observed by us in West Africa in regions where the diet contains a large amount of vitamin A is believed to be highly significant (Table 1). An attempt was made to ascertain, both by assays and by clinical trials, whether vitamin A deficiency was implicated or not. In a rural community in the Plateau province of North Nigeria, where the intake of vitamin A was marginal, the sera of several cases were assayed. All the subjects came from neighbouring villages except the last (Serial No. 1,291). One patient had disciform degeneration and another had syphilitic chorio-retinitis. Table II shows that the two non-onchocerciasis cases had a marginal level of vitamin A, whereas only one of the posterior lesion cases (Serial No. 1,291) could be placed in that category. The last patient was examined at a different time of year, when vitamin A in plenty had reappeared in the diet, and this offers a ready explanation of the anomaly.

The situation at this point may be summarized as follows:

(1) The posterior segmental lesion is found in subjects with a low I.D.F. (mean I.D.F. 11), but is not found in non-onchocerciasis areas.

(2) Cases with the posterior lesion have a low nodule mean (0.9).

(3) The posterior lesion is not found in areas where an abundance of vitamin A is present in the diet.

TABLE II

Serial No.	Vitamin A (i.u./100 ml. plasma)	Lesion
773	88	Disciform degeneration of macula
829	30	P.S.L.
830	10	P.S.L.
831	10	P.S.L.
845	20	P.S.L.
872	25	P.S.L.
879	80	Syphilitic chorio-retinitis
883	45	P.S.L.
900	20	P.S.L.
1,029	42	P.S.L.
1,291	88	P.S.L.

A value of vitamin A below 70 i.u. per 100 ml. plasma is taken as sub-normal.

TABLE III

Serial No.	Visual Acuity						Lesion	Improvement in Visual Acuity
	Before Treatment		After One Week		After One Month			
	R	L	R	L	R	L		
772	H.M.	C.F.	6/36	6/36	6/36	6/12	P.S.L.	Yes
789	6/24	6/24	6/18	6/18	6/18	6/18	P.S.L.	Yes
829	6/60	6/60	6/24	6/12	6/12	6/12	P.S.L.	Yes
830	H.M.	H.M.	6/60	6/60	6/60	6/60	P.S.L.	Yes
831	C.F.	C.F.	No change				P.S.L.	No
837	6/9	6/9	No change		6/12	6/12	Syphilitic neuro-retinitis	Worse
845	H.M.	H.M.	6/60	6/60	6/60	6/60	P.S.L.	Yes
924	6/36	H.M.	No change				Disciform degeneration of macula	No
930	H.M.	H.M.	No change				Disciform degeneration of macula	No
1,291	6/60	6/60	6/36	6/18	6/18	6/18	P.S.L.	Yes
1,306	6/36	H.M.	No change		Did not return		P.S.L.	No
1,338	6/36	6/36	6/9	6/6	6/6	6/6	P.S.L.	Yes

Treatment consisted of 165,000 i.u. vitamin A daily, prescribed as 5 capsules Crooke's vitamin A.

(4) It was established in a group of subjects in an area where the intake of vitamin A was (on European standards) marginal, that cases exhibiting the lesion had a lower quantity of vitamin A in the plasma than might be expected.

It seemed reasonable, then, to carry out clinical trials with massive vitamin A therapy to ascertain whether or not any recovery in the visual acuity occurred. Details of the trials are given in Table III.

The lack of success of such therapy in the control cases need not be emphasized; it was only to be expected. In four cases exhibiting the lesion, the recovery of vision could be classified as almost complete, and in three as partial. When the effect of the therapy is analysed, some interesting facts emerge. The best results were naturally obtained in cases with early lesions, i.e. those with a history of night-blindness, who showed early sclerotic changes around the disc with or without oedema or pigmentary disturbance at the macula, but partial success can be attained even when the ophthalmoscopic picture reveals a fairly gross lesion. Little or no improvement was achieved in two cases where peripheral pigmentary corpusculation existed, and the improvement was slight in those where atrophic cupping of the disc was present; these two appearances would seem to be contra-indications to therapy. It is difficult in this small series to generalize as to when or when not it is worth while attempting treatment with vitamin A, but it may be said that, where the visible choroidal blood vessels are red in colour, there lies the

greatest hope of recovery. Gross macular upset and optic atrophy do not appear to militate against the achievement of some improvement. The effect of these massive doses of vitamin A was dramatic in some cases and produced quite a sensation in the village concerned. For example, one man (829) who had taken to begging, returned to his farm, and when we visited the village a year later during the rains when the farmers were busy in their fields, he was working happily among them. Another case (772), who had been completely blind, returned to market as a petty trader, where he would certainly need to use his eyes. A third (1,291), gardener to a European, who was witness to his visual recovery, gained a new lease on his job.

Where possible, we controlled the degree of the hemeralopia with a portable dark adaptometer. The set was standardized on an arbitrary scale at a figure for normal eyes of 2.2 after 30 minutes in total darkness. In early cases the figure obtained lay between 4 and 5; in some cases the brightness of the flash had to be doubled before it was seen; in others (even a few of the early cases) the test could not be performed at all. It was impossible to carry about a heavy apparatus like the Goldmann Adaptometer so our results were comparative only. Although resolution of the anatomical changes did not occur, the dark-adaptation returned to normal in early treated cases.

The Snellen charts for illiterate subjects (Landolt C and E) were used for testing visual acuity. It could be argued

that the results of these tests varied according to the part of the functioning retina which was directed towards the letters. It was not possible to carry out adequate perimetry with this type of subject to ascertain which parts were healthy, but every effort was made to eliminate such a source of fallacy at the initial examination. This was done by encouraging head-tilting, and the results here showed fairly conclusively that only a little if any variation in the acuity occurred at all (letters not lines). On subsequent examinations the same practice was carried out, so that the subjects were given every chance to see the charts under equal conditions on each occasion of testing. Guessing was soon discovered. Objectively, there was no doubt when recovery of vision had been obtained. Whether such recovery was maintained, we do not know apart from the three cases quoted above. We are satisfied that no improvement occurs in the visual acuity after a course of any filaricides; only with vitamin A. Diethyl-carbamazine does not alter the condition in any respect.

CONCLUSIONS AS TO THE PATHOGENESIS

It is now possible to consider the probable course of events in the evolution of the degenerative lesion. It seems likely that the initial symptom of night-blindness depends upon a vitamin A deficiency. It may be that the first appearance of retinal pigmentary disturbance in the area between the macula and the disc (juxtapapillary) is related to the high incidence of rods in that area; the rods are most dense directly below the papilla, being about 170,000/sq. mm. in this region. It is interesting to note that Ramalingaswami, Leach and Sriramachari[15] found structural changes in the rods and cones and pigment epithelium of the retina in monkeys placed on a diet deficient in vitamin A. It is permitted to accept in the light of this interesting paper that, as a result of a gross vitamin A deficiency, the retinal pigment epithelium and the visual receptors of the human eye may be similarly affected. This is given indirect support by Hume and Krebs[16], who showed that cone function as well as rod function was affected in a group of human volunteers deprived of vitamin A. Wald, Brown and Smith[17] showed that the carotenoid components of the rhodopsin and iodopsin systems are identical. Iodopsin is now known to be vitamin A aldehyde. At the same time as these retinal changes occur in the posterior degenerative lesion, the choroidal vessels are equally badly hit. This is more difficult to explain. A vascular degeneration may be induced by a circulating

intoxicant, as in diabetes. Clinically, the vascular changes of the degenerative lesion under discussion could be explained in the same way. What filarial source of intoxicant might there be? In view of the low I.D.F. in these cases and in view of the fact that in some of them no mf. have been found either in the skin or in the eye, mf. as a source are unlikely. The only other source of toxin is the adult worm. This is not outside the bounds of possibility. It has been mentioned earlier that the average number of nodule sites (nodule mean) in these cases is low (0.9), and that we found many cases without any palpable nodule; it is thus possible to argue that it is only free adult worms which permit the circulation of such a toxin. The occurrence of free adult worms has been demonstrated post mortem by van den Berghe[18] and others. Here, too, vitamin A deficiency may play a part. The nodule mean where the posterior lesion is never seen in rain-forest villages (vitamin A being plentiful in the diet) was found by us to be 4.0 even where the density quotient was less than 5. Might not a deficiency of vitamin A, therefore, be in some way responsible for the low nodule mean, leaving a proportionately high number of adult worms free in the tissues? The factors which determine the effect of the nutritional status on parasitic infestations are complex; yet Moore[19], after reviewing the contradictory literature, suggests that the migration of parasites or of their

larvae may be facilitated by defective barriers in animals deficient in vitamin A. There is some evidence for this. It may well be, then, that the nodulation of adult filariae Onchocerca volvulus is to some extent determined by the vitamin A content of the diet: the more vitamin A the greater the number of nodules; the less the greater the number of free worms. A toxin liberated by adult worms lying free in the tissues is much more likely to achieve a reasonably high titre in the blood than if it were liberated within the comparatively avascular and densely fibrous-walled nodule. That would be one possible explanation for the absence of the degenerative lesion from rain-forest country. If a toxin liberated by free adult worms exists, it is well known that the complexity of the chorio-capillaris not infrequently results in a high local titre of any circulating poison. Owing to the huge surface area offered for absorption, retinal damage results. How these toxic substances act is obscure, of course, and it is still debated whether all of them are essentially neurotoxic or some of them act primarily upon the choroidal or retinal blood vessels causing a secondary neuronic degeneration through vasoconstriction.

In primary choroidal sclerosis the vascular changes precede and induce the pigmentary changes, but Ramalingaswami and his colleagues did not discuss the choroidal vessels although the retinal pigment was altered as a result of vitamin A deficiency. Leach[20] says that changes in the walls of the choroidal vessels,

especially the choriocapillaris, were seen in sections taken from the eyes of monkeys deficient in vitamin A in this study, but there were no changes in the retinal vessels. This may be seen in the original illustrations to the paper. Leach is the first, however, to agree that such important observations require confirmation. There is no real evidence that the vascular changes precede the pigmentary in the posterior lesion.

The degenerative lesion of onchocerciasis seems to differ from a primary choroidal sclerosis in that a vitamin A deficiency is an ever-present factor. There is, therefore, no alternative but to postulate at least two aetiological factors. These two, of course, could be linked: a filarial toxin may interfere in vitamin A metabolism as well as affect the vessels. In this way a vicious circle is set up, all the more likely to appear in subjects already somewhat deficient in the vitamin. There is some evidence to support the first hypothesis. Eveleth, Goldsby, Bolin and Bolin[21] have made preliminary studies on the conversion of carotene in sheep infested with filariform larvae after deprivation of vitamin A. A better conversion was observed in one non-infested sheep than in three which were infested. In experiments on guinea pigs, animals which were infested with D. filaria were found 8 months later to have reserves averaging 2.5 i.u./g., as compared with 23 i.u./g. in control animals not so infested. Animals infested with D. viviparus also had lower reserves than control animals (Soliman[22]).

Several factors suggest that the pigment epithelium plays a vital role in the rod and cone degeneration. Popper and Greenberg[23] demonstrated the presence of vitamin A in this epithelium, and suggested that the vitamin is altered within it as it passes from the circulation to the receptors. Damage to the epithelium could occur secondary to a choroidal sclerosis of the type described here as being possibly induced by a filarial toxin, and would thereby lead to inhibition of the vitamin A metabolism with a resultant adverse effect on the rods and cones; the epithelial changes, on the other hand, might be simply a primary degeneration in a vitamin A deficient subject as a result of the generalized deficiency. In neither of these events is it necessary to postulate a direct competitive effect by a toxin to explain the breakdown in the vitamin A metabolism. It would be enough if the epithelium itself was made deficient in vitamin A. It is well known that the more rapid the destructive process the greater will be the deposition of retinal pigment in new locations. In Ridley's drawing of the degenerative lesion in his monograph, and in the excellent retinographs of Boithias[24], accumulation of the retinal pigment is slight if the size of the area involved is taken into account. This supports the view that the condition is insidious and non-inflammatory, which all the better fits the pathogenetic possibilities just suggested, rather than the death of mf. <u>in situ</u>.

To sum up, it seems likely that as a result of vitamin A deficiency - either with or without additional interference in vitamin A metabolism by a filarial toxin - there will be destruction of the retinal pigment epithelium which in turn will lead to degeneration of the retinal receptors. Simultaneously a choroidal sclerosis develops, probably as the result of a filarial toxin, for it is not as yet proven that vitamin A deficiency alone can lead to such a change. The vascular sclerosis in its turn gives a greater impetus to degeneration of the retinal pigment epithelium, thereby further aggravating the break-down in the vitamin A metabolism. The restriction of the lesion in the first instance to the papillo-macular area, where the choriocapillaris is most dense, lends support to the belief that a toxin does play a part in producing the vascular changes. It should not be impossible to isolate such a toxin, if it exists. The hypothesis put forward rests partly on circumstantial evidence, but in the absence of a better argument it affords some basis on which to work in the future.

While this explanation goes as far as possible in the light of our present knowledge, it leaves us wondering why only 5% of the many subjects whose bodies almost certainly contain free adult filariae, and who are deficient in vitamin A, appear to suffer from the ocular lesion. Many might consider that 5% is a reasonable percentage in any large biological series; others might ponder on the probability of there being a third factor

involved. In this connexion, it is interesting to read of the toxin present in rye germ, the ill-effects of which on the spinal cord could be prevented by vitamin A (Mellanby[25]); cereals constitute the main item of food. There are also other possibilities: there may be some reason why the carotene which is ingested is not converted into vitamin A; the blood carotenoids were estimated in only two of our cases (1,029 and 1,291); both figures were abnormally high despite the level of vitamin A. We do not know if this is the rule; it may be an important observation, or merely misleading. Wald and Hubbard[26] have shown that the conversion of retinene to vitamin A is a coupled reduction for which cozymase acts as a coenzyme, and fructose diphosphate can act as a substrate. The cycle is dependent on the existence of an adequate supply of nicotinic acid, which is contained in cozymase. While there are ample amounts of nicotinic acid in the diets of the people of Northern Nigeria (Nicol[27]) there is a variable degree of deficiency in riboflavine; it is believed that a close relationship exists between these two respiratory enzyme vitamins, so that cozymase activity may well be interfered with in riboflavin deficiency. It is in directions such as these, perhaps, that further light may be thrown on this fascinating subject.

SUMMARY

1. Two types of onchocercal lesion in the posterior uvea are shown to exist: one is due to the death of microfilariae in or adjacent to the choroid, and is inflammatory in type. The other is a degenerative lesion associated with a low degree of infection in the individual and a low nodule mean; its pathogenesis is not understood.

2. The sera from ten cases suffering from this lesion were found to have a low vitamin A content (about 25 i.u./100 ml. plasma). Recovery of vision (almost complete or partial in seven cases of the posterior degenerative lesion) followed the administration of 165,000 i.u. vitamin A daily. Improvement, when it occurred, began at the end of one week. In addition, the dark adaptation in early cases so treated returned to normal. There was no resolution of the anatomical abnormalities. Where atrophic cupping, peripheral corpusculation, or orange-white sclerosis of the larger choroidal blood vessels existed, no improvement resulted.

3. Assessment of possible factors in the aetiology suggests that a combination of vitamin A deficiency with a toxin liberated by free adult worms could explain its onset. This belief is supported by the fact that the lesion does not occur in areas where vitamin A insufficiency in the dietary or onchocerciasis endemicity exist by themselves, but only when the two are associated.

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PART IV.

THE PATHOLOGY

The pathological observations are presented here in two sections: in the first the material, techniques, and microscopic appearances of the parasite during the stages of its dissolution are described; the section concludes with certain original observations as to the routes preferred by mf. O. volvulus in entering the tissues of the eye.

The second section consists of a description of the histopathology of the different ocular lesions. Throughout this section one must be constantly aware of the presence of the parasites, or of fragments of them, either alive at the time the eye was excised (as the staining will show) or, being dead, in different stages of disorganisation, for it is that background, as much as that of the structural patterns formed, which makes the topic unique. By first stressing the changing appearances of the parasite body after it dies, and the places where it is most likely to be found in the eye, the writer hopes the reader will have created the necessary background for himself before the actual pathology is considered.

SECTION I

I. Material

The observations were carried out on 12 eyeballs and 37 pieces of tissue; for various reasons another 8 eyeballs were rejected in the final assessment, for it was essential to use material that was clinically above criticism and without any complicating feature whatever. The 12 best and most characteristic eyes only were selected. They included acute, subacute and chronic stages of the various clinical lesions of onchocerciasis. The same strictures were applied to the biopsy material.

II. Techniques

Some of our specimens were sent to the Department of Pathology in the Institute of Ophthalmology, London, where celloidin embedding was performed, and routine sections (stained with haematoxylin and eosin) supplied. Others (the majority) were sent to the Department of Histology in the University Laboratory of Physiology, Oxford, where low-viscosity nitrocellulose embedding was carried out. On the writer's return from Africa he was kindly given permission to use the facilities in Oxford, and a great variety of staining techniques was undertaken; the London blocks were permitted to be brought to Oxford and further sections cut there at leisure. Those who have carried

out research will understand how appreciative any worker is when a colleague generously gives him of his time and service. The writer owes a great debt of gratitude to Dr. Ashton of London and Mr. Leach of Oxford in this connection. The latter elaborated a method of bleaching the pigment in the heavily-pigmented negroid eye without affecting the staining, and this proved of great value (Chesterman and Leach[1]). Considering that the eyeballs after the writer excised them in the bush had to be rushed to the nearest airport - perhaps four hundred miles away over difficult terrain - they were in remarkably good condition when they reached the United Kingdom; only occasionally were there signs of hardening. The specimens were fixed in Bouin for two days and despatched in 70% alcohol. Biopsies were sent the same way, but were paraffin-embedded.

The photomicrography was carried out partly in Oxford by E. H. Leach and partly in India by the author.

Routine stains used included Phloxine tartrazine, Mallory, Masson, van Gieson, Weigert's and Periodic acid Schiff.

III. The dissolution of mf. O. volvulus in the human eye and its effect on the tissues

1. Introduction

Here we shall describe the microscopic changes which occur in mf. O. volvulus (apparently after its death) in the tissues of the eye. We shall also attempt to associate these changes with

the local reactions appearing in the vicinity of the parasite.

The cornea is the tissue best suited to observations of this kind, especially in pigmented eyes. Because the parasites and inflammatory cells are required to travel through a comparatively simple avascular structure, the changes can be viewed as in a culture medium. Recently and lightly infected eyes are usually more informative than old heavily infected ones as the latter present a complicated picture in which acute and chronic tissue changes overlap, and both living and dead mf. in every stage of dissolution exist side by side.

The observations reported here are presented with some diffidence for fragments of a microfilaria are difficult to photograph, the whole seldom being in focus. The absence of colour further weakens the pictorial evidence so necessary in work of this kind. However, although these are insoluble defects, they are after all only relative ones.

2. Historical

To date it has been presumed that it is the products of disintegration of mf. O. volvulus which produce inflammatory changes in the human eye. Robles[2] and Calderon[3] suggested that a toxin secreted by the adults or the larvae when alive was responsible for the pathological picture. Later Shafi[4] and Vogel[5] inclined to the same view; the former, who is often quoted, made no study of onchocerciasis, simply drawing a parallel

with helminthic parasites. Strong[6] suggested the reaction was at least partly due "to the passage of large numbers of mf. through the lymphatic vessels of the eye and the subsequent dilatation and oedema which results." Later[7] he stated "at the same time it would appear probable that the mf. through their metabolism, or their death and destruction in the tissues, might also exert a pathological effect." The finding of mf. in every ocular tissue logically proves nothing except that the adults are probably not implicated. We cannot even be sure of this. Hissette[8] summed the position up carefully thus: (1) there is a slight and slow reaction to the living mf., and (2) a clear and appreciable reaction to the dead larvae. The former, as has been suggested by Strong, might be due simply to the passage of the parasites in number; equally well it might be due to a toxin which they or the adults secreted; the latter, as Hissette believed, is most likely to be caused by the disintegrative products of the larvae. These conclusions, nevertheless, are all equivocal.

What is surprising is that no pathologist to date has been able to associate the reactions of the tissues with microscopic changes in the mf. bodies. Hissette came very near to this for he was aware that some mf. stain well, every nucleus being clearly outlined, whereas others appear to lose their affinity to the stain, the nuclei becoming masked. He assumed the former were alive at the time the eye was excised, and the latter dead. Unfortunately he did not attempt or was unable to associate these two different

appearances of the parasite with the processes of inflammation.

3. Staining techniques of choice

Haematoxylin and eosin and phloxine tartrazine were the two techniques which proved most valuable for this study.

4. Structural changes in the parasite during its dissolution

Where the body outline is uniformly cylindrical, the nuclei well-stained and clear-cut, the rest of the soma unstained (H. and E.) or only faintly stained (Phlox.Tartr.), the microfilaria can be said to have been alive at the moment the eye was excised; in a recently invaded eye (i.e. with no inflammatory reaction visible) this is the typical appearance.

The changed appearances of the mf. as it is absorbed, presumably after death, have been classified as under:-

Stage 1: Increased affinity of the soma to the stain occurs. This we have called 'somatic staining'.

Stage 2: The nuclei then become indistinct, the difference in colour intesity between them and the soma becoming less and less until the whole stains uniformly. The body characteristically is irregularly swollen. This stage we have called 'ballooning'.

Stage 3: The body now breaks up abruptly into fragments. This stage we have called 'fragmentation'.

Stage 4: Finally, in proportion as the fragments become smaller and fewer, the surrounding tissue stains diffusely blue (H. and E.) or pinkish-blue (Phlox.Tartr.). We have called this the stage of 'diffusion and absorption' as that is what appears to be happening.

These changes are probably those of autolysis, for at no time did we find them associated with the presence of macrophages or giant cells.

5. Association of stages in parasite dissolution with local changes in the tissues

No reaction was seen until 'ballooning' occurred (Stage 2). A few eosinophil leucocytes were invariably closely applied to the parasite body at this stage; with fragmentation (Stage 3) their number increases enormously, and a few round cells can be seen. The local cyto-architecture probably determines the number and dispersal of the inflammatory cells. In the potentially-wide space between the epithelium and Bowman's membrane they cluster round the parasite in large numbers; in the more dense substantia propria the inflammatory cells, required to pass along the narrow interstitial spaces, are not so closely applied to the disintegrating body. At the start of Stage 4 the picture is variable; sometimes eosinophil leucocytes dominate (they are always present), sometimes fibroblasts; the plasma cells and lymphocytes have also increased in number. Coinciding with the arrival of these migratory cells into the cornea, new capillaries grow towards the parasite body: during Stage 4 the vessels are often already close to the inflammatory focus. As the limbal area has usually been the site of earlier more advanced inflammation, there is a reservoir of the different cells within easy access.

By the time the mf. fragments have become completely absorbed the number of plasma cells invariably equals or exceeds the eosinophil. On the whole, however, it is the fibroblasts of the stroma which are most conspicuous, supporting the view that we are dealing with a reactive fibrosis induced by the stimulus of non-

necrotising toxins derived from the parasite body as a result of its dissolution.

It is not possible to say how long the process of dissolution takes. The inferences are that it lasts only for a few days. The acute reaction may continue for some days longer; although the nature of the inflammatory cells is more in keeping with a chronic lesion even where the clinical signs were acute, that is a commonly-found anomaly. There seems little doubt that after the parasite body has disintegrated the toxic products linger on diffusing very slowly from the primary focus into the neighbouring tissues. In this way adjacent parts may be affected without necessarily being the locus of a dead parasite; such areas it is believed may be distinguished by the frequent absence of eosinophil leucocytes; a single capillary associated with a few fibroblasts and plasma cells is a common finding at some distance from a parasite in Stage 3 or 4 of dissolution. It is impossible of course to prove that no parasite died in such small areas of pannus; that many arise as a result of the diffusion of toxic products from a nearby focus is a personal view.

There was no strong evidence of a transitory stage between ballooning and fragmentation; that is why fragmentation is described as occurring "abruptly". Possibly Stage 4 might come in for some criticism; the faint staining of the tissues which characterises it, the writer has not seen in any other condition except perhaps calcification in an old keratitis. The association

in an infected eye of a local aggregation of eosinophil leucocytes, fibroblasts, and plasma cells with diffuse staining of the tissues in which these cells lie is highly suggestive.

One concludes that mf. O. volvulus is a good example of a perfectly adapted parasite, and that the inflammatory changes observed in the cornea in this study were caused by the products of dissolution of their bodies, and not by any exotoxin secreted by them or the adult worms when alive.

6. Summary

(1) Structural alterations of four types, seen in mf. O. volvulus in sections of the human eye stained with Haematoxylin and eosin and Phloxine tartrazine, are described. It is believed they represent four stages in the dissolution of the mf. body.

(2) The local reaction of the tissues in relation to each of the four stages is reported.

(3) The living parasite appears to induce no tissue reaction. After its death, however, eosinophil leucocytes, lymphocytes, plasma cells and fibroblasts appear in the vicinity of the disintegrating body roughly in that order and relative proportion; new capillaries grow towards the focus. At no stage in the process are polyblasts or giant cells seen. When dissolution is complete the proportionate representation of these cells is reversed.

(4) It seems probable that after the visible processes of dissolution are complete ultra-microscopic toxins in solution are

left to diffuse slowly into and affect neighbouring tissues and structures. This can be recognised by a diffuse basophil staining of the tissues in which inflammatory cells, characteristic of stage 4, lie.

IV. The movements of the parasites into and within the eye

1. Introduction

It is generally believed that the mf. of O. volvulus must enter the tissues of the eye from the lids, whence they can pass under the palpebral and bulbar conjunctiva to penetrate the eyeball at the corneoscleral junction; thereafter all the ocular tissues are readily accessible. Histopathological evidence of their presence in each of the ocular tissues has been afforded in the past except in the case of the retina, although only one worker has demonstrated the parasites in the optic nerve (Giaquinto Mira[9]), and no photomicrograph has, to our knowledge, as yet been published of the latter finding.

2. Bleaching

Bleaching the pigment was of incalculable value in this study.

3. Observations

(1) Lids to outer coats of eyeball: The mf. in a case with a high I.D.F. (Rodger and Brown[10]) can always be found in great numbers in the lids. Palpebral and bulbar conjunctival biopsies

in such cases reveal the same picture. Indeed, as has been suggested elsewhere (Rodger[11]) skin biopsies of the lid are as revealing as conjunctival biopsies. In these patients the anterior segment of the eye is invariably heavily invaded (cornea, corneoscleral junction, and root of iris) as are the extra-ocular muscles, the loose episcleral tissue below Tenon's Capsule and the outer fibres of the sclera. The latter is the usual site of mf. as far as the sclera is concerned; most photomicrographs reporting mf. in this tissue in fact reveal them either in the episclera or just inside the outer coat; only rarely have we found mf. in the substance of the sclera itself, except anteriorly at the limbus where the structure is looser. In view of the resistance of the sclera proper it is not surprising the mf. do not penetrate it readily.

(2) Outer coats of eyeball to inner: There is a quantitative decrease usually in the number of parasites found in the uvea in heavily-infected eyes as we move back from the limbus to the optic nerve. This is such a common finding it suggests that, in such eyes, the scleral barrier is a very real one except anteriorly. In other words it appears that the mf. penetrate wherever the tissues are least resistant; that is to say the routes most likely to be taken are from under bulbar conjunctiva into the cornea between its epithelium and Bowman's membrane, in a posterior direction through the corneoscleral junctional fibres to the angle

and hence into the uvea
of the eye, or into the corneal stroma, in that order. That the most readily accessible route is above Bowman's membrane is borne out by the observations we have made on nearly 3,000 heavily-infected eyes with the ocular biomicroscope, where we saw subepithelial corneal mf. many times but seldom if ever stromal; moreover it is the classic site of pannus onchocercosus. Para. 4 below enlarges on the possible anterior routes.

(3) Inner coats of eyeballs to intra-ocular fluids: It was not possible to find any evidence in the sections suggesting the mode of passage of mf. into the aqueous humour. It seems likely, nevertheless, that when they reach the angle of the eye they will break through the thin uveal scaffolding forming the ligamentum pectinatum comparatively easily, and that when they are present in the iris they will emerge through its fine anterior endothelium with even less difficulty. We do not believe mf. can pass from the retina directly into the vitreous, as where they were present we saw no sign of that; but it is possible they might. More probably, in our view, the parasites break through the anterior face of the vitreous gel, which affords no barrier at all, having previously passed from the anterior chamber into the posterior.

(4) Passage via the adventitial sheaths of blood vessels: The presence in some eyes of a restricted anterior invasion associated with a restricted but definite choroidal invasion suggested that some parasites must pass into the choroid by another route as a

quantitative decrease from cornea to optic nerve is to be expected in an invasion from the anterior segment backwards. Because we were fairly sure that the hard wall of the sclera afforded an almost impassible barrier, the possibility was considered that the mf. might pass down the loose fibrous sheaths of the penetrating equatorial vessels. A certain amount of evidence of this was found in serial sections. At the same time it was abundantly demonstrated that the adventitial sheaths of the perforating anterior ciliary vessels were even more favoured by the parasites as an easy means of passing from the outer eye to the inner, thereby adding to the routes of entry into the eye at or around the limbus. Theoretically, although it is possible for mf. to reach the choroid without invasion of the anterior segment, as it will depend on orbital invasion which is unlikely to happen except where the parasites are also present in force at the limbus, choroidal invasion probably occurs only rarely as an isolated phenomenon. Our clinical experiences support this belief.

(5) Invasion of the retina by the mf. of O. volvulus: In the course of the last-mentioned observations the original discovery was made that mf. invade the retina. The parasites apparently are able to reach this structure by forcing a passage through the pigment epithelium for there was evidence of such a break-through. There was no sign, however, of a break in the continuity of the membrane of Bruch. So apparently they must either break through from the ciliary body between the pigment

epithelium and the cuticular lamina of Bruch or pass from the vitreous directly into the retina. One of two mf. observed lay in the sheath of a retinal blood vessel with its head projecting into the lumen in the direction of the optic disc. The attitude and position suggest it had not passed up into the retina via the vascular sheath of a retinal vessel (unlikely although not impossible) but had entered the sheath from within the retina, and was in the process of forcing its way along the loose adventitial tissue. The position of its head appearently within the lumen of the vessel may be an artefact. The distinct staining suggests this parasite was alive at the time of the operation.

Photomicrographs give a rough indication only of the size of such mf. by comparison with the retinal nuclei. The only other unsheathed species which volvulus might be confused with is mf. perstans, although the latter is smaller. Factors other than size, which anyway is an unsatisfactory standard where only a fragment of the parasite is visible, depend upon the part of the body that can be made out. In the more complete of the two mf. seen in the retina the head and half the body is visible, so that by its shape and the distribution of nuclei identification can be made with confidence, apart altogether from the evidence of its size. In neither of the two retinal mf. was the tail visible, but the evidence supplied by the caudal nuclei is not important where the other characteristics described above exist. More convincing

still, perhaps, is the fact that both patients had an I.D.F. of 31 and that in none of 10 thick blood-films examined in each were blood-borne mf. found. In short all the evidence led to the one conclusion, that these mf. in the human retina were volvulus.

(6) Invasion of the optic nerve: Although finding in each of two subjects a solitary mf. in the optic nerve substance there was no evidence to show (as is likely) that they burrowed into the nerve from the orbit along the septal nutrient vessel sheaths or (less probably), through the border tissue of the Elschnig from the choroid or sclera. In each of the two positive cases there was massive orbital invasion, so invasion via the blood vessels of the nerve is quite a probability.

4. Summary

(1) A study of the possible routes of entry of mf. O. volvulus into the different ocular tissues was carried out.

(2) Serial sections were made after low-viscosity nitrocellulose embedding; paraffin was used in the case of the lids. The uveal melanin was bleached in most instances before the sections were stained.

(3) It was concluded that the most common route taken by the mf. is from under the bulbar conjunctiva into the cornea between the epithelium and Bowman's membrane, followed by passage from the limbus to the angle or into the corneal stroma - in that order. The sclera appears to offer a strong barrier to their passage, except at its anterior extremity where its fibres mingle

with the corneal lamellae, or where it is pierced by blood vessels (as at the equator).

(4) It was also discovered that the parasites travel by the adventitial sheaths of the perforating blood vessels at the limbus, and (perhaps) by those of the nutrient vessels of the optic nerve.

(5) It is believed that the presence of mf. O. volvulus in the retina described here is an original finding, and that the photomicrographs of mf. within the retina, vitreous and optic nerve are the first ever to be reproduced.

SECTION II

The pathology of all the ocular manifestations attributed to onchocerciasis is given here and this section contains microscopic proof of the writer's claim that the posterior segmental lesions are of two types.

The following are the conditions described:

1. 'Bung' eye
2. Limbitis
3. Superficial punctate keratitis
4. Sclerosing keratitis
5. Anterior uveitis
6. Posterior uveitis
7. Chorio-retinal degeneration (the posterior degenerative lesion of onchocerciasis)
8. Optic atrophy.

1. 'BUNG' EYE

This colourful descriptive term was coined by Owen and Henessey[12] to describe a (permanent) swelling of the upper lids commonly found in association with ocular onchocerciasis. The usual explanation of the phenomenon has been that oedema is the cause.

Macroscopically there is oedema of the dermis in which many engorged vessels are present. The subcutaneous tissue, however, showed in the four cases collected that deep to the dermis simple bilateral lobulated lipomata were present. It is interesting to note that a lipomatous change in onchocercomata is a common finding elsewhere in the body. The writer himself had one such excised from over his ribs!

Microscopic appearances:

The epithelium of the lid in the early stages of invasion is of the normal stratified squamous pigmented type. There is no evidence of invasion of the epidermis by capillaries. In the dermis, which is oedematous, many foci of chronic inflammatory cells exist. A great number of mf. fragments may be found, some of them in the interstitial tissue of the underlying limpoma. Changes in the elastic are negligible. In longstanding cases, on the other hand, the epithelium has become atrophic and the subepithelial elastic lost. The blood vessels reveal swelling and proliferation of the endothelium and in many instances this has led to occlusion of the lumen. The internal elastic laminae stain faintly, and there are signs of reduplication of vessels. The fibroblasts have proliferated somewhat, especially near the vessels. Mf. volvulus were present in the lipomata in all our cases.

One concludes, although admittedly from somewhat slight evidence, that the toxic products of the dead mf. bodies probably act as a stimulant to the adipose tissue of the lid with the subsequent development of a lipoma. This bears a parallel to the increased activity of the fibroblasts in the stroma. The cause of the epithelial atrophy, the elastic degeneration (after the initial proliferation) and the vascular anomalies may also be toxic; more probably the toxic effect involves the bloodvessels first, and most severely, so that the anoxia resulting from their subsequent

occlusion is responsible for the epithelial and elastic atrophy.

2. LIMBITIS

There is hyperaemia and chemosis of the bulbar conjunctiva and sclera at the limbus. The adjacent sector of the cornea frequently exhibits subepithelial opacification. Mf. are always present in biopsies of the overlying conjunctiva.

Microscopic appearances:

There is a thick and pigmented conjunctival epithelium. Pigment is present in places in clear cells at all levels; this is to be expected in the negroid eye, of course, and is normal. There is no suggestion that the epithelium is keratinised, or even truly stratified, but there are one or two extra layers of polyhedral cells.

The underlying limbal lymphatics and vessels are characteristically somewhat dilated and new capillaries have sprouted here and there into the epithelium of the conjunctiva. In the subepithelial tissue there is a not inconsiderable infiltration of plasma cells along with a few eosinophil leucocytes and lymphocytes. Even in the early stages the subepithelial elasticum seems to be affected and in contrast to the skin of the lid is soon lost from the area; in old cases the deep elastic as well as the vascular laminae are wholly destroyed.

Oedema of the substantia is marked and there is evidence of increased fibroblastic activity, especially around the larger vessels. Where a limbitis was associated clinically with a 'nodule' at the limbus, the latter always turned out to be a pinguecula. In some of the biopsies, a few capillaries were found projecting into the corneal tissue, usually but not always running between Bowman's membrane and the epithelium; they appeared in the crescent of corneal opacification. The latter was not due to oedema and an overflow of inflammatory cells, as it seemed clinically, but to fibrosis; a few inflammatory cells were present, but too few to account for the clinical picture.

The biopsies were all taken from 3 or 9 o'clock, close to the limbus, and included a small piece of the adjacent cornea. The question arises as to whether or not the changes found, especially in the case of the conjunctival epithelium, were due to the disease or to exposure, heat and malnutrition, all undoubted factors in the tropics. A peripheral ring of corneal opacification running round the entire circumference of the limbus has been described by the writer elsewhere (Rodger[13]). At first sight it appears to be an arcus senilis. Microscopically it differs: it is never interrupted; it involves the anterior part of the stroma only; nor does it stain with Sudan III. It consists of fibroblasts and a few chronic inflammatory cells, which pass under the limbus without any lucid interval. The writer has called this ring an arcus tropicalis, being convinced

that it is caused by the climatic and dietary conditions prevailing in the tropics. However, in onchocercal limbitis, the corneal opacification corresponding to the arc of the limbus which is inflamed is superimposed in advance of any arcus tropicalis. Moreover, although it is difficult to conclude what the normal appearance of the epithelium may be in this part of the bulb, it appears, in comparison with eyes not suffering from a limbitis, that we have in the latter a true metaplasia of the epithelium due to the parasites. About the remainder of the pathological changes there can be no doubts: the vascular reaction is characteristic, as are the changes rung between proliferation and degeneration.

3. SUPERFICIAL PUNCTATE KERATITIS

The opacities lie on either side of Bowman's membrane, and only occasionally more deeply in the stroma. The writer has used the descriptive terms 'morula', 'patch', and 'halo' to describe the commonly present opacities as viewed with the slitlamp (Rodger[14]). In the active stage of the keratitis, whatever its cause, it is the 'morula' type which is found; this consists of a round collection of discrete greyish-yellow dots like a mulberry. In onchocerciasis this collection is less likely to be round, being generally polyhedral and is usually much bigger in size than those due to a virus; punctate opacities can vary from 0.25 mm. to 2 mm., the

larger being described as nummular by some workers; in most cases of onchocerciasis the greater diameter is the rule. The common appearance, then, is of a yellowish-grey opacity contiguous with one or other side of Bowman's zone, usually polyhedral and about 2 mm. in diameter. As the disease continues the opacities are lost in the greater involvement of the cornea consequent upon the development of a sclerosing keratitis. They may coalesce with each other or become part of the generalised keratitis, submerged in the advancing pannus. In cases supposedly self-limiting, the punctate keratitis undergoes healing. As there is some doubt about the diagnosis and pathogenesis of the punctate lesions we cannot say for sure that the opacities due to onchocerciasis heal; in those supposed to be due to onchocerciasis, nevertheless, the writer has seen the individual dots of the 'morula' fuse to form a uniform 'patch', which in turn started to clear from the centre so that it came to look like a 'halo', whence it continued to fade until no trace remained. Persistence in any shape or form on the other hand is common. Finally, it should be noted that this sequence of events is non-specific, being equally relevant to any of the other types of punctate keratitis so commonly found in the tropics.

Microscopic appearances:

Although we have excised many corneal opacities of the three descriptive types, the intention at the time was to ascertain whether or not mf. were present in them; the procedure adopted was to stain the excised opacity in bulk (it was surrounded by a margin of healthy cornea) and examine it on the flat. Parasites were never found. Where living mf. were seen with the slit lamp on several occasions the piece of cornea containing the parasite was excised, and subsequently blocked and sectioned; here no inflammatory reaction was ever observed. Only in two patients were punctate opacities excised, blocked and sectioned and stained variously; one of these cases suffered from onchocerciasis. As the opacity was of the patch type it enables us to give a good microscopic description of a healed opacity in a quiet eye. Opacities more characteristic of acute or chronic onchocerciasis were present in several whole eyeballs excised primarily for other reasons, but identification of individual ones is not easy. The microscopic appearances now to be described have been built up from all these sources of information. It must be stated, however, at the outset that the writer is presenting a hypothetical sequence of events as to the course taken in punctate keratitis, each one of which in itself is accurate, but the pattern of the whole not necessarily so.

When a parasite finds its way below Bowman's membrane and dies there, it has already been shown in Section I that it

attracts to the spot in great abundance several types of inflammatory cell such as the eosinophil leucocyte, the plasma cell and to a lesser degree the lymphocyte. When the parasite passes above Bowman's membrane, exactly the same sequence of events happens. There is nothing in this state of affairs which is likely to produce the minute discrete opacities typical of a classic punctate keratitis (as the photomicrographs show), nor has any other evidence been obtained which suggests it. The classic viral punctate keratides so commonly found in association with onchocerciasis are those, we believe, where the opacity is round and small; but there is nothing round and small in the collection of inflammatory cells we discovered in the acute onchocercal eyes we examined, as serial sections well show. This supports our view that the punctate keratitis of onchocerciasis (in its acute stage particularly) only bears a superficial resemblance to the viral types, the opacities being on the whole larger and denser as well as less round.

The large opacity formed around the dead parasite as it fragments during the acute phase of the keratitis will in the end either vanish into the advancing bulk of an associated pannus, when its individuality will go, or the nature of its infiltrate will change, and the cells forming the opacity ultimately become absorbed. One would expect when the locus is below Bowman's membrane that the accompanying fibrosis will leave a permanent

scar, and alternatively, when above the membrane, that there will be no residuum. This is not the case, for an opacity clinically demonstrable as the latter we have found to depend on structural changes in the epithelium only.

Where, then, a parasite dies above Bowman's membrane, the early appearances are those due to the local aggregation of eosinophil leucocytes and the fragmenting body; the nature of the infiltrate then slowly changes until plasma cells displace the eosinophil; the close association of the toxins with the epithelium apparently causes it to atrophy; the cells become disordered and flattened; the nuclei are pyknotic and their alignment generally horizontal; PAS does not stain the muco-proteins of the basement membrane; pigment granules invade the cells at all levels, sitting like caps on the outer side of the nuclei, occupying the external part of the cell body. When the last of the inflammatory cells has become absorbed, it is these epithelial changes alone which persist.

If, on the other hand, the parasite dies in the stroma of the cornea below Bowman's membrane, although the changing nature of the inflammatory cells follows the same course, there is the extra complication of the stimulating effect the toxins have on the fibroblasts; being at a greater distance from the toxins (on the far side of Bowman's membrane), the epithelial cells do not this time become atrophic but are like the presumably tougher fibroblasts stimulated to proliferate. As a result the

epithelium becomes hypertrophic, its nuclei staining darkly, but otherwise normal. In addition the basement membrane stains densely with PAS; it is thicker than normal and is disordered and granular. In the region of the parasite's supposed grave in the stroma, once the inflammatory cells have disappeared and the lesion quietened down, all that remains is an excessive collocation of disorientated collagen fibres which stain heavily with PAS; the hyperplastic epithelium lies directly above. Thus it is that persistant opacities exist in the quiet eye both above and below Bowman's membrane, although unfortunately there is nothing specific in the appearances to distinguish them in the late stages from corneal opacities of other origin.

4. SCLEROSING KERATITIS

The essential changes here are those of repair, the dominant feature pannus formation. In a transparent tissue like the cornea the damage results from the stimulating effect of the disease on the corneal fibroblasts. Loss of their transparency, it seems, depends not so much upon an alteration of the planes in which they lie as of their chemical nature.

The classic site of pannus onchocercosus is between Bowman's membrane and the epithelium in the situation of pannus degenerativus. There is some disagreement about the constitution of the latter; some workers say it is rich in vessels and round cells, others that it has few or no vessels and is unassociated

with inflammatory cells. Whatever the opinions held the pannus of onchocerciasis should never be confused with panmus degenerativus, as it is distinctive in character. In advanced cases pannus onchocercosus is also found in the stroma; here it is in all respects identical with the pannus found above Bowman's membrane. The nature of the infiltrate distinguishes it from pannus eczematosus. Sometimes it advances far in towards the apex from the periphery, at other times it exists as islands in the stroma apparently isolated from the limbal vasculature, if that is possible. These interstitial patches are usually only 10-50 μ thick. The posterior third of the stroma never was found to be involved.

The keratitis of onchocerciasis may be associated with a punctate keratitis; it is always associated with a limbitis, marked congestion of the limbal vessels being a feature. The pannus never originates in the upper half of the cornea; it usually starts at 3 or 9 o'clock in the form of invading tongues; sometimes it commences as an apron, advancing into the cornea from below towards its apex. Fusion of the tongues or of the tongues and apron, when both are present, is the rule, and ultimately leads to total opacification of the cornea. With the slit lamp a tongue of pannus consists of three zones as follows, reading from the limbus inwards: a pigmented, a densely-white and a grey zone (Rodger[15]).

Microscopic appearances:

The early changes in the cornea when a parasite dies therein have already been described in this thesis. Pannus formation is a development of the last phase mentioned there. Thereafter, when the characteristic sclerosing keratitis has commenced, new capillaries, fibroblasts, and inflammatory cells, of which plasma cells are most abundant and eosinophil leucocytes always present, can be seen. The classic site of the pannus, as we have seen clinically, is above Bowman's membrane, although it may also exist below. This membrane does not seem to be readily destroyed; it is only in the more advanced cases that this happens. It tends rather to be pushed out of the way, until the fibroblasts by force of numbers break it down and its continuity is lost. The pannus pushes itself into the cornea in the shape of a wedge, so that its innermost advancing portion is the most thin. This explains the inner of the three zones, the grey, seen clinically, for here the optical density is least; above a certain thickness of pannus no light is transmitted at all and the colour changes to white. This is the middle zone. The basal pigmented zone is so designated because the epithelium takes up pigment. The distribution of the latter will be described below along with the epithelial changes. The elastic fibres disappear throughout the stroma wherever it is involved in the inflammatory reaction; the writer has never been able to observe changes either in Descemet's membrane or in the

stromal elastic tissue lying next it, although with distortions due to pannus formation the former may seem to be changed clinically.

The epithelium of the cornea early on becomes hyperplastic and the nuclei stain moredarkly above the area of pannus. Metaplasia follows until in the end early keratinisation is seen. Pigment in the corneal epithelium exists at all levels in clear cells, but the greatest accumulation of it is to be found in the wing and basal cells, the granules occupying the outer halves of the cell bodies, as we saw in the conjunctiva. This hyperpigmentation appears to be a (melanoblastic) property of the resting epithelial cell rather than an invasion by chromatophores or the result of excessive production of melanin by known limbal melanoblasts. In longstanding cases epithelial pigmentation may be almost total. As the keratitis advances into a chronic or quiescent phase, the epithelial hypertrophy diminishes and atrophic thinning developes. The surface then becomes uneven because the processes of atrophy do not occur equally over the entire cornea. It is at this stage that Bowman's membrane usually fragments, and calcification of the membrane and the adjacent stroma commences. In old cases of onchocercal endophthalmitis, epithelial bullae probably result from secondary glaucoma, almost certainly being due to pockets of fluid (keratitis bullosa or vesiculata) which later become filled in with fibrous tissue.

The vessels of the pannus persist for a very long time, the proliferating fibroblasts lying more or less parallel to them;

it is the nature of the infiltrate which changes. In the early stages, as we have seen, eosinophil leucocytes are found among the plasma cells; earlier still, before pannus developed, the former were the dominant cells lying beside the disintegrating body of the parasite; they are the first to decrease. In an advanced keratitis, therefore, the infiltrate consists of plasma cells, an occasional group of lymphocytes, plasma cytoid cells in not inconsiderable numbers, and only a few eosinophil leucocytes; a good place to look first for the latter is in the congested blood-vessels of the pannus along the periphery of the bloodstream. No classic macrophage or giant cell was seen in the infiltrated tissue of the cornea nor did we find any Russell bodies in this structure, although they are common in the uvea. As in endemic areas repeated invasion of the eye is to be expected, so a complicated picture usually arises where acute and chronic changes overlap, and this may well explain why in longstanding cases eosinophil leucocytes are still present; in healed areas, they might be expected to disappear if it were not for this. They never dominate the picture, however, as they do initially in the acute stage, except locally where a parasite in the early stages of dissolution exists.

As mentioned earlier, in association with this sclerosing keratitis there is marked congestion of the limbal vessels, which are invariably cuffed with plasma cells. As invasion of the eye by mf. via the limbal route is going on all the time, and as the life of the parasite is a short one, this is to be expected. Here, too,

fibroblastic repair is a feature, so that adhesions between the conjunctiva and the bulb at the limbus are common. The internal elastic lamina of the limbal vessels becomes defective, and the scleral elasticum also stains badly. Parasite bodies are found in a heavily-infected eye throughout all parts of the cornea in different stages of dissolution, and present a remarkable if somewhat confusing picture. We have found as many as ten in one section (7 μ) of cornea.

5. ANTERIOR UVEITIS

The microscopic appearances of the iris and ciliary body will be described under this single clinical term, although where there is onchocercal disease of these structures the anterior choroid is usually affected in consequence; in fact in severe cases the entire choroid will be affected, the condition being one of an endophthalmitis. We obtained slight but authentic evidence, nevertheless, that the posterior uvea can be attacked alone in the absence of anterior involvement, and so for this reason will discuss choroidal changes separately under the heading of posterior uveitis.

The classic anterior uveitis of onchocerciasis exhibits occlusion of the pupil, frequent adhesions at the angle of the anterior chamber or at the posterior corneal face, and quite often a mass of tissue lying in the position one associates with hypopyon.

In acute eyes these inflammatory exudates are soft and yellow, in long-standing they are tough, white and fibrinous. The latter lead to distortions of the pupil, characteristically in a downward direction so that it becomes pear-shaped. Where the occluding membrane becomes wholly or partially absorbed there is nothing specific in the clinical picture, so that it is harder to diagnose than, say, the keratitis. It is in the writer's view the acute phase which determines the prognosis; he has written elsewhere (Rodger[15]) supplying evidence that such acute attacks occur, although this hitherto had been denied; the truth of the matter is that the course of the disease is punctuated by acute attacks of very short duration. If such an attack is severe, the inflammatory exudate will be great, and there is less chance of absorption occurring. The factors which determine the amount and degree of exudation are complex, and have been discussed in the previous part of this thesis. When we take all these facts into consideration it is clear that to classify the lesion as either 'non-granulomatous' or 'granulomatous' is not possible; a short mild attack where the exudate is absorbed leaving few signs behind (characteristic of the former) is just as common as a long-standing one punctuated with acute phases which lead to occlusion or some other gross residuum (characteristic of the latter). In the description which now follows, the course of events is traced microscopically from the first acute attack to the resting phase in the old quiescent blind eye.

Microscopic appearances:

If it is the extra-iridal exudate which dominates the clinical picture it should be the nature of the infiltrate within the tissue which dominates the microscopic picture - as it did in the case of the keratitis, where a high significance may be placed on the presence of eosinophil leucocytes beside disintegrating parasites. In fact, so dense is the pigment in the African uvea that there is no question that it is the pigment changes which are the most striking. We also found them the most interesting.

The toxic products of the mf. bodies lead to great activity on the part of the pigment cells. In the first instance, it is those of the posterior pigment epithelium which are stimulated. Even in apparently healthy eyes a few of these cells can be seen to have broken away and passed into the iris stroma; usually they lie not far from the maternal epithelium, but some pass close to the anterior face of the iris. Characteristic of the cell is the appearance which has been described as 'clumped'. The clump cell, whether free or fixed, is the melanoblast of the iris. The stimulus of the first parasitic toxins causes many of them to break away immediately into the iris stroma, a tendency most marked at the root; at the same time they step up the manufacture of pigment granules; these are shed into the tissues. We have never been able to see how the granules come to be liberated; the cell membrane does not seem to rupture; the granules appear to move out through the surface in a manner impossible to understand. Whatever the mechanism, as

the uveitis increases in intensity, vast numbers of these granules can be seen lying free in the stroma. In this way the second pigment cell of the iris, the chromatophore, comes into the picture. The latter can be seen at this time very actively reproducing themselves; mitotic figures are common, being more readily seen in the less dense chromatophore than in the very dark-coloured clump cell. As soon as the chromatophore is formed it starts taking up the surplus granules. Clinically the lesion is still acute when the chromatophores themselves withdraw their processes and adopt the shape, if not the colour, of the clump cell. It seems probable that in the period between being released by the clump cell and picked up by the chromatophore the granules undergo a change, probably a chemical one; this would explain why their size is greater in the melanoblast than in the chromatophore. The lighter colour and the smaller size always enables us to distinguish the latter from the former, even after each has become clumped.

Both cells tend to attach themselves to the walls of the bloodvessels, but in view of the great proliferation of pigment cells and vessels this may not be purposeful. We have found transitional forms between the amoeboid- and the clump-shaped chromatophore in acute eyes, so there is no doubt what is happening. Later on in the course of the uveitis they become increasingly dark in colour until it is impossible to distinguish them from epithelial clump cells; one gets the impression, nevertheless, that the melanoblast always remains the larger. In addition to the greatly

increased activity of the epithelial clump cells within the stroma in raising the production of pigment (which makes one wonder 'why?'), and of the chromatophores in multiplying and scavenging the granules, both types of cell gradually assemble along the anterior face of the iris; they also concentrate increasingly at the pupillary margin among and in the neighbourhood of the inner circle capillaries and smooth muscle fibres. As a result of this activity in the acute and subacute stages the picture presented is of an iris etched in black with a large polka-dot design within it.

In the established chronic anterior unveitis, the pigment epithelial cells which have not migrated into the stroma have become so swollen that they now form what appears to be a continuous black band of pigment; in bleached sections they look like a line of compressed cushions; each of course is simply a fixed clump cell. This is characteristic of a chronic anterior uveitis; in places several μ long, usually near the root whence the earliest and greatest migration occurs, the pigment epithelium is totally depigmented, and the few residual cells have atrophied. There is usually a membrane adherent posteriorly to the epithelium at these places, in the fibrin of which chronic inflammatory cells are entangled. A similar cushioning occurs in the cells which have massed along the anterior face, although here the fused band of pigment is not so smooth in outline, nor is there any evidence of depigmentation until much later. Clump cells of both types still abound in the stroma, but there is in places usually evidence now

of a return to normality, for the processes of some of the chromatophores have started to reappear; the pigment within, however, is still dense.

When the processes of healing are complete and the eye becomes quiet, the most striking microscopic feature is the greatly decreased intensity of the pigment in the chromatophores. Once more they can be recognised by their elongated processes, but they are paler than in health and are compressed, lying parallel with the surface. The greater area covered now by these elongated cells as compared with the small spherical shapes when they were clumped gives them the appearance of sardines packed into the iris, layer upon layer. The row of pigment cells along the anterior face has become thin and in places depigmented, as we saw happened earlier in the case of the posterior epithelium; the appearance of the latter is much the same. Here and there an occasional clump cell can still be seen in the stroma, its dark colour in striking contrast to the other pale chromatophores, which helps to remind us of the inflammatory storm that has now subsided. Many of the capillaries are heavily coated with pigment, the lumina often being occluded. There are no longer any signs of mitosis; no longer do surplus granules lie everywhere; no longer are collections of clump cells found in every available corner; one gets the vivid impression that a party is over. Clinically the iris is faded and limp.

The description of the pigment changes in the iris should be supplemented by those in the ciliary body which are less dramatic

but somewhat similar. The pigmented and the nonpigmented epithelium are both involved. There is some migration of the cells into the stroma of the processes, but not a great deal; the more usual route is on to the surface of the ciliary epithelium. Here the liberated granules lie in a cyclitic membrane where they are picked up and ingested by wandering cells. Local proliferation in places is associated, as in the case of the iris, with loss of pigment in others.

Subsequent to such complications as posterior synechiae, where there has been gross proliferation and migration of pigment into the pupillary area, there is likely to result ectropion of the posterior epithelium or depigmentation of the fringe.

If the pigment changes strike the eye most readily, the vascular changes are almost as remarkable. In the early acute phase the vessels become heavily congested, the walls dilate, inflammatory cells - of which by far the greatest number are eosinophil leucocytes - can be seen lining the endothelial walls, and reduplication of vessels commences. The latter is most prominent at the inner pupillary margin, which swells to twice its resting size as a result.

When the anterior uveitis becomes established and passes from the subacute to chronic phase, the vessels begin to show evidence of poisoning. The endothelium swells and proliferates; the elastic lamina reduplicates itself but takes less and less stain. The ciliary vessels have an internal elastic lamina of the usual type, but in the iris it is never as clean-cut as elsewhere,

even in healthy eyes; now, however, it stains very faintly if at all. The space normally present in the wall of the iris artery disappears as it is filled in with hyaline material. In the ciliary body, on the other hand, fibrosis of the arteries not hyalinisation occurs; this does no more than reflect the essential difference in the structure of the two vessels, which in turn reflects their different functions. In old cases, where the uveitis is quiescent, these changes are even more striking: the lumen of the iris artery or arteriole is closed, a few heavily-staining endothelial nuclei only remaining, and the rest of the vessel is hyalinised; no elastic fibres remain in the wall. In the case of the ciliary vessels many of them are completely sclerosed. The absence of vessels in the chronic anterior uveitis is as striking as the abundance of them was in the acute.

The condition being described is characterised clinically by the exudate produced. In the early acute stages this exudate pours out from all the surfaces of the anterior uvea: into the anterior and posterior chambers, into the vitreous, over the ciliary processes and the pars plana, even above the anterior part of the retina; it also is present within the structures involved. As a result it covers the pupil, blocking the angle, forming posterior and anterior synechiae, a cyclitic membrane and a supraretinal pellicle. Not surprisingly the health of the lens suffers so that secondary cataracts are extremely common.

From the start there is a lot of fibrin present in the exudate; even in eyes where the pathology suggests we are dealing with a chronic rather than an acute condition there is fibrin to be seen in the exudate. This supports the observation that repeated small acute attacks occur. The exudate stains pale red with eosin, pale blue with the trichrome stains. The fibrin is less easily observed within the stroma of the iris and ciliary body. When the exudate is examined microscopically in the chambers of the eye, it is seen to contain ghost cells (empty red corpuscles), fibroblasts, several plasma cells, a few lymphocytes and several monocytes. Only rarely was an eosinophil leucocyte found in the exudate of the ocular chambers. In the advanced lesion the exudate has collected on the surfaces of the iris and the ciliary body. Fragments of parasites can be seen along with fibroblasts, plasma cells, lymphocytes (usually with a clearly-outlined cytoplasm) and monocytes. The latter often show the elongated processes of an amoeboid phase. The presence of monocytes in the free exudate is as striking as the absence of eosinophil leucocytes. An occasional haemoglobin body can be seen alongside the ghost cells and fibrin bodies may be found occasionally. Over the ciliary processes free pigment granules lie, some of them having already been ingested by monocytes. In the healed condition organisation of the exudate leads to the deformities already described, such as blockage of the angle with its resultant effect on intraocular tension. In the vitreous the residual exudate in the chronic stage is albuminous; there are many red cells

and ghost cells present along with lymphocytes, plasma cells and monocytes, some of the latter being amoeboid, some round. The area occupied by the exudate, as we would expect, is now quite small.

Although the nature of the infiltrate within the iris and ciliary body is rather overclouded by the pigmentary and vascular changes, it is highly significant. In the acute eye there is a high proportion of eosinophil leucocytes. Plasma cells also appear early on. In the established lesion, subacute or chronic, plasma cells more and more dominate the picture, and plasma cytoid cells, Russell bodies both intracellular or free in the stroma, are common; the so-called unicellular Russell body, which we understand is the last stage in the process of degeneration of the plasma cell, is also found. Although present in both anterior uveal structures, the Russell bodies were much more common in the iris. As the inflammatory reaction becomes quiescent the eosinophil almost completely disappears; the plasma cells, plasma cytoid cells and Russell bodies which appear indicate what is happening. Rarely a small 'nodule' of lymphocytes may be seen. One new cell appears in the late stages, and that is a large acidophil cell with two or more nuclei, in the cytoplasm of which are several vacuoles and granules, some of the latter pigmented. Three or four such cells invariably were/present in each half iris in each section, in chronic quiescent eyes. By reason of their size, four times that of a plasma cell, and the fact that their red cytoplasm was so distinct in the atrophic depigmented uvea, they are quite conspicuous.

Clearly a histiocyte, the size of these cells corresponds closely to that of a clump cell. In our view it is probably a variant of the latter. This is a type of cell about the origin of which it is impossible to be dogmatic.

Such, then, are the changes which occur in the anterior uveitis of onchocerciasis. In brief, to collate the points we have treated separately, in the acute phase the pigment cells proliferate, the arteries and arterioles multiply, a fibrinous exudate in which red cells and inflammatory cells appear is poured out within and without the structures concerned, and in the interstitial tissue the dominant cell is the eosinophil. As the condition settles into the chronic stage the pigment cells fuse into a solid band on both faces of the iris, the clump cells continue to multiply and get darker, the exudate starts to become organised, the vessels degenerate (the iris vessels becoming hyalinised), and the eosinophil leucocyte is replaced by the plasma cell. Russell bodies and hyaline spherules are now common. After healing, when the eye is quiet (and blind usually) the pigment is pale, the iris chromatophores, thin and drawn out, lie parallel with the surface, parts of the posterior epithelium including that covering the ciliary body are depigmented, many vessels are hyalinised (in the iris) or sclerosed (in the ciliary body), deformities due to the organised exudate are common, and among the plasma cells which remain are found several large histiocytes intent on clearing up the debris.

6. POSTERIOR UVEITIS

The term posterior uveitis is in our view a correct one, for it is an inflammation of the choroid, exogenous and exudative, due to the death of mf. volvulus in this structure. Severe affections of the choroid always involve the retina, and in onchocerciasis this is no exception. We have never found a single case, however, where the retina was involved in the absence of choroidal inflammation, or where it was affected first, although the reverse is common. True, we have found parasites in the retina, and if they die there before dying in the choroid a primary retinitis would ensue, but that we believe is a rare occasion. As a rule the retina is involved in the inflammatory reaction in small patches, a fact which can be observed macroscopically. It is possible that these areas of chorioretinitis occur only in the immediate vicinity of where a parasite has died in the choroid, the titre of toxin being sufficiently great at such places to affect the retina; the same products of intoxication on the other hand will spread readily in both directions within the choroid to enlarge the uveal lesion laterally. It is difficult to explain how else these islands of chorioretinal fusion arise. With massive invasion of course the entire uveal tract and retina may be involved; there will be an endophthalmitis.

Above it has been mentioned that an anterior uveitis is usually associated with some degree of posterior inflammation. Early on this may be due to the diffusion of toxins backwards into

the choroid; this would explain the presence of chronic inflammatory cells in the anterior choroid in the absence of mf. Once the parasites have entered the iris and the ciliary body, however, they are almost certain to pass posteriorly into the choroid in the end, when a primary posterior uveitis will commence. The cells characteristic of the new acute reaction will necessarily mingle with those of the subacute or chronic anterior inflammation, which has passed back earlier into the choroid. As a result it is as difficult to establish the existence of an acute phase in this lesion microscopically as it is clinically, where the fundus can seldom be clearly seen owing to the frequency with which the anterior segment is simultaneously involved.

Finally, we have to consider the possibility of the posterior uvea being invaded by the parasites without anterior segmental invasion. This prospect was put forward clinically as a possibility in an earlier paper[15]; it was raised again when we described the passage of the mf. down the vascular sheaths of the equatorial vessels (Rodger[16]); now one of the excised eyes furnishes proof of it. It is a nonspecific uveitis with which we are dealing, and may be confused at some stages with the degenerative lesion of the choroid and retina found in this same disease, which will be discussed next. Although there is no doubt that a primary posterior uveitis occurs, we hold to our previously expressed conclusion that such a lesion is seen very rarely as an isolated phenomenon. In general, then, a posterior uveitis is associated

with an anterior uveitis, the retina is involved secondarily and then only in patches, and it is rarely that a posterior uveitis by itself is found. In some eyes with an anterior lesion a few chronic inflammatory cells are present in the posterior uvea, in others the uveal invasion by these cells is marked, in still others living parasites exist in the area that is infiltrated, and in others again living parasites are found outside the infiltrated area altogether in what is apparently healthy choroid posterior to the equator. These findings underline the chronological and topographical chain of events leading to the development of the posterior uveitis. Macroscopically the choroidal vessels are greatly congested, and the exudate filling the vitreous cavity is noticeably present.

Microscopic appearances:

The pigmentary changes although striking do not dominate the picture in the posterior uvea. In the negro eye the choroidal pigment is darker than the retinal unlike the white races; the opposite state of affairs, as we saw, exists in the iris, where the pigment corresponding to the retinal (the posterior pigment epithelium) is darker than the pigment corresponding to the choroidal (the chromatophore of the iris stroma). This is important when the question of invasion of the retina by unidentified pigment arises. At the height of the inflammation in the anterior uvea, both types of cell become equally dark; this is also true of the posterior

segment. A second point of similarity between the two areas is that the pigment granule formed by the retinal melanoblast is bigger than the granule contained in the choroidal. These facts do not seem to have been reported before; nor do they correspond to what has been described in the European eye. As the retinal pigment is affected early on in an onchocercal uveitis they will be described along with those occurring in the choroid.

The granules of retinal pigment are characteristically spindle-shaped. They lie within the outer processes of the rods and cones, the base of the epithelial cell being devoid of pigment in its outer part; the granules, present in its inner part, are more elliptical than those in the processes, but are not completely round as some writers have said. The nuclei of the retinal pigment epithelium are of two types, suggesting that two types of cell are present: one is darker and flatter than the other. It is believed that the former manufactures the inner cuticular layer of the membrane of Bruch, while the other type of cell with the paler, rounder nucleus is concerned wholly with the manufacture of pigment. The changes which develop consequent on the death of parasites in the uvea support this view. Taking the posterior segment of the eye as a whole, disruption of the retinal pigment epithelium occurs only adjacent to an inflammatory focus in the choroid. This is an important means of distinguishing between the exudative posterior lesion and the degenerative one. Coinciding with the reaction of the retinal pigment is that of the choroidal; here the uvea is

affected over broader stretches and disturbance of the pigment can be seen in all parts.

The first change we observed was that the spindles of pigment in the processes of the retinal pigment cells withdrew from between the outer limbs of the rods and cones into the cell body, mixing with those already there. As they do so they themselves become rounder, corresponding to the granules of the base. At first sight these changes seem to occur in every cell in that area, but as they become clumped under further provocation, and break away into the retina, so the second type of cell, that with the flatter darker nucleus, becomes exposed. The latter are now visible as a single continuous layer of flat cells, maintaining in some way the continuity of the epithelium despite the breaks made by the pigment cells which have broken away. The clump cells of the retinal pigment do not wander far during the initial acute phase, usually being found lying near-by between the rods and cones. They tend to form small groups, attaching themselves to each other. The choroidal pigment cells meanwhile have moved imperceptibly inwards towards the choriocapillaris, which is pigment-free even in the negroid eye. These cells are so dark that it is difficult to assess the relative size of the granules except at the outer fringe. As was seen in the anterior segment the granules leave the clump cells of the retina without apparently breaking the cell body, but none apparently is liberated outwards into the choroid. The membrane of Bruch

probably prevents it. The increase in density of the uveal pigment posteriorly, therefore, would appear to depend upon the tendency of the choroidal pigment cells to make more pigment and accumulate in masses rather than on taking up surplus granules released by the retinal cells.

As the condition advances the retinal pigment gets darker until the colour corresponds to the very dark choroidal pigment. At this stage, unless the size of the granules can be assessed, there is no way of concluding by this means whether or not choroidal pigment has passed into the retina. In fact it never does because although the continuity of the cuticular lamina of the membrane of Bruch is broken in severe cases, the elastic lamina never is, not even in an onchocercal endophthalmitis. In longstanding cases the cuticular layer becomes locally hyalinised with the production of colloid bodies. In addition the retinal clump cells migrate into the nervous layer in chronic cases, and in places the vessels are surrounded by pigment granules, which either have been liberated by the cells or left behind when the pigment cells disintegrated. The distribution of pigment around the retinal vessels is dissimilar to that observed in retinitis pigmentosa; it does not attach itself by preference to veins, but to arteries, and lies indiscriminately among the ectodermal glia and mesodermal adventitia, forming large plaques of pigment, seldom seen with the ophthalmoscope, in the shape of bone corpuscles.

When the eye becomes quiet, the choroidal pigment cells start to elongate, as we saw in the iris, and lie parallel with the membrane of Bruch; they retain their dark colour and tendency to stay close together. The part of the choroid corresponding to Haller's layer of vessels is frequently now devoid of pigment cells. The retinal pigment is most frequently seen at this stage in small rounded aggregations lying among the rods and cones. These changes are most striking where the greatest infiltration and destruction of tissue has occurred in the choroid. Such areas are disseminated although sometimes confluent as well.

The inflammatory exudate of posterior uveitis is found within the choroid, between the choroid and the retina (in places), and on the surface of the retina. It is acidophil with much fibrin in it until the chronic stages are reached. The same type of inflammatory cell can be seen in this condition as were described in the anterior uveitis; eosinophil leucocytes early on dominate, later plasma cells. Plasma cytoid cells and Russell bodies are not uncommon but we never found large histiocytes as we did in the iris.

The vascular changes also reflect those found anteriorly, and in a structure like the choroid are of course important. Early on the endothelium of the congested vessels become swollen. Eosinophil leucocytes are present in large numbers in the paraxial stream. The endothelial cells then proliferate both in arteries and veins, especially the former. Hyperplasia of the media and adventitia commences until the process of sclerosis leads to complete

occlusion. The internal elastic lamina of the arteries is not affected until late on when it stains faintly or not at all. The choriocapillaris is not affected first; even in chronic cases it is patent except where the greatest destruction has occurred. Sclerosis when present is striking but by no means widespread.

In the vicinity of the greatest destruction of the uveal tissue, probably the site of a parasite's death, the retina is grossly affected. Where a parasite dies within the retina such destruction would be greater still. The rods and cones in these areas are quickly destroyed by the toxins, the bipolar cells diminish in number, the ganglion cells become distorted, and finally, in this order, the rod and cone granules (which appear to be the most resistant of all) become reduced. Gliosis occurs in the later stages. Thus when the lesion has healed, a gliosed retinal patch lies side by side with a fibrosed choroidal. This has, in our opinion, been wrongly described as an area of chorio-retinal fusion. There is no actual fusion, for even in the most severely affected areas, as we showed above, the membrane of Bruch remains intact. The plasma cells in the uvea which are present for a long time in such areas, resemble the rod and cone granules giving the impression that the latter have invaded the choroid; this may have encouraged the idea that actual fusion occurs.

We have described what is essentially a nonspecific posterior uveitis. It is the association of such a condition with anterior segmental lesions typical of onchocerciasis which

establishes the diagnosis. We have, nevertheless, at no time failed to find in such eyes living or dead mf. in the choroid.

7. THE POSTERIOR DEGENERATIVE LESION OF ONCHOCERCIASIS

It was first suggested by the writer that this condition is a degenerative one, not an inflammatory (Rodger[17]). When coupled with the observation that an (inflammatory) posterior uveitis also occurs in onchocerciasis much of the confusion which has ruled in the past can be eliminated (Rodger[18]). The fact of the matter is that ever since an affection of the choroid and retina in this disease was recognised by Bryant[19] and Hissette[20], the posterior segmental changes have been a controversial topic; while we have established without doubt that mf. die in the posterior uvea and produce an inflammatory condition there, which may or may not involve the retina, the pathogenesis of the degenerative lesion has not yet been explained satisfactorily. An earlier part of this thesis attempts to do so. The important thing to remember is that the degenerative lesion is not associated with the presence of parasites in the eye, yet is only found in onchocerciasis patients.

The fact that so vague a descriptive term should be given to the lesion might appear to underline a certain lack of confidence on our part in the pathogenesis we ourselves have suggested. This is not true. In classifying it we are up against a difficulty inherent in ophthalmology, the classification of which includes many misnomers, that remain unchanged because they are colloquialisms.

The most common error has been in classifying a disease as inflammatory when it is in fact not associated with any of the phenomena of inflammation. The usual alternative has been to rename these etymologically-incorrect terms as "pathies" or degenerations, although these are heterogeneous groups based on inconsistent criteria. The lesion in question could be described as an atrophy, but is it an atrophy? Do atrophy and degeneration clinically mean different things? Atrophy may occur as a result of the action of toxins, we know, and in this lesion we have suggested intoxication plays a part, which might lead to arterial spasm or to vascular degeneration and atrophy of the rods and cones. Avitaminosis A may also play a part, and the word atrophy scarcely covers that; degeneration would be better. Then again we may be wrong on all counts. There may be some explanation other than an intoxication coupled with vitamin A deficiency. The lesion might be classified as a disorder of metabolism, or of circulation, or even turn full-circle and prove to be a mild inflammation after all. In ophthalmology each of these possibilities would determine the ultimate title; so we have called it simply 'the posterior degenerative lesion of onchocerciasis', leaving it to other workers to confirm or disprove our proposals as to the pathogenesis before committing ourselves to anything more definite. Clinically we would be justified in calling it a central areolar circumscribed choroidoretinal atrophy, for that is what it looks like. Such conditions may be familial in

origin, but we have eschewed this explanation because in a primitive community you can never prove it. Our experiences suggest that inbreeding in the endemic onchocerciasis areas of Africa is a myth encouraged only by the more flamboyant of the anthropologists and sociologists, who crowd there from the west.

In the condition we are describing there is no exudate, little infiltration, no sign of eosinophilia, no parasite in the eye, no anterior onchocercal lesion with which to tie it in (except rarely and then as a secondary complication). There are no areas of choroidoretinal inflammation disseminated here and there, just a simple straight-forward atrophy of the retina and the choroid, all of one piece, with a clear-cut margin, within which, as a result of pigmentary degeneration, the choroidal vessels can be seen. It is strange the two conditions have been confused for so long, yet although the difference between them is so obvious microscopically, clinically pigmentary upset and exposure of choroidal vessels dominate each.

Microscopic appearances:

The earliest changes (if not the most striking) involve the retinal pigment epithelium and the choriocapillaris. Neither of them coincide topographically, so it is impossible to say which occurs first, and it is unlikely that one causes the other.

The first area affected usually lies between the disc and the macula, although in some eyes the changes are observed simultaneously in both parts. A reduction of the number of patent

loops in the choriocapillaris is not so commonly seen early on as the pigmentary disturbance, but is the rule in the area next the papilla. The changes in the retinal pigment are those of depigmentation. The thinning-out process is best likened to the effect that would be produced if every second stake in a fence were removed. This corresponds to what we have described with the ophthalmoscope as 'freckling of the fundus'. It is a genuine loss of colour for the outline of the spindle-shaped granules can be seen devoid of pigment; as a result the choroidal vessels can early on be viewed with the ophthalmoscope, although at first they will be healthy. The macula, which we have said may or may not illustrate these changes is usually oedematous; the latter vanishes later on when the macula becomes increasingly involved in the atrophic processes.

In the established case where there is no longer any doubt clinically as to the diagnosis, certain areas of the retina reveal proliferation of the pigment cells; they become clumped and either fuse together into a long thick band of pigment (usually at the periphery of the lesion) or break away into the nervous layers of the retina where they form small aggregations or even attach themselves round the retinal vessels. Here too, nevertheless, colourless granules can be seen. Secondary "retinitis pigmentosa" occurs in 5% of all posterior cases. It is a term that is open to error. We have never seen the fine bone corpuscle appearance typical of a primary retinitis pigmentosa, nor does the distribution

of the pigment around the vessels correspond. It is usually much darker and more clumped, and we prefer for this reason the term 'pigment corpusculation', which can be modified by adjectives to suit all occasions. Around the vessels, the swollen pigment cells lie in all three coats of the arteries up to and touching the internal elastic lamina; nor are hyaline changes in the smaller vessels seen as in primary retinitis pigmentosa; and the choriocapillaris is always affected somewhere. In none of these respects is the posterior lesion similar to a familial retinitis pigmentosa.

We have described the activity of the retinal pigment epithelium at length for it seems to us to be of the highest importance, coinciding clinically, as it does, with hemeralopia. The changes in the choroidal pigment are less marked; the cells tend to migrate into the pigment-free choriocapillaris, but they do not aggregate very much, which was a feature of onchocercal uveitis; even in the areas of worst degeneration it is found in small patches, distributed equally throughout this structure, each patch consisting of two or three sausage-shaped cells strung together, and parallel with the surface. Wolff[21] calls them both chromatophore and melanoblast, which is a contradiction in terms at first sight. Yet there is no way of explaining, as we did in the case of the iris, how they obtain the contained pigment. The nearest melanoblasts are in the retina separated by the impervious lamina vitrea as we showed when describing the inflammatory lesion. Wolff is almost certainly right, for in the

degenerative type the elastic lamina of Bruch always stains well. It is a complete barrier between the two pigments.

Continuing the description of an established case, coinciding with the retinal and choroidal pigmentary disturbances just described is a striking degeneration of the retina. The rods and cones are totally destroyed and their attached nuclei disappear. The bipolar cells become reduced in number, and some fall out of line. They never completely disappear, being the only survivors. The ganglion cells degenerate imperceptibly, and there is no gliosis; the retina simply dissolves away, figuratively speaking. The affected area commences abruptly with the sudden disappearance of the rods, cones and their nuclei. This gives the lesion its characteristic punched-out appearance. As we approach the disc, all that remains in what is the oldest part of the lesion is a thin layer of bipolar cells, two cells thick very often. The same appearance is found on the other side of the disc (for this is an areolar lesion) and as we move away from the optic nerve head we finally come to the same raised margin, as healthy retina appears once again. This margin is frequently accentuated microscopically (concealed clinically) by a heaping-up of the retinal pigment cells, which become very dark and expanded. The membrane of Bruch over the entire area maintains its continuity; nor have we ever seen infiltration of the retina with chronic inflammatory cells.

The choroidal changes which coincide are equally distinctive. There is no congestion of the vessels, no exudate, and the marked hyperplasia of all the coats of the arteries as well as the elastic changes in the vessel walls seen in the exudative lesion are absent; moreover infiltration with plasma cells is only half as great. The fibrocytes, which constitute the adventitial sheaths and the syncytium of the stroma in which the vessels lie, insidiously overgrow the choriocapillaris, arterioles, and arteries of the posterior uvea. The veins which for the greater part lie in the outer side of the choroid do not seem to be so greatly involved. It appears that what we are dealing with is a reactive fibrosis of a very slow and subtle type. This is the sort of thing which could happen as a result of an ischaemia from prolonged arterial spasm, like that caused by an intoxicant. A cursory glance gives the impression that no sclerosis of vessels exists, for none of the whorled vessels typical of arteriosclerosis is to be seen. It is only on closer inspection that one becomes aware that not only has the choriocapillaris completely disappeared in the degenerate area but that most of the arteries have as well. Earlier we have noted some hyperplasia of the intimal coat, but now the intima is not to be seen; the elastic lamina is also lost. There are no monocytes or giant cells in the established case but plasma cytoid cells, lymphocytes and an occasional Russell body may be found among the plasma cells; the lymphocytes sometimes collect into fairly large 'nodules'.

The final question to be answered is whether or not the retinal degeneration occurs as a result of the choroidal sclerosis. The pigment changes and the rod and cone degeneration, however, are not necessarily associated with an overlying sclerosis of the choriocapillaris, so we tend to the view that the two are not at first any way related. When the blood supply of the choroid, however, becomes seriously reduced, then probably the whole retina is quickly involved. The comparative resistance of the bipolar cells must depend upon the nutrition obtained from the retinal vessels themselves.

8. OPTIC ATROPHY

At a meeting of the Royal Society of Tropical Medicine and Hygiene in London in January, 1958, when onchocerciasis was discussed, the following statements were made:

1. Iritis does not cause cataract.
2. Optic atrophy and retinal degeneration are not manifestations of onchocerciasis.
3. As mf. volvulus are not found in the bloodstream it is difficult to understand how they gain access to the optic nerve and the choroid.
4. Onchocerciasis is not a serious cause of blindness.

It is a good thing for the fair name of the eminent Society concerned that most of the speakers did not agree with these wild statements. The present thesis supplies most of the answers, which had been suspected by field workers in many instances, and in others already known and recognised. Optic atrophy,

nevertheless, is one condition which we agree is doubtful. Workers, such as Toulant[22] with whom I have had long discussions, consider that onchocerciasis produces a characteristic optic atrophy which may be recognised by sheathing of the arteries; there is nothing more to their arguments than this, which seem rather slender. No eye where an isolated optic atrophy existed was examined microscopically in the present studies, so there is no new evidence to confirm or disprove that a primary optic atrophy can occur as a result of this disease. On epidemiological grounds we have shown elsewhere (Rodger[23]) that it is unlikely. On pathological grounds we have also shown that it is unlikely, in Section I of this part of the thesis. However, in a heavily infected eye, there is no doubt that the optic nerve can be and is invaded. The photomicrograph presented here proves it. Giaquinto has also shown mf. in the nerve substance, as previously mentioned[9]. In a posterior uveitis and in the posterior degenerative lesion, atrophy of the nerve is certain to happen sooner or later as a secondary phenomenon; where the parasites in the former condition pass into and die in the nerve itself, then the lesion will in a sense be a primary inflammation. Whether this happens in the absence of other lesions in the eye, we cannot say. We don't think so. Atrophic cupping is the rule in the case of the posterior degenerative lesion, but in the uveitis, where anterior and posterior lesions are frequently conjoined, glaucomatous cupping is common, the angle of the eye being blocked by the

organised exudate. Sheathing at the disc is variable, and in our opinion occurs more often in the inflammatory than in the degenerative lesion. The microscopic appearances confirm most of these generalisations.

Microscopic appearances:

In the early stages of the posterior uveitis the nerve head does not appear to be affected. In an established (acute to subacute) case there is definite oedema of all the tissues of the papilla. It even involves the adjacent retina. The swollen disc protrudes into the vitreous; this is not accompanied by very much infiltration with inflammatory cells, although the retinal and posterior ciliary vessels show an unusually high proportion of eosinophil leucocytes then. In addition all the vascular coats exhibit signs of hyperplasia at a fairly early stage in the condition, and within the nerve itself the nutrient vessels are engorged.

Later, in the chronic stage, there is dense infiltration of the septa and papilla with plasma cells, lymphocytes and several eosinophils, the former of which are widely distributed. They can even be seen lying on the surface of the disc in a pellicle of fibroglial tissue which has formed there around the roots of the vessels; they are the dominant cells in perivascular infiltration of the small vessels making up part of the circle of Zinn. The melanoblasts of the retina and the pigment cells of the choroid now migrate and accumulate at the edge of the disc usually above the level

of the lamina cribrosa. In one eye we found them forming a mantle around a retinal vein. Early gliosis has also commenced, and it is noticeable that it is the paler of the glial nuclei which is involved in proliferating. The oedema so prominent earlier at this stage is gone.

In the healed quiescent posterior uveitis, glaucomatous cupping is the first thing to meet the eye, the lesion being usually associated with an anterior one. The septal vessels have become largely sclerosed as to a certain extent have the retinal and posterior ciliary arteries. The nerve fibres are atrophic and the process of gliosis now heavily involves the nerve proper as well as the adjacent retina.

In the posterior degenerative lesion the picture is quite different. We have never found the disc oedematous, nor have we seen glaucomatous cupping; an atrophic cupping is more likely to be present. Eosinophil leucocytes at all stages are conspicuous by their absence, not even being seen within the lumen of the vessels, which is a useful way of spotting them quickly. Sclerosis of the retinal and posterior ciliary arteries does not seem to occur. In advanced cases there is a slight to moderate infiltration with plasma cells. The nerve itself when the condition is advanced becomes atrophic and gliosed to such an extent that the septal vessels have largely disappeared. While the choroidal pigment may be somewhat heaped at the disc margin, more usually it is not.

As for the retinal pigment adjacent to the tissue it is among the first to become colourless.

From these pathological observations it would seem that the appearance of the optic nerve should be of great importance in the clinical diagnosis of a posterior lesion, especially early on. However, early cases are rarely seen, so, although it has been reported, we have never witnessed a papillitis. The optic nerve head undoubtedly is more useful in indicating which of the two posterior lesions we are dealing with in advanced cases. It is doubtful if either a papillitis or an optic atrophy ever occurs apart from a coexisting posterior lesion. After all on what grounds could such primary conditions be diagnosed as being due to onchocerciasis where found alone? The picture is quite non-specific.

But this is dangerous ground on which we are treading; that the optic nerve in onchocerciasis can exhibit a papillitis or an atrophy we have demonstrated, although in every case along with and apparently secondary to a coexisting lesion in the posterior uvea and retina; theoretically a primary affection could also occur. That has to be admitted.

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PART V.

CONCLUDING

The names of Ochoterena[1], Strong[2], Hissette[3,4] and Rodhain[5] are among the most renowned in the history of the pathology of onchocerciasis. Of British workers, Bryant[6] reported on two eyes exhibiting the posterior segmental lesion which he called 'Sudan Blindness'; Hughes[7] reported on one eye obtained at autopsy; Ridley[8], who was unlucky and obtained no eyes, did British ophthalmology a service, nevertheless, by reviewing the subject at a time when it was badly needed. This review has certain limitations based on the fact that it is a summarised version, part of a monograph on the entire subject of onchocerciasis. In the circumstances it would seem we were most fortunate to obtain the amount of material we did, for unlike most other workers no eye was bought, or obtained at autopsy; each was excised surgically, after selection, with full permission of the patient.

What, briefly, has emerged from this thesis? All the evidence suggests that the dead bodies of mf. volvulus act as chemical poisons. Animal parasites are frequently living irritants, acting by virtue of toxins which they excrete, or by the mechanical irritation they excite. The adult O. volvulus may come into the former category as far as the degenerative lesion is concerned, but in all the other ocular manifestations it is almost certainly not involved. It is well adapted. The mf. are also well adapted during their lifetime. After they die, nevertheless,

the products of their dissolution cause a violent but brief local reaction; the subsequent dilution and dispersal of the toxic products by the tissue fluid leads to a comparatively mild chronic phase. As the processes of tolerance set in, so subsequent mf. deaths lead to less and less severe acute attacks, but prolong the duration of the irritation. For the greater part in the course of this disease, therefore, the toxins will act as stimulants to growth, different cells being activated to varying extents - rather than necrotisers. Thus initially we have a destructive lesion which is followed by insidious changes furthering those processes which lead to blindness unless tolerance occurs first.

Helminthic parasites are usually engulfed when they die by macrophages. In onchocerciasis as far as the mf. are concerned these cells are conspicuous by their absence, and when a few do appear it is only in the late stages after the parasite has been absorbed; it is the dead tissues they are probably scavenging, especially debris within the chambers of the eye. This makes the condition unique. It also means the parasite bodies must be quickly fragmented and absorbed, although the products of disintegration seem to linger on for a while.

The cells which are present during the acute phase are eosinophil leucocytes, which conforms with the general idea that they are attracted by the products of animals' parasites. These cells appear in the affected tissues quickly and disappear just

as quickly early in the disease, although naturally an eosinophilia of the usual order in parasite infestations can always be found even in chronic infections. The fact that plasma cells and lymphocytes appear in large numbers, following the acute reaction, strengthens the view that antibody formation occurs. Boyd[9] believes that both these cells are concerned with the development of a complete or partial immunity, which, in fact, our experiments demonstrate. That we were unable to demonstrate the transfer of antibodies in the guinea pig or rabbit does not weaken the absolute results achieved in the second group of animal experiments and in man (especially) where a noxious dose of dead mf. injected under the conjunctiva produced a violent local reaction in non-infected subjects; on the other hand, in subjects who appeared to be immune to infection - by reason of the fact that parasites were present in their eyes in great abundance in the absence of any ill-effect - similar injections led to no reaction whatever. This seems to the writer to be an original observation of the highest importance.

As has been stated above, a great many of the catastrophes in ocular onchocerciasis result from the processes of stimulation, or repair. This particularly affects the fibrocytes, the vessels and the pigment cells. It is the growth of these cells into the cornea which turns it permanently opaque. The posterior uvea and the optic nerve suffer in the same way as a result of the

proliferation of glia and fibrous tissue. On the other hand, the exudate which occurs in the acute eye is, if anything, even more prone to lead to blindness as it becomes organised. In the case of the degenerative lesion, where we don't seem to be dealing with an exogenous infection, sclerosis of the choroid is probably the dominant factor, whatever its origin.

These studies suggest how we should tackle the problem of ocular onchocerciasis therapeutically. The quicker the acute attack is brought under control, the fewer the complications (Rodger[10]); the question of an acquired immunity on the other hand needs fuller investigation. It is unlikely, where mf. are found in the conjunctival biopsies and seen in the cornea and anterior chamber of the ocular biomicroscope in eyes that are perfectly quiet and reveal no signs of a previous attack, that none of the parasites (as many as 50 have been counted in such eyes) has died at the time of examination. It is even less likely that the invasion is a recent one. We have seen too many cases for this to be tenable. The only explanation is that these eyes have acquired tolerance. But the idea of complete or partial immunity (tolerance) gains credence beyond these arguments when we consider the fact that very few at all of those who <u>have</u> ocular lesions go blind. In short, the circle of reasoning always returns to the question of antibodies. As the latter consist largely of gamma-globulins their formation must depend upon the general protein reserve of the body. This

is universally low, although to varying degrees, in onchocerciasis areas. But who is to say what the threshold values may be of the processes which determine the degree of immunity? There are too many imponderables here to make it a fruitful line of conjecture at the moment, not until our experimental conclusions have been confirmed by somebody else at any rate.

The greatest controversy at present revolves around the pathogenesis of the posterior segmental lesion. The view expressed here, that there are two conditions, is clinically sound, and the evidence is incontrovertible that while one is caused by the death of mf. in the uvea, the other is not; it is only the pathogenesis of the latter lesion which remains in doubt. The possibility that it is caused by a toxin secreted by the adult worms combined with a vitamin A deficiency was based on evidence built up carefully from a number of findings, statistical, epidemiological, physical, nutritional and pathological; but it is still somewhat equivocal; nor, we admit, are the therapeutic trials very convincing. It may be that it is the death of the adults which releases (as a product of disintegration) a toxin poisoning the choroid and retina, the only difference between this conjecture and what we know happens in the case of the mf. being that the adults die outside the eyeball. For an adequate titre of toxin to arise this way one supposes several adult worms would have to die about the same time; from what we know of the natural history of onchocerciasis such a theory fits in well

with the age of onset of the posterior degenerative lesion which coincides in point of time with the expected deaths of the first adults originally placed in the body about the age of 5. Such then are the facts and figments.

In conclusion, it must be said that we hope this thesis may make onchocerciasis more credible to those who have not worked in endemic areas and are (not unnaturally) usually unconvinced at the enormity of the problem; it is also hoped that these studies may help to advance our knowledge to a stage where the ocular complications can be prevented, and a large under-privileged proportion of mankind given release from what can be called - without any suspicion of melodrama - a living death. A blind man in Africa is comparable to a mentally-defective child in an impoverished family in the west; and there is no Health Service to take care of him.

Kumaon Hills, India. 1st June, 1958.

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APPENDIX A

Photographs 1 to 94

EXPERIMENTAL MATERIAL

Fig. 1: Rabbit 12. Subconjunctival injection of living mf. repeated after a fortnight and killed two days after second injection.

Corneal involvement is restricted to the most superficial lamellae of the stroma immediately adjacent to the limbus. The complete absence of eosinophil leucocytes is a notable feature. Subconjunctival oedema and cellular infiltration was slight. X 410.

Fig. 2: Rabbit 11. Treated exactly as above (Rabbit 12) but dead mf. were used.

The subconjunctival stroma shows a diffuse cellular infiltration which consists predominantly of eosinophil leucocytes, which have formed in one area a well-demarkated and localised aggregation immediately under the epithelium adjacent to the limbus. Fragments of mf. are present among the cells. This is the acute phase of an onchocercal limbitis. X 260.

Fig. 3: Rabbit 23. 8 weekly subconjunctival injections of dead mf. and then killed day after last injection.

An inflammatory pannus passes for 1-2 mm. into the cornea from the limbus on one side of the section lying close under the epithelium. The limbal region is vascularised and contains among the vessels eosinophil leucocytes and plasma cells. This is an onchocercal limbitis with a commencing sclerosing keratitis.

X 125.

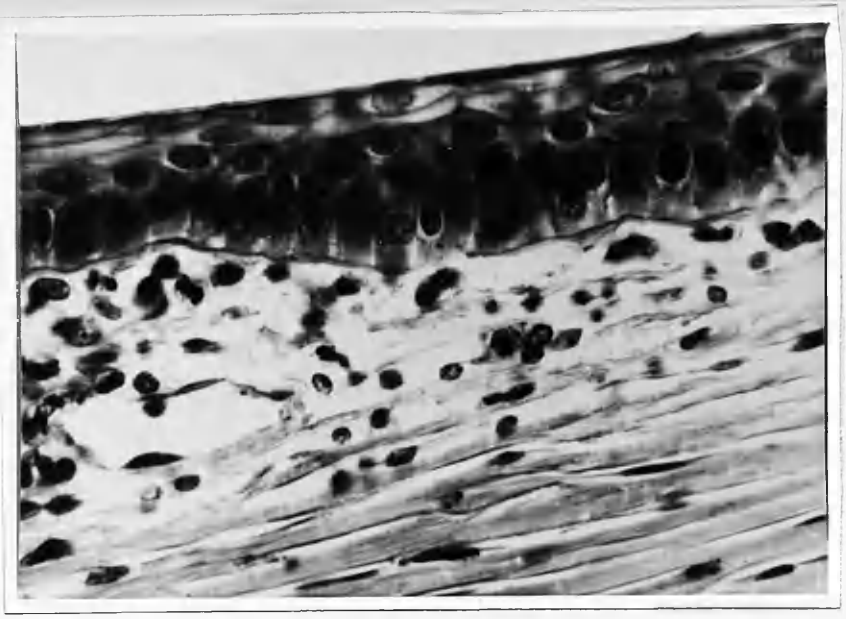


Fig. 1

Fig. 2

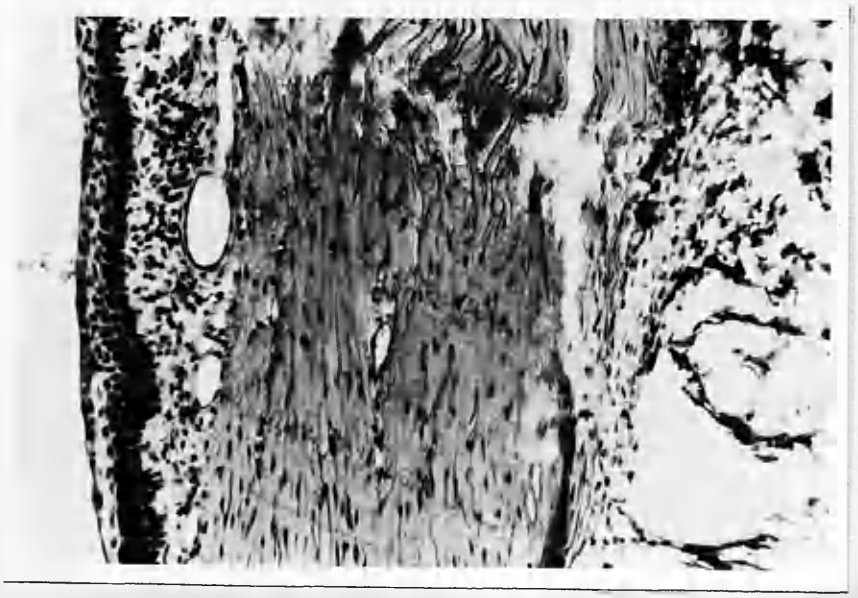


Fig. 3

Fig. 4: Rabbit 8. Intracorneal injections of dead mf. repeated 5 days later. The animal was killed 10 days after the second injection.

An inflammatory reaction can be seen involving half the stroma and consisting of an organising subacute keratitis in which eosinophil leucocytes, lymphocytes and plasma cells are present. New vessels have grown out from the periphery quickly to the area of the injection which lay about 5 mm. from the limbus. This is a sclerosing keratitis associated with interstitial areas of inflammation. X 125.

Fig. 5: Rabbit 14. Dead mf. placed in the Anterior Chamber and iris and animal killed 2 days after injection.

The episcleral tissues and peripheral corneal stroma show an inflammatory infiltration with numerous eosinophil leucocytes in it. It is suggested in the text that this reaction is due to fluid from the suspension of dead mf. having been released during the passage of the needle. The picture is one of a commencing sclerosing keratitis in a keratouveitis, exactly comparable to what is seen in man. X 75.

Fig. 6: Rabbit 14. As above. High power view of angle of eye.

The filtration angle is encircled with eosinophil leucocytes, some monocytes and a few plasma cells. There is abundant fibrinous exudate in the anterior chamber. Blockage of the angle of the eye is a common complication of a keratouveitis or anterior uveitis. X 260.

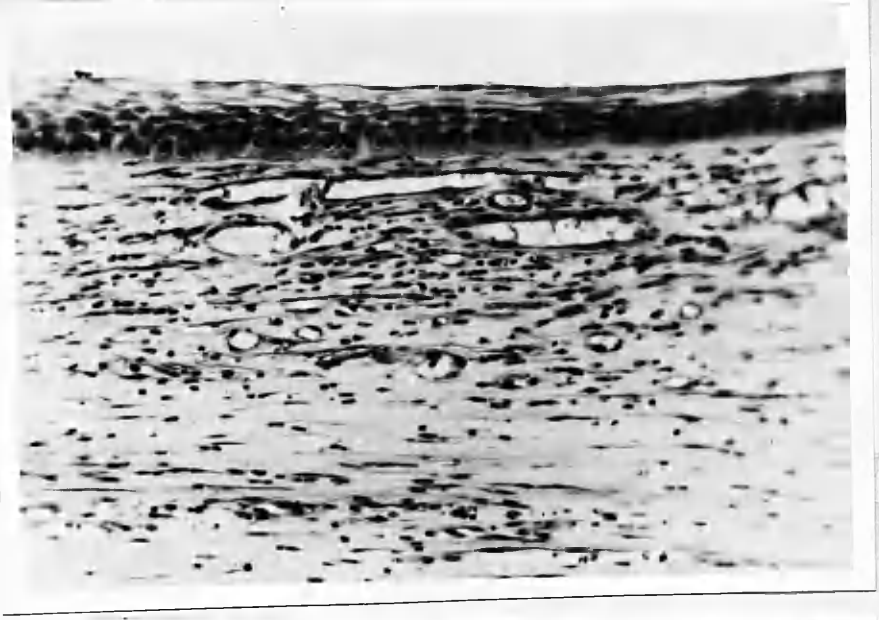


Fig. 4

Fig. 5

Fig. 6

Fig. 7: Rabbit 14. As above. High power view of iris.

Over the surface of the iris there has formed a fine organising membrane. There is a diffuse eosinophilia on the iris vessels, the endothelium of which is swollen, and in places occludes the lumen. The stroma exhibits a fibrinous exudate in which many eosinophils are present. The pigment cells have moved forward to the anterior face forming a solid barrier underneath the surface exudate. This is an onchocercal anterior uveitis. X 520.

Fig. 8: Rabbit 3. Dead mf. injected into the iris directly (a heavy load) and the animal killed 6 weeks later, when the condition was subacute to chronic.

The filtration angle is blocked with organised exudate in which many plasma cells and a few monocytes and eosinophils are present. In the root of the iris a few hyaline spherules can be seen. This is the classic picture of onchocercal anterior uveitis in man. X 125.

Fig. 9: Rabbit 3. As above.

An organised membrane occludes the pupil. X 28.

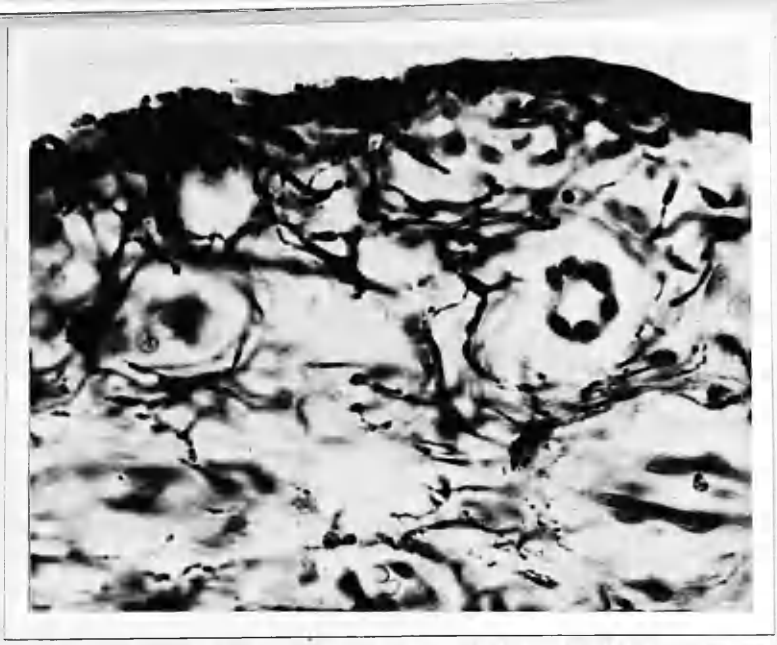


Fig. 7.

Fig. 8

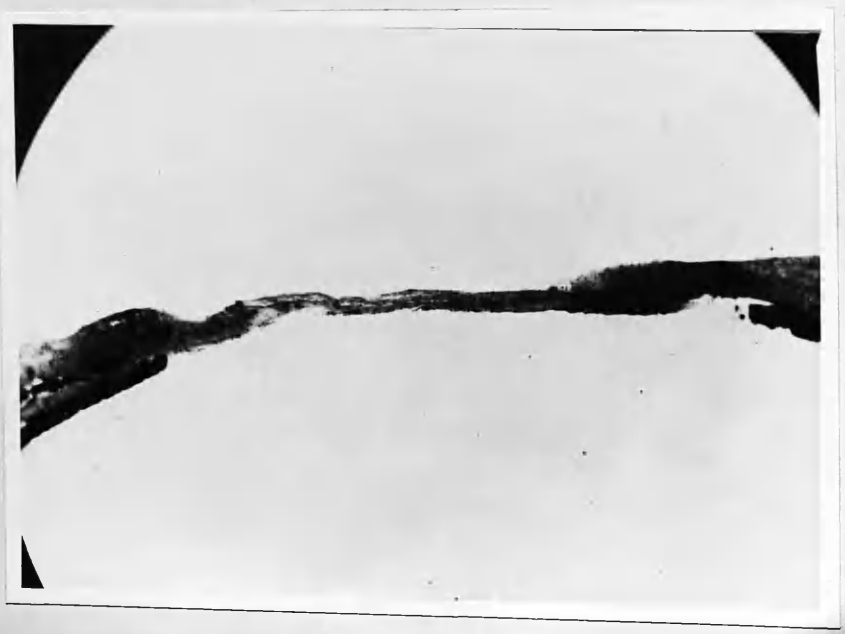


Fig. 9

Fig. 10: Rabbit 21. Same treatment as above (Rabbit 3).

Fibrous tissue has proliferated within the iris stroma. The vascular endothelium is swollen and some vessels occluded. There is a chronic inflammatory cell infiltration throughout; the posterior pigment epithelium forms a solid band on the posterior face but the band of chromatophores along the anterior face is breaking down and thinning out, signifying healing. This is characteristic of the iris in a chronic anterior uveitis. X 125.

Fig. 11: Rabbit 33. Subcutaneous injections of dead mf. were given weekly for two months in an attempt to poison the retina or the choroid.

No abnormality of the posterior segment of the eye exists. This specimen is stained with Masson, unlike the others all of which are stained with Haematoxylin and eosin. X 260.

HUMAN MATERIAL

Fig. 12: Mf. volvulus in skin biopsy 1 by 2 mm. Mayer's haemalum. X 55.

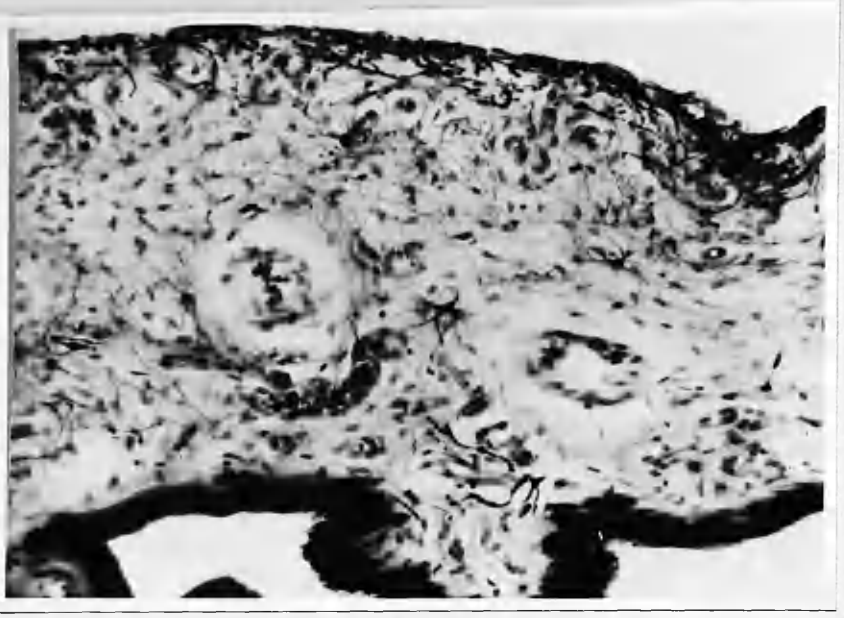
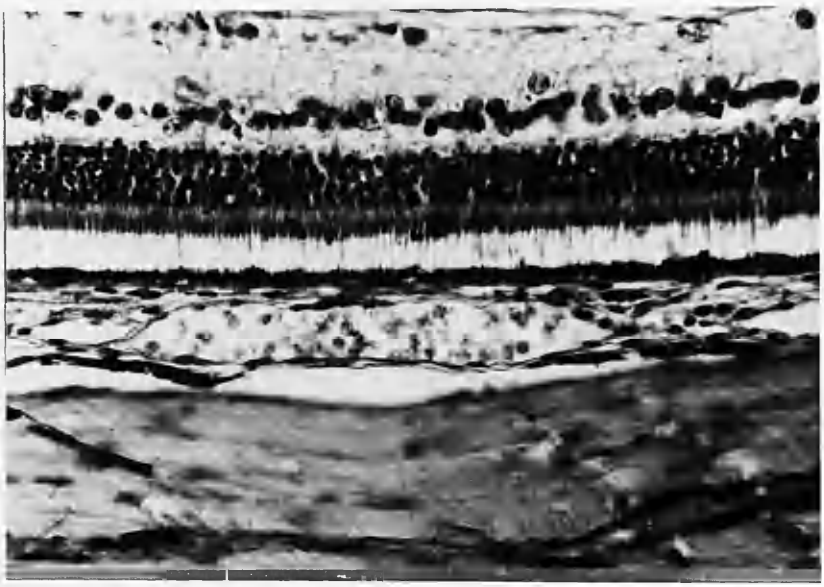


Fig. 10



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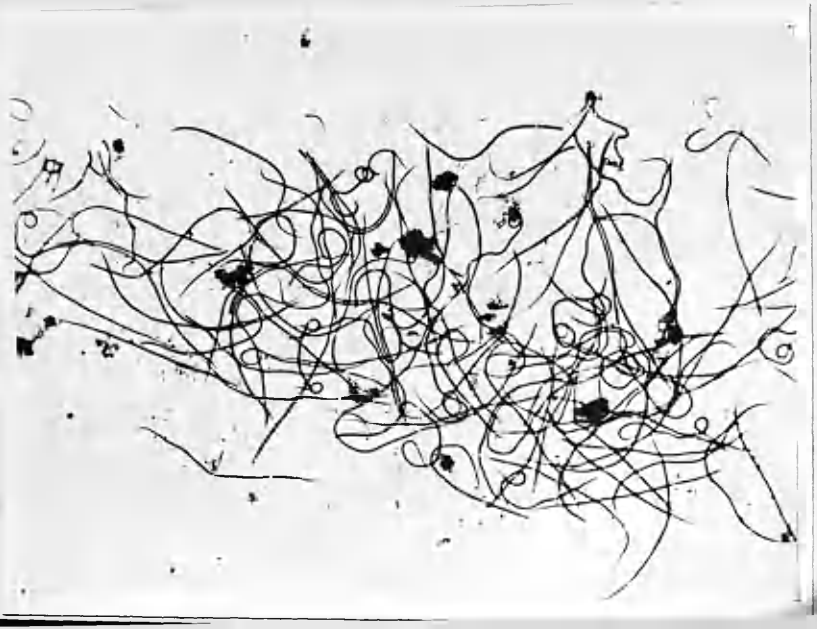
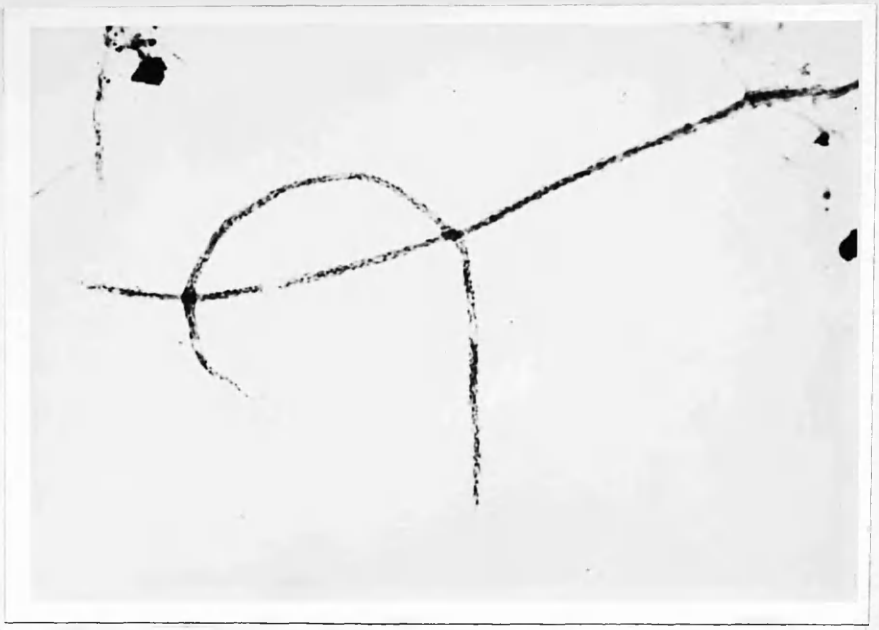


Fig. 12

Fig. 13: Mf. volvulus. Mayer's haemalum. X 220.

Fig. 14: Mf. streptocerca. Mayer's haemalum. X 220.

Fig. 15: Mf. perstans. Giemsa (R66). X 220.



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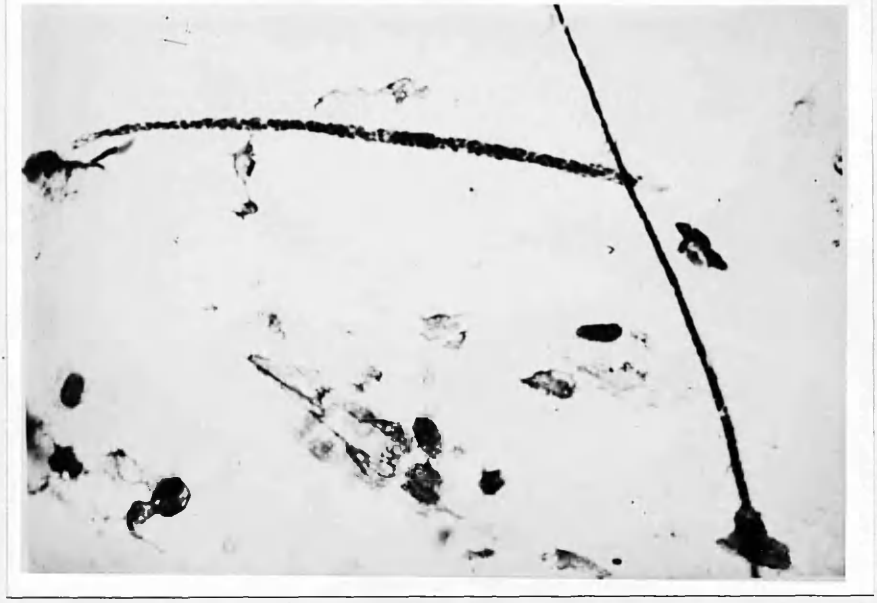

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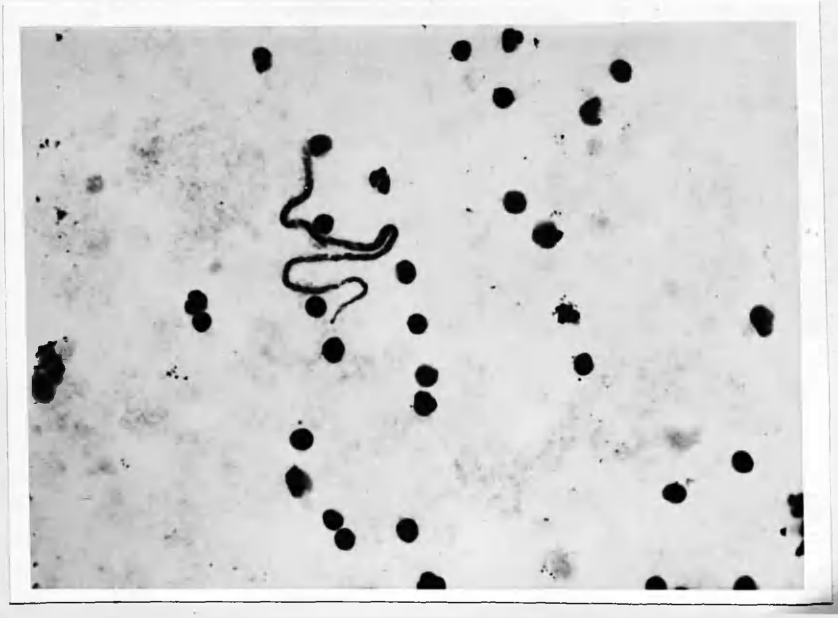


Fig. 15

Fig. 16: Mf. bancrofti. Ehrlich's haematoxylin. X 220.

Fig. 17: Mf. loa. Ehrlich's haematoxylin. X 220.

Fig. 18: Mf. volvulus in lid. H & E. X 300.

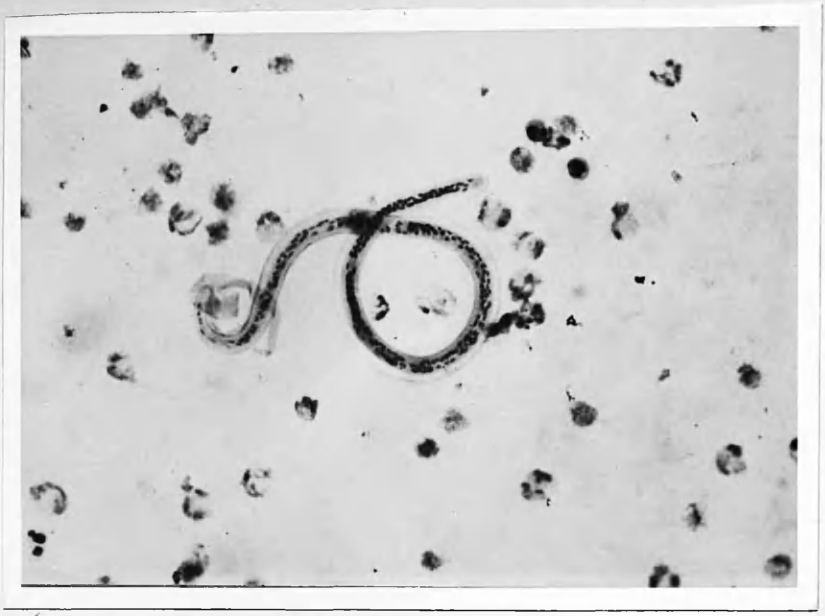
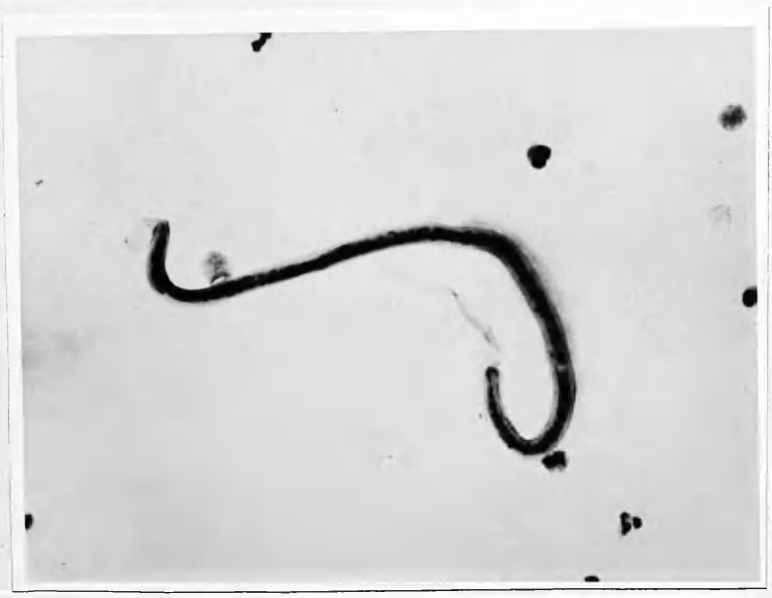


Fig. 16



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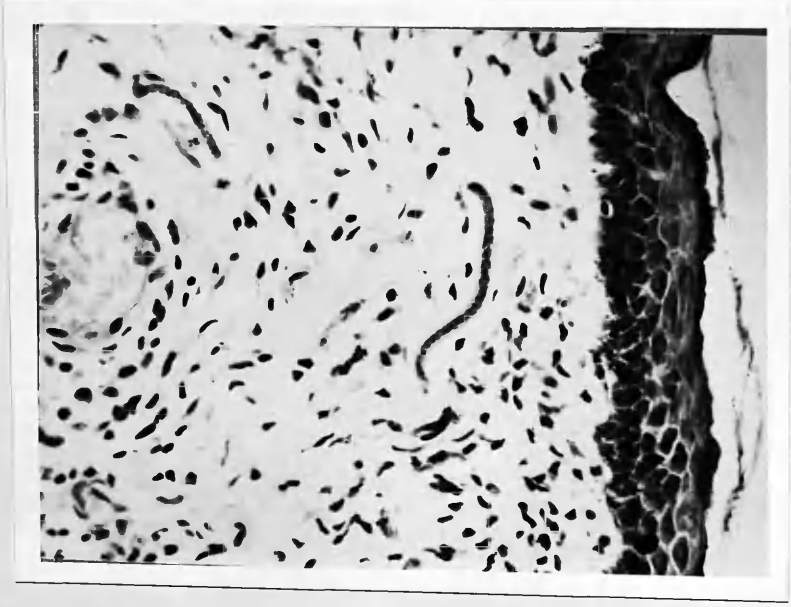
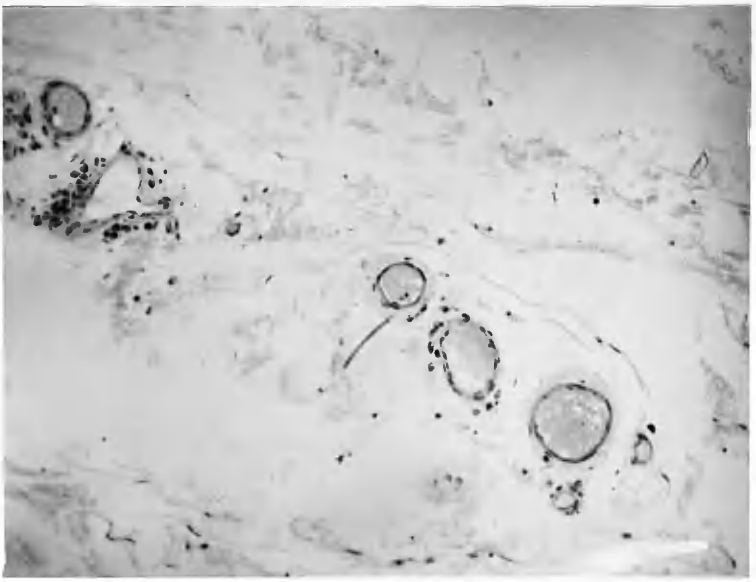


Fig. 18

Fig. 19: Mf. volvulus in lipoma (Bung eye). H & E. X 110.

Fig. 20: Bung eye - simple lobulated lipoma. H & E. X 110.

Fig. 21: Mf. volvulus in subconjunctival tissue. H & E. X 280.



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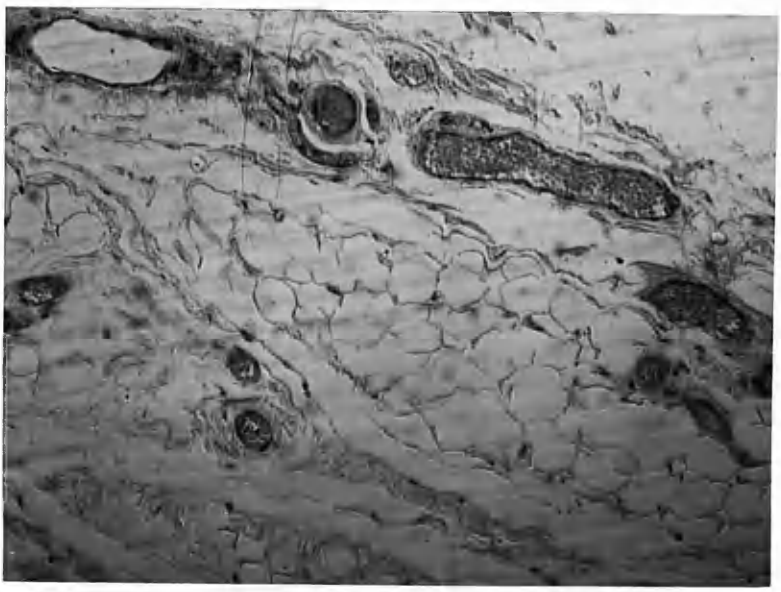


Fig. 20

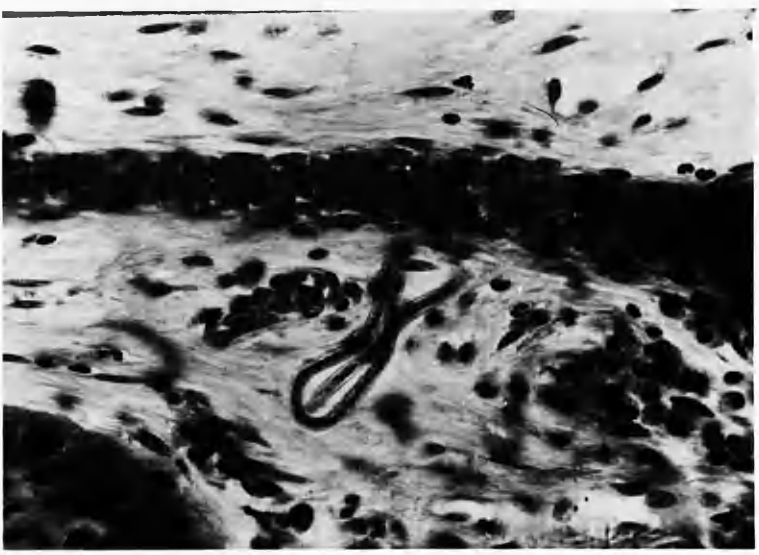


Fig. 21

Fig. 22: Mf. in corneal pannus. Masson. X 220.

Fig. 23: Mf. volvulus passing down in sheath of perforating anterior ciliary vessels. Masson. X 220.

Fig. 24: Mf. volvulus in anterior chamber. Phloxine Tartrazine. X 220.

Fig. 22

Fig. 23

Fig. 24

Fig. 25: Mf. volvulus in iris. Phloxine Tartrazine. X 500.

Fig. 26: Mf. volvulus in vitreous. Masson. X 440.

Fig. 27: Mf. volvulus in ciliary body. Phloxine Tartrazine. X 500.

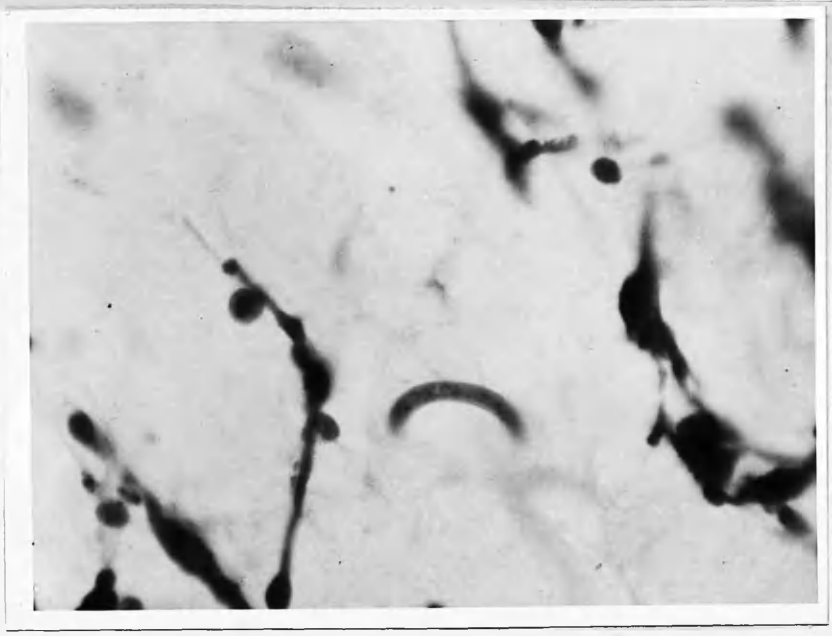


Fig. 25

Fig. 26

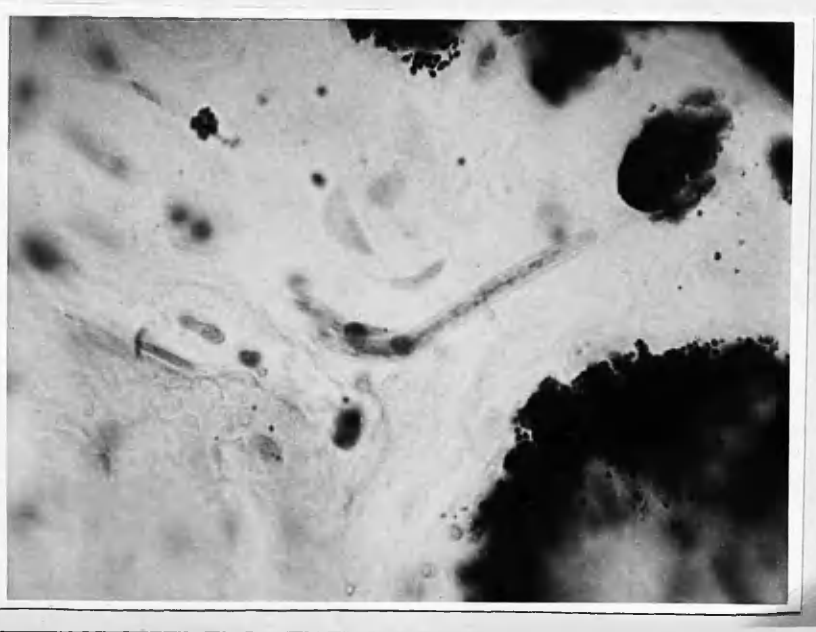


Fig. 27

Fig. 28: Mf. volvulus in choroid. Masson. X 350.

Fig. 29: Mf. volvulus in retina. Phloxine Tartrazine. X 440.

Fig. 30: Mf. volvulus in optic nerve. Phloxine Tartrazine. X 440.

Fig. 28

Fig. 29

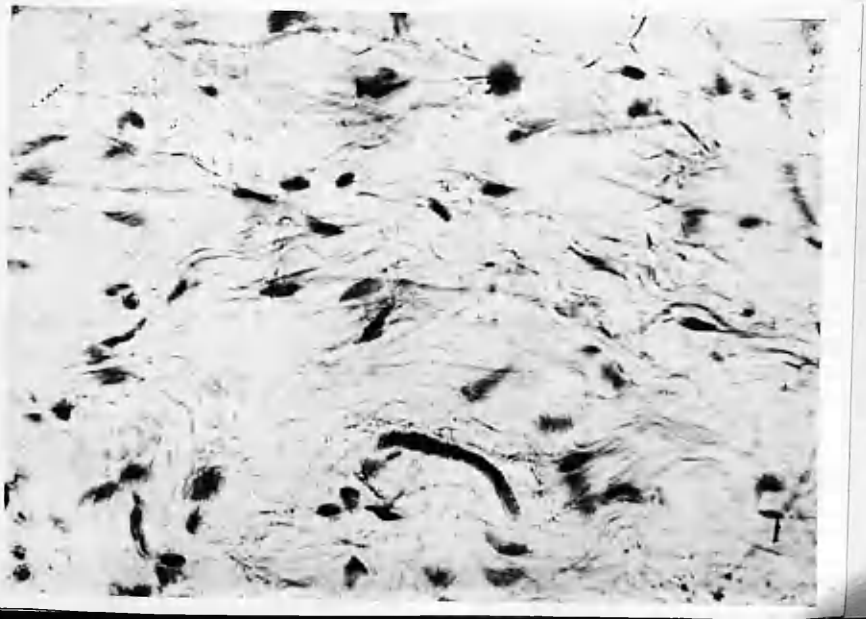


Fig. 30

Fig. 31: Living mf. volvulus in cornea. H & E. X 500.

Fig. 32: Mf. volvulus in cornea, somatic staining. Phloxine Tartrazine. X 500.

Fig. 33: Mf. volvulus in cornea, ballooning. H & E. X 500.

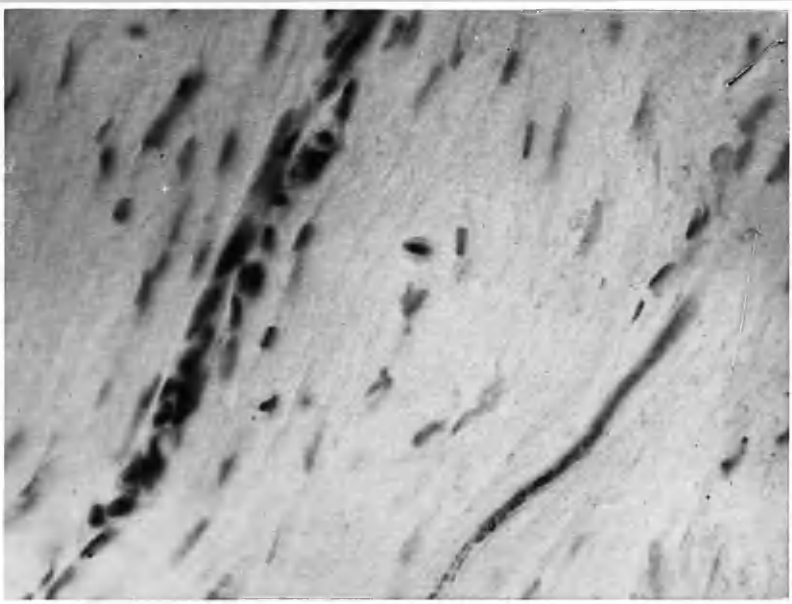
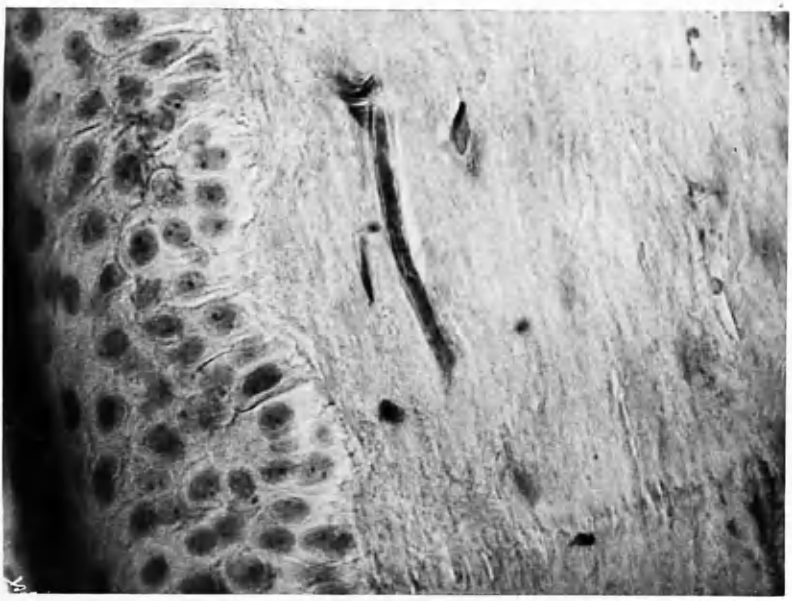


Fig. 31



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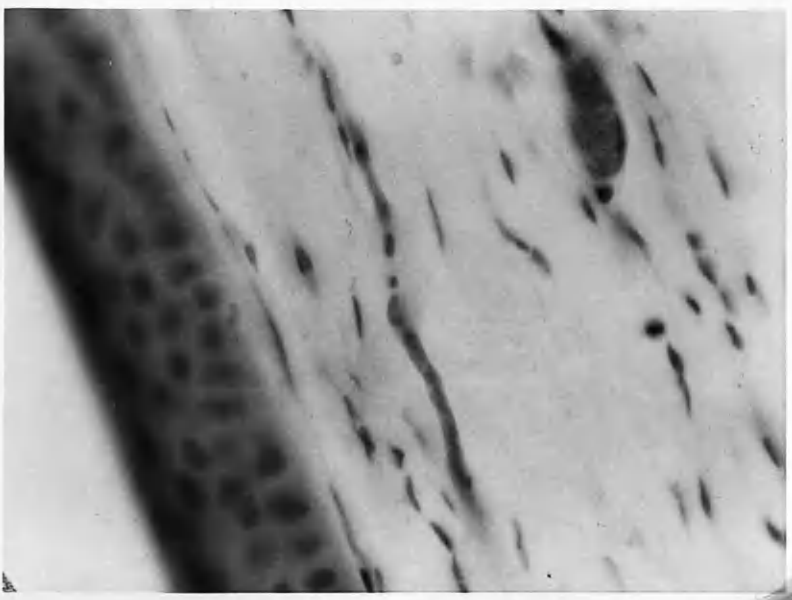


Fig. 33

Fig. 34: Mf. volvulus in cornea, fragmentation. H & E. X 500.

Fig. 35: Mf. volvulus in cornea, dissolution and absorption. H & E. X 220.

Fig. 36: Patch type of punctate corneal opacity. H & E. X 150.

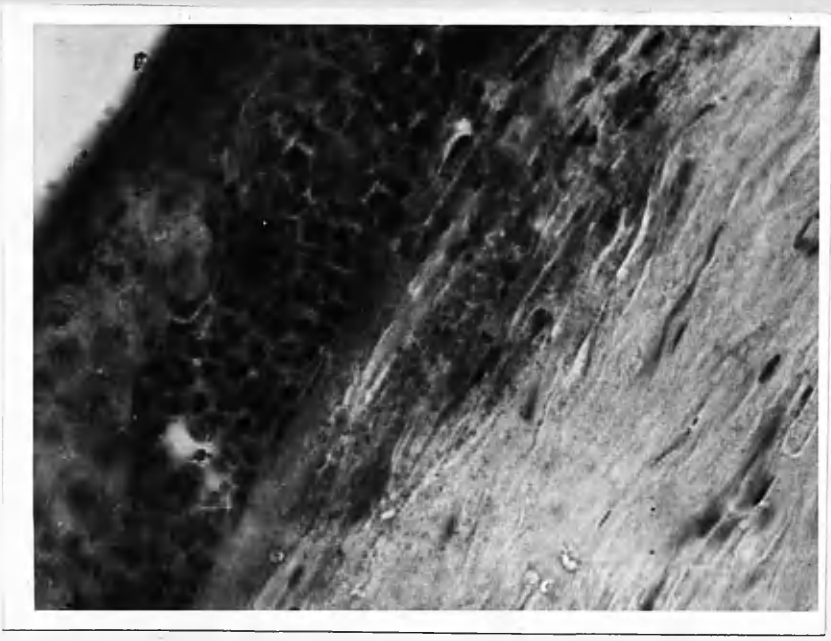


Fig. 34

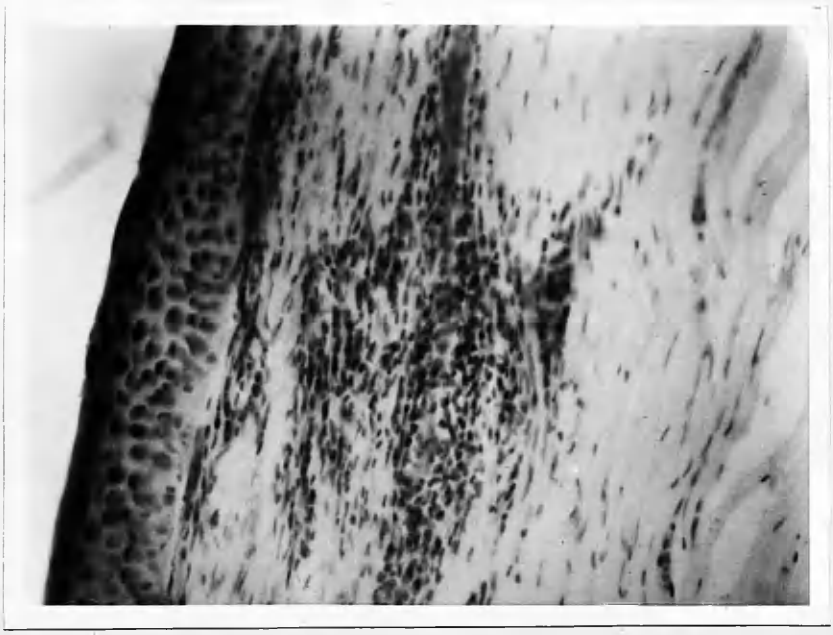


Fig. 35

Fig. 36

Fig. 37: Punctate corneal opacity in onchocerciasis subject. H & E. X 150.

Fig. 38: Mf. volvulus in epithelial cyst in cornea. H & E. X 220.

Fig. 39: Acute limbitis. Two mf. volvulus (living) can be seen. H & E. X 30.

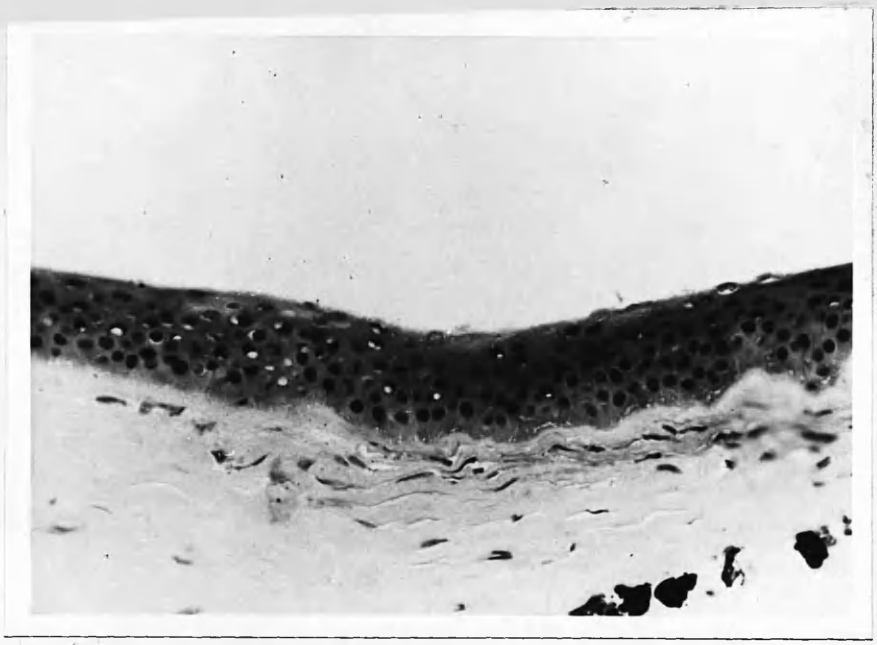


Fig. 37

Fig. 38

Fig. 39

Fig. 40: Pannus onchocercosus. Phloxine Tartrazine. X 410.

Fig. 41: Sclerosing keratitis, pigment in epithelium at base of pannus (zone 3). Unstained. X 110.

Fig. 42: Chronic quiescent keratouveitis with marked interstitial keratitis. Masson. X 30.

Fig. 40

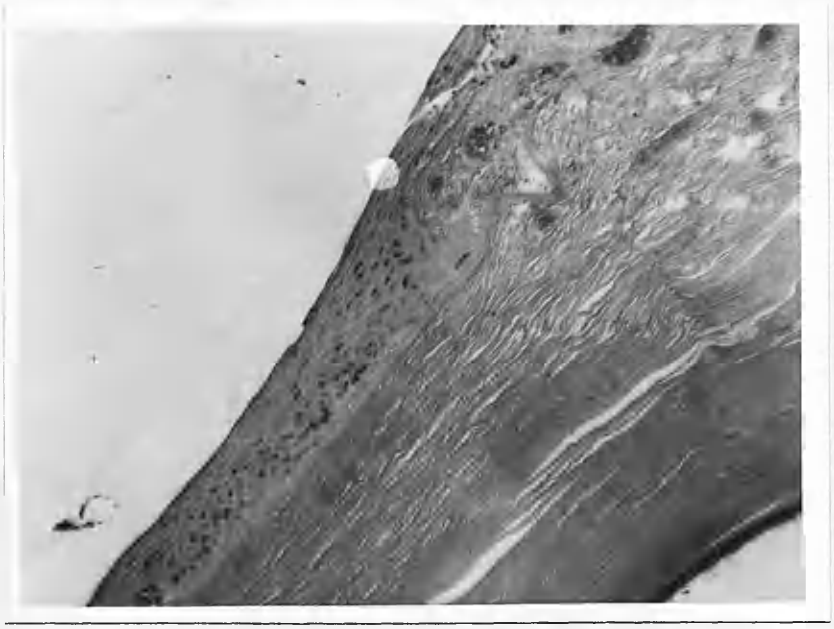


Fig. 41

Fig. 42

Fig. 43: Bleached negroid iris to show average distribution of clump cells in apparently healthy eye. Phloxine Tartrazine. X 110.

Fig. 44: Early migration of pigment from root of iris in acute anterior uveitis. Masson. X 110.

Fig. 45: Established subacute anterior uveitis to show wide distribution of pigment cells in stroma. Masson. X 110.

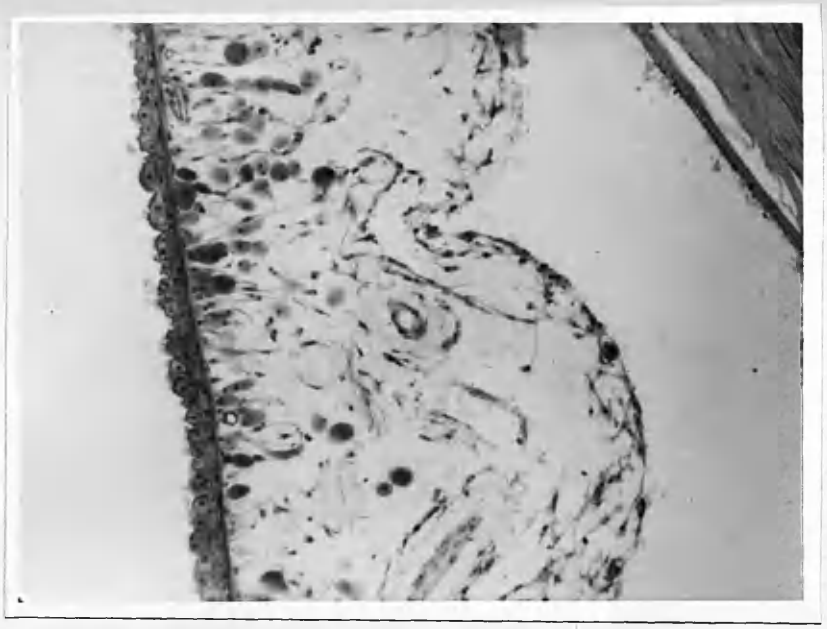


Fig. 43

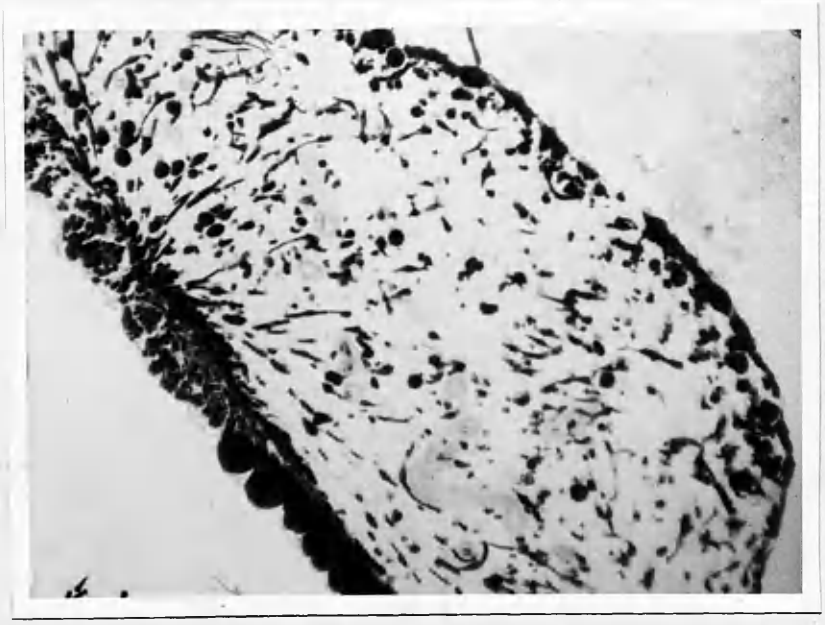


Fig. 44

Fig. 45

Fig. 46: Acute anterior uveitis with dense clumping. Part of a mf. is present. Masson. X 400.

Fig. 47: Pigment mantles on iris capillaries in anterior uveitis. H & E. X 500.

Fig. 48: Bleached melanoblasts of iris surrounding blood vessel in acute anterior uveitis, early stages. Phloxine Tartrazine. X 500.

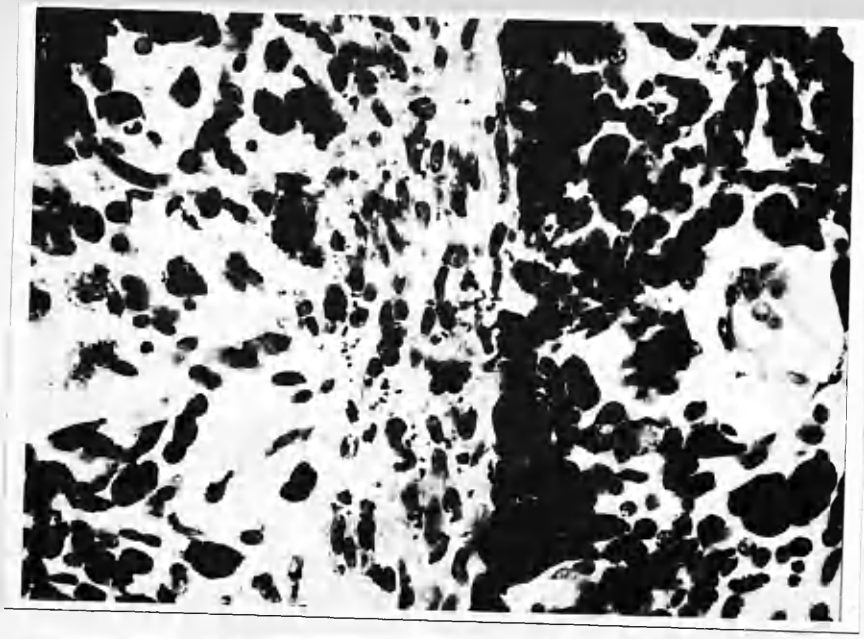


Fig. 46

Fig. 47

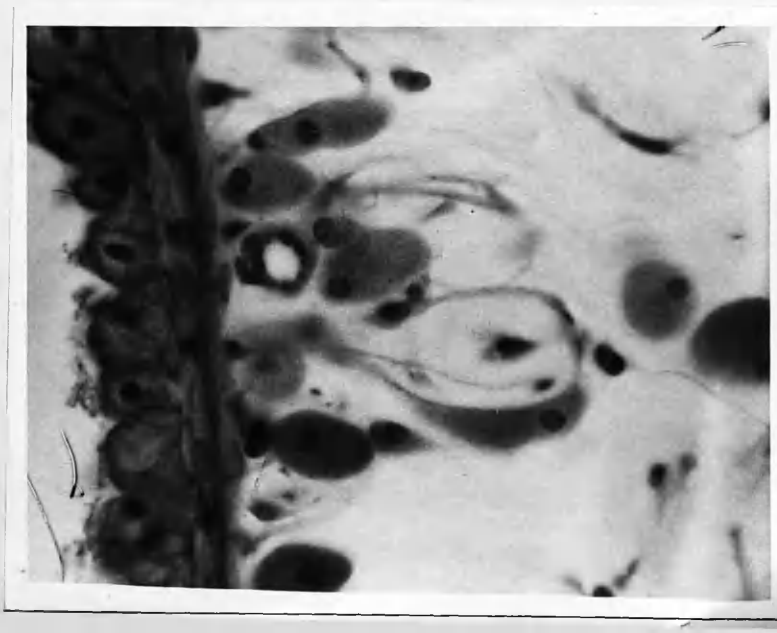


Fig. 48

Fig. 49: Healthy iris blood vessel with characteristic space in wall. Mallory. X 500.

Fig. 50: Hyalinised blood vessels in advanced anterior uveitis and aggregation of pigment cells, the round granules of which can be made out. Mallory. X 500.

Fig. 51: Acute anterior uveitis, well established, showing swelling of vascular endothelium and reduplication of arterioles, as well as hyperactivity of pigment. Masson. X 500.

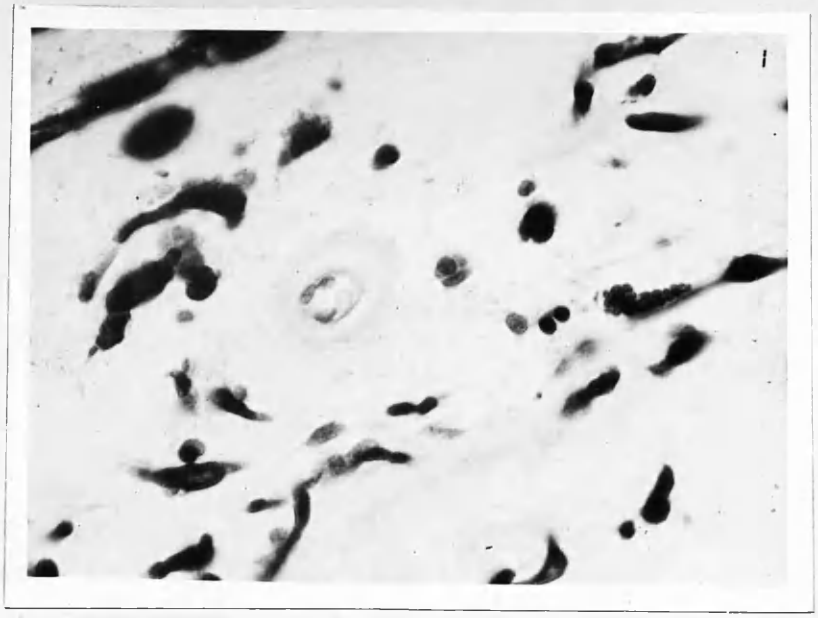


Fig. 49

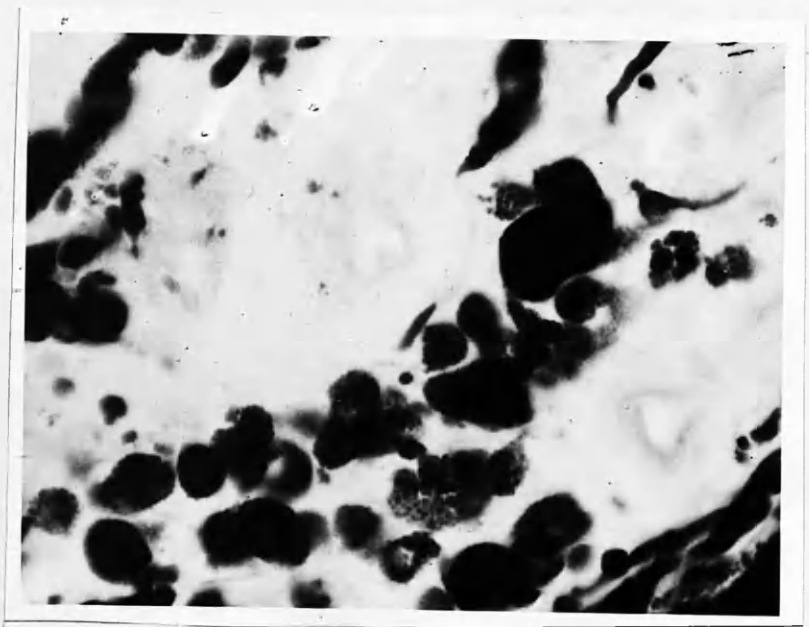


Fig. 50

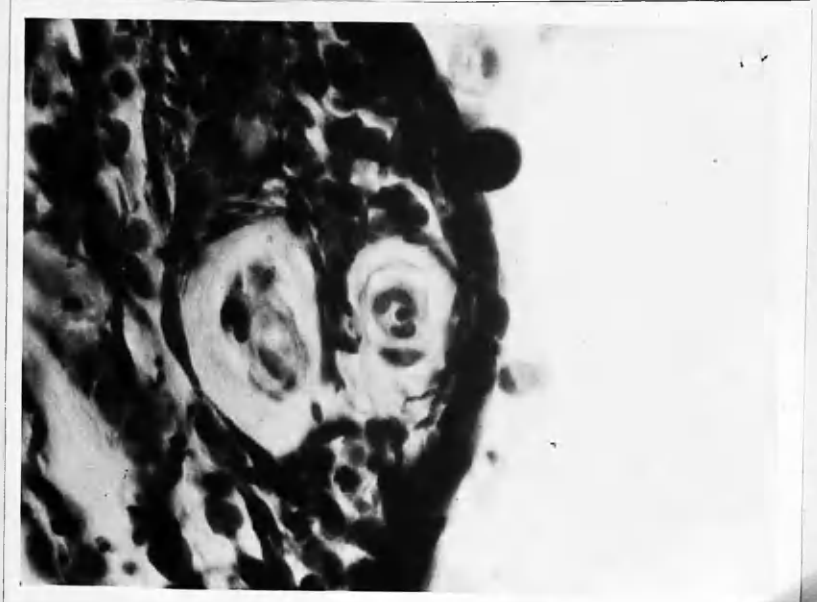


Fig. 51

Fig. 52: Chronic anterior uveitis. Plasma cells dominate in the infiltrate. Also seen are an intracellular Russell body and free pigment granules. Masson. X 500.

Fig. 53: High power view of clump cells in iris shedding pigment granules. Mallory. X 860.

Fig. 54: Healing anterior uveitis, iris stroma shows persistent plasma cells, a single hyaline spherule, and signs of declumping of pigment. H & E. X 260.

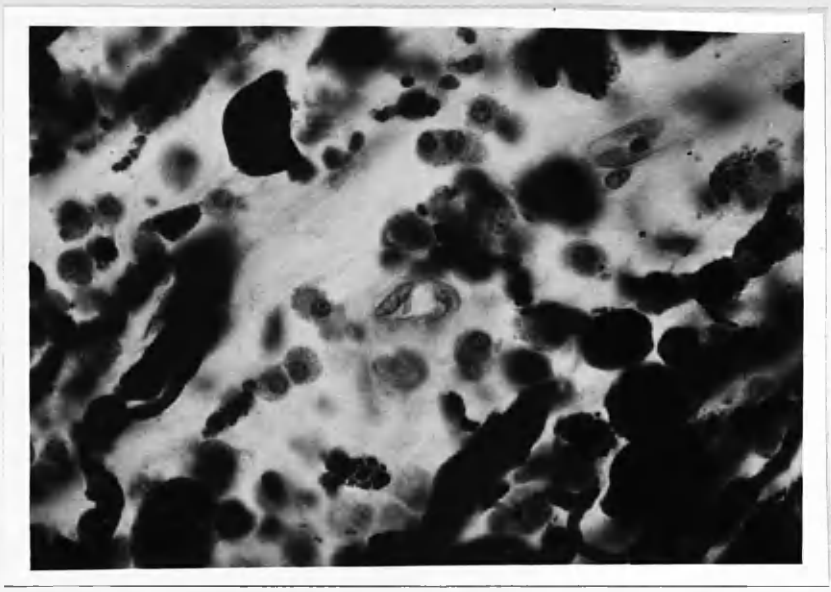


Fig. 52

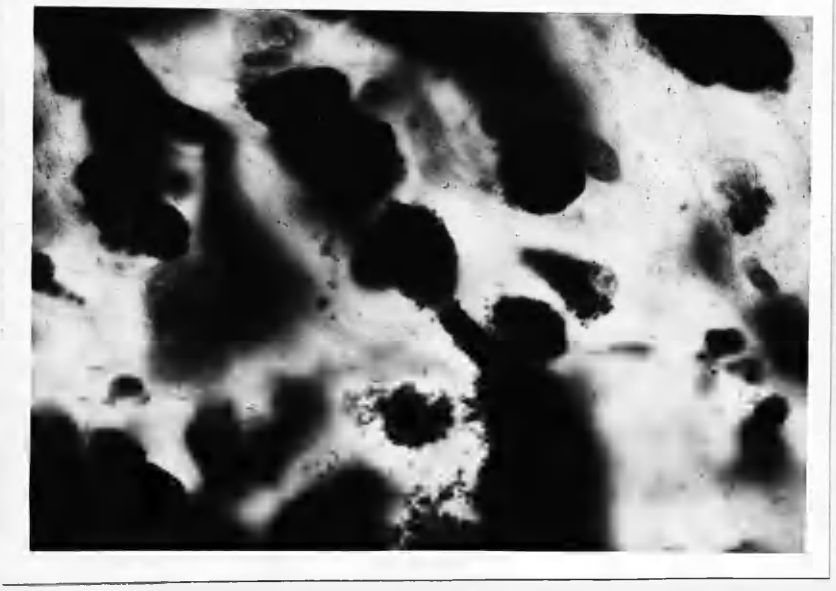


Fig. 53

Fig. 54

Fig. 55: Healed chronic anterior uveitis. Masson. X 110.

Fig. 56: Healed anterior uveitis showing posterior synechiae at pupillary margin. Masson. X 180.

Fig. 57: Healed anterior uveitis with organised pupillary membrane. H & E. X 110.

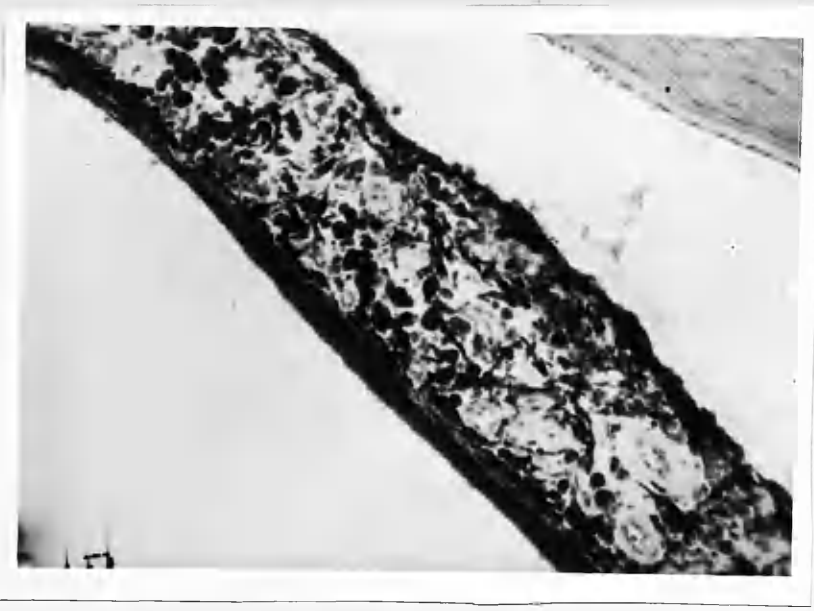


Fig. 55

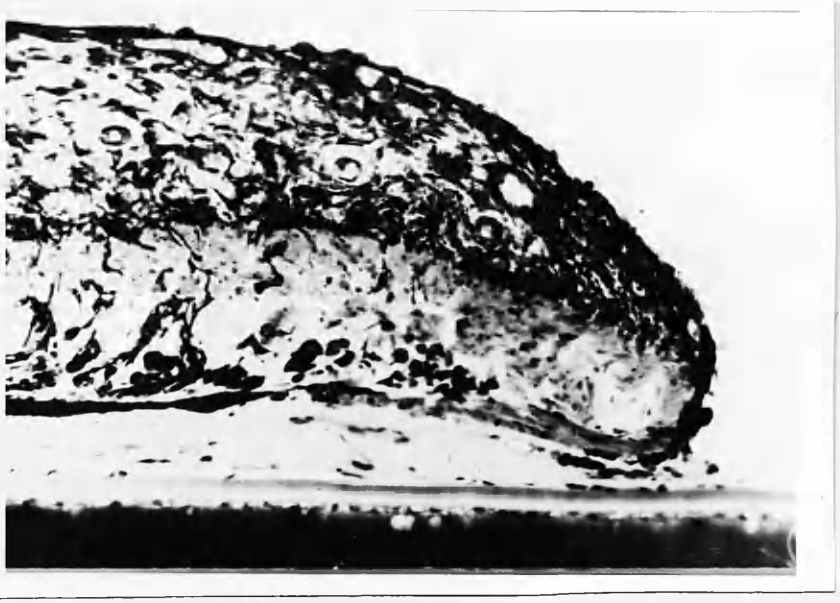


Fig. 56

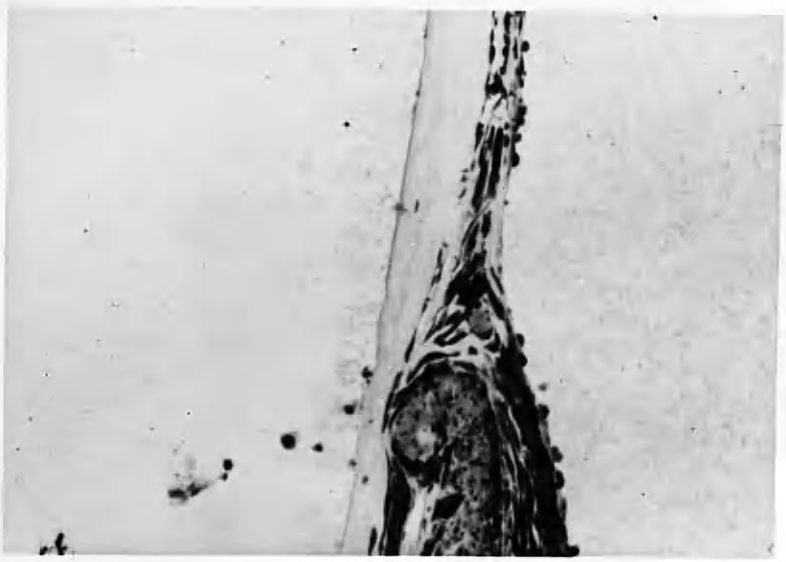


Fig. 57

Fig. 58: Occlusio pupillae in old quiescent anterior uveitis. Masson. X 80.

Fig. 59: Blockage of filtration angle in healed anterior uveitis. Masson. X 110.

Fig. 60: Acute phase of anterior uveitis showing exudate in anterior part of vitreous, as endophthalmitis develops, with amoeboid lymphocytes and monocytes present. H & E. X 220.

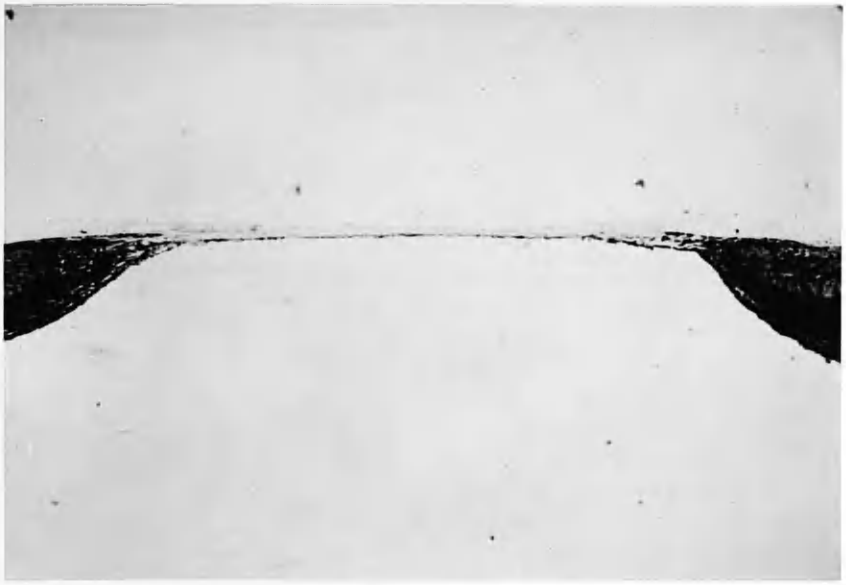


Fig. 58

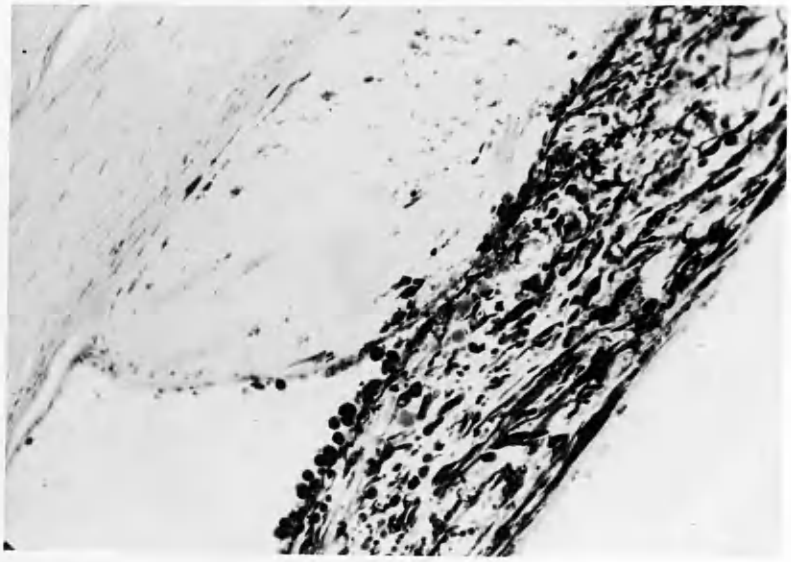


Fig. 59

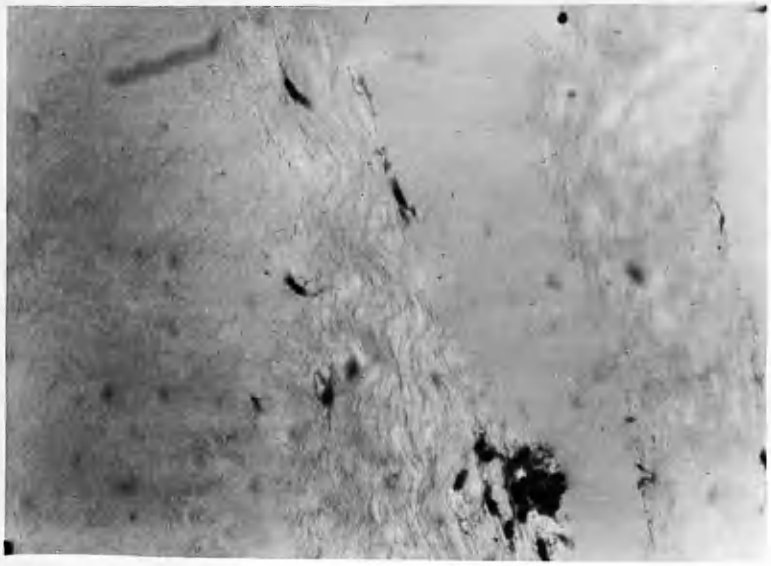


Fig. 60

Fig. 61: Sclerosis of ciliary artery in anterior uveitis with chronic inflammatory cell infiltration of stroma. Mallory. X 500.

Fig. 62: Mf. volvulus in choroid. Early invasion of choroid following an anterior uveitis. Masson. X 180.

Fig. 63: Early signs of activity of retinal and choroidal pigment in posterior exudative uveitis. Masson. X 220.

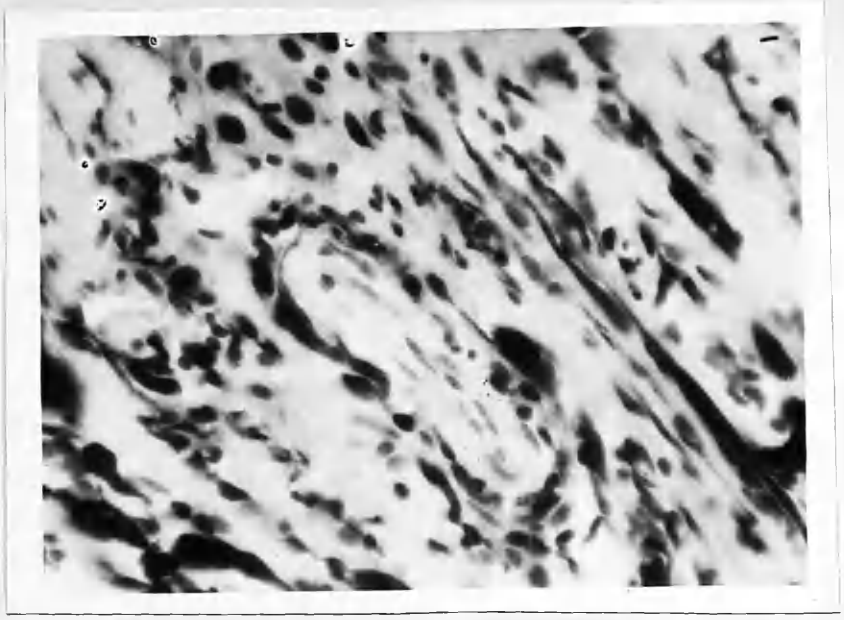


Fig. 61

Fig. 62

Fig. 63

Fig. 64: Aggregation of retinal pigment and migration of choroidal forward into choriocapillaris in posterior exudative uveitis. Masson. X 500.

Fig. 65: Posterior exudative uveitis. The cuticulum-secreting cells of the pigment epithelium are clearly seen where the pigment cells have broken away. Bruch's membrane is visible below, intact. Masson. X 500.

Fig. 66: Old healed case of posterior exudative uveitis with migration of retinal pigment into retina and clumping of residual cells. The area has now been badly affected. Phloxine tartrazine. X 140.

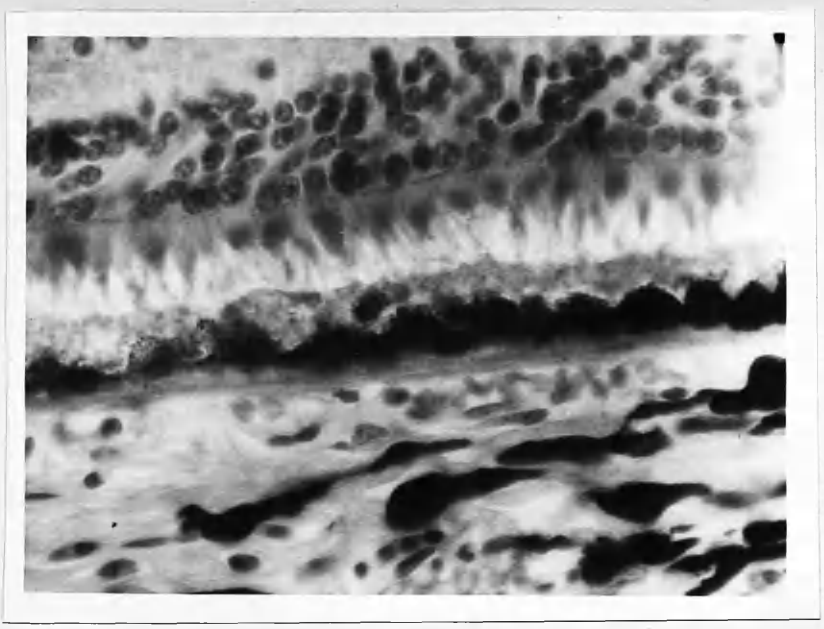


Fig. 64

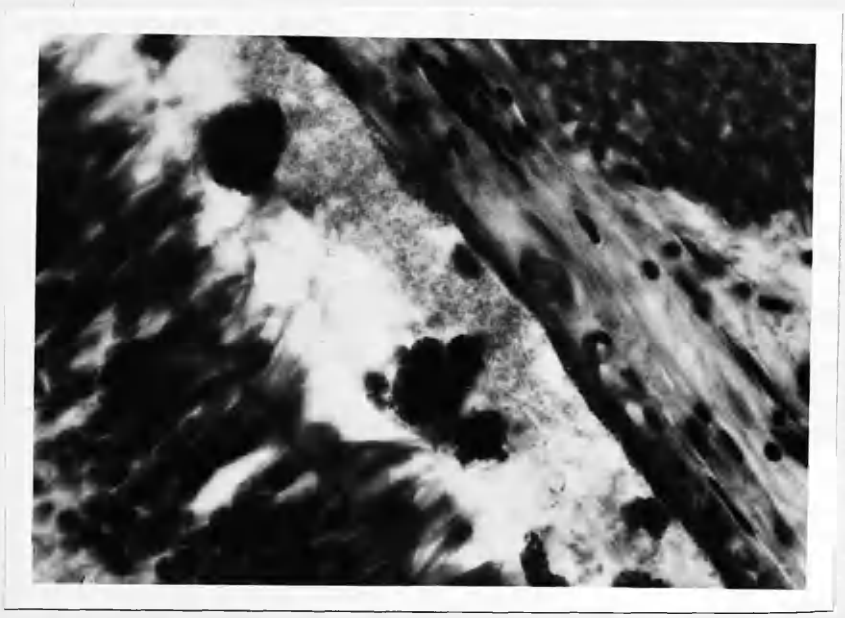


Fig. 65

Fig. 66

Fig. 67: Acute phase of posterior uveitis with choroidal congestion, reduplication of vascular intima and many eosinophils in the blood vessels. Masson. X 220.

Fig. 68: Advanced stage in posterior exudative uveitis with marked choroidal sclerosis, infiltration with plasma cells, a Russell body, and break-up of retinal pigment associated with inward migration of the choroidal. Masson. X 220.

Fig. 69: The same as Fig. 68, adjacent. While the choriocapillaris is still patent, the overlying retina has started to degenerate over a portion of choroid that is badly affected. Masson. X 220.

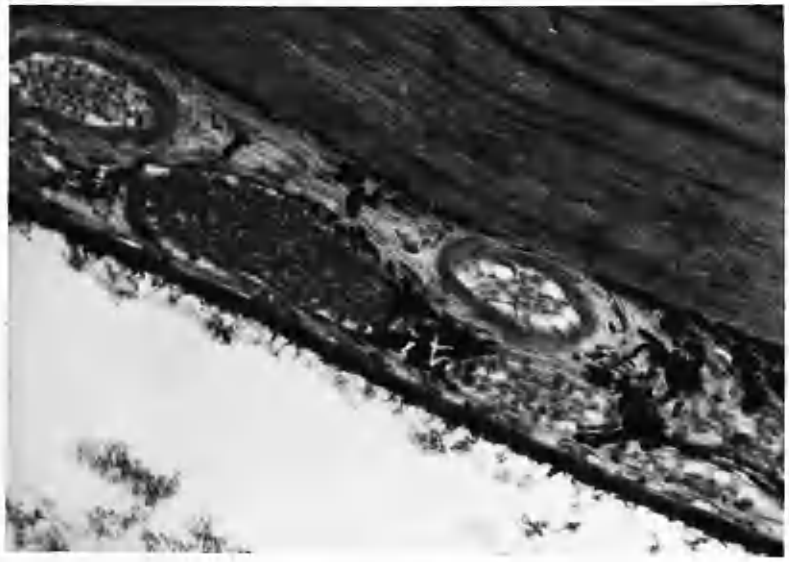


Fig. 67

Fig. 68

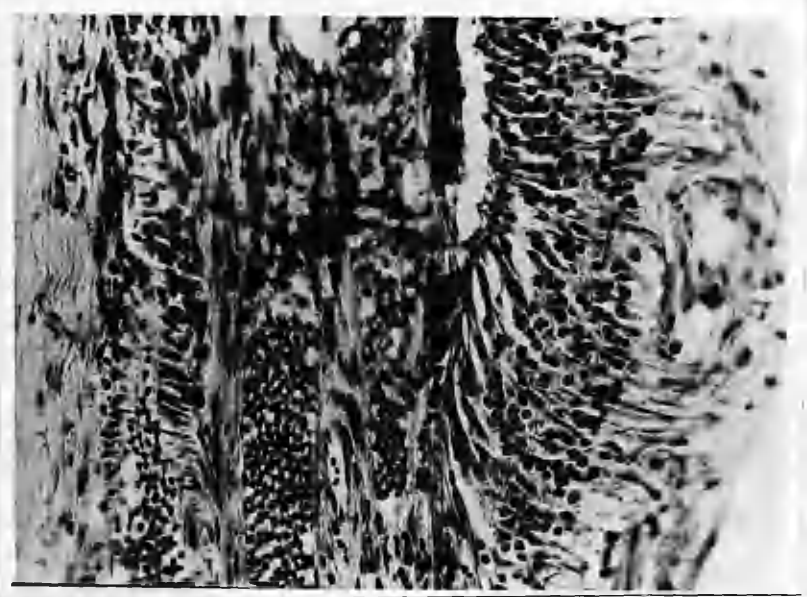


Fig. 69

Fig. 70: Exudate on the surface of the retina in a posterior exudative uveitis, showing fibroblasts. Masson. X 280.

Fig. 71: The same in another eye showing amoeboid lymphocytes and a macrophage. Masson. X 280.

Fig. 72: Subacute posterior uveitis with a nodule of lymphocytes in the choroid. This is uncommon. The overlying retina is completely destroyed, only a few bipolar cells persisting. The membrane of Bruch is highly resistant and is well seen. Masson. X 220.

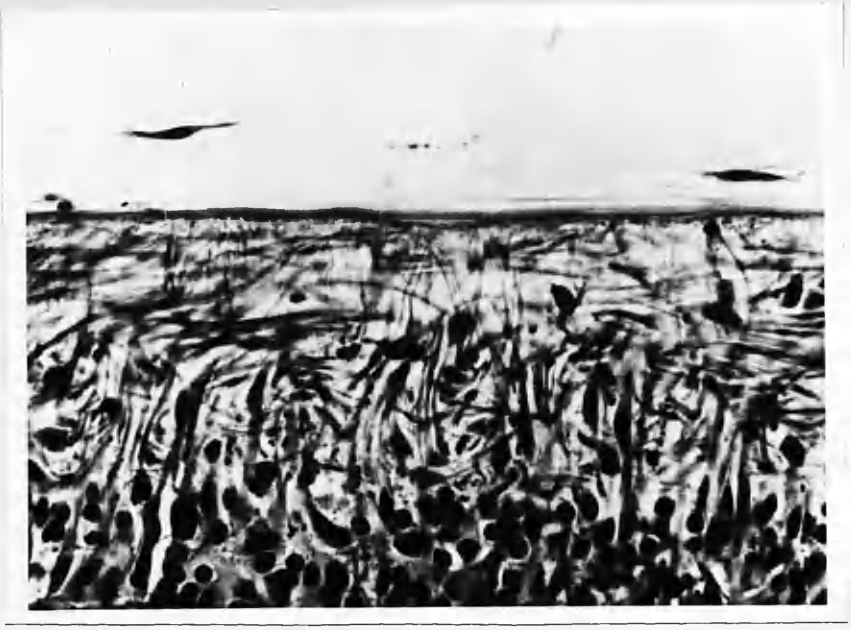


Fig. 70

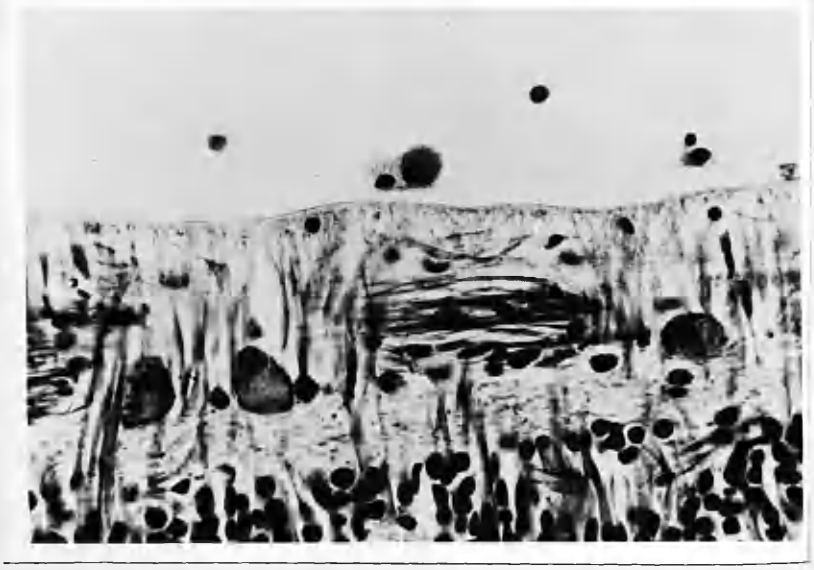


Fig. 71

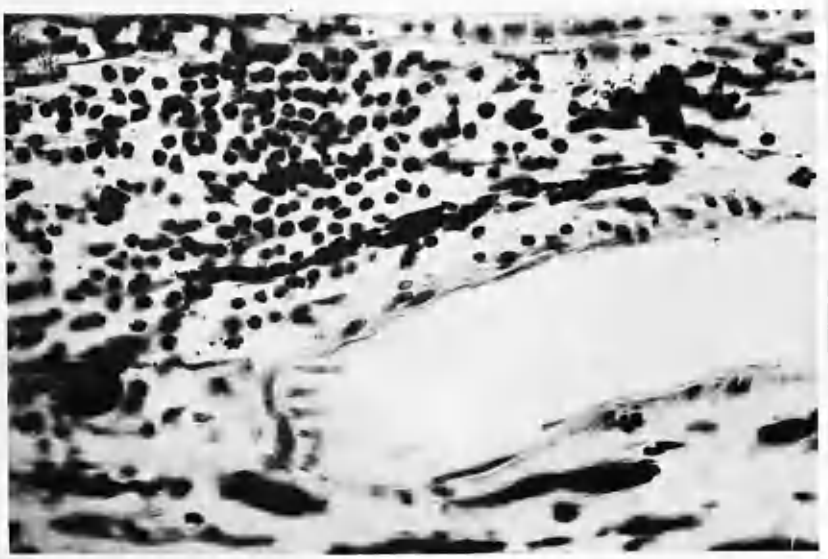


Fig. 72

Fig. 73: Plasma cell infiltration is characteristic of the posterior exudative uveitis. Mallory. X 220.

Fig. 74: Chronic posterior uveitis showing patch of chorio-retinal degeneration with heavy infiltration of choroid by plasma cells and congestion of vessels. Mallory. X 90.

Fig. 75: Another of the same. Mallory. X 90.

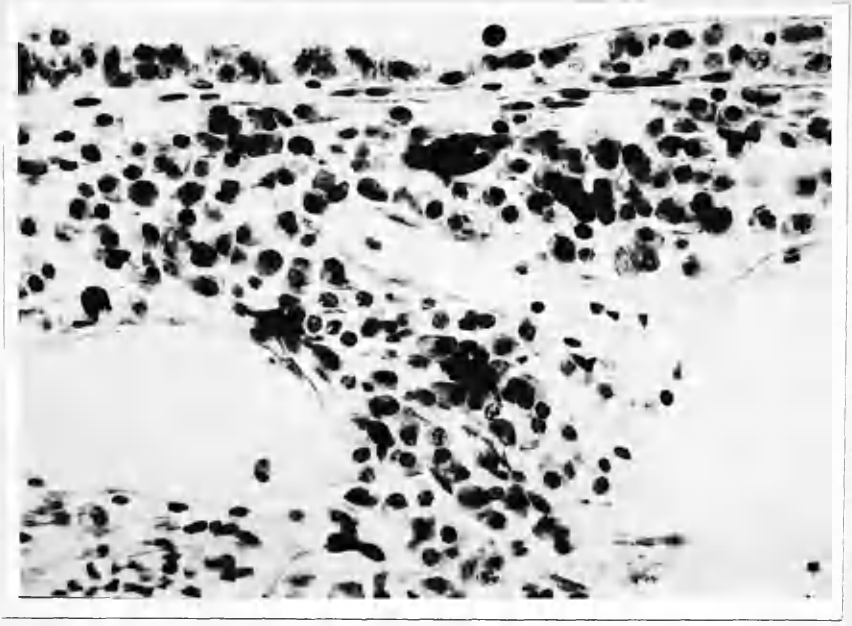


Fig. 73

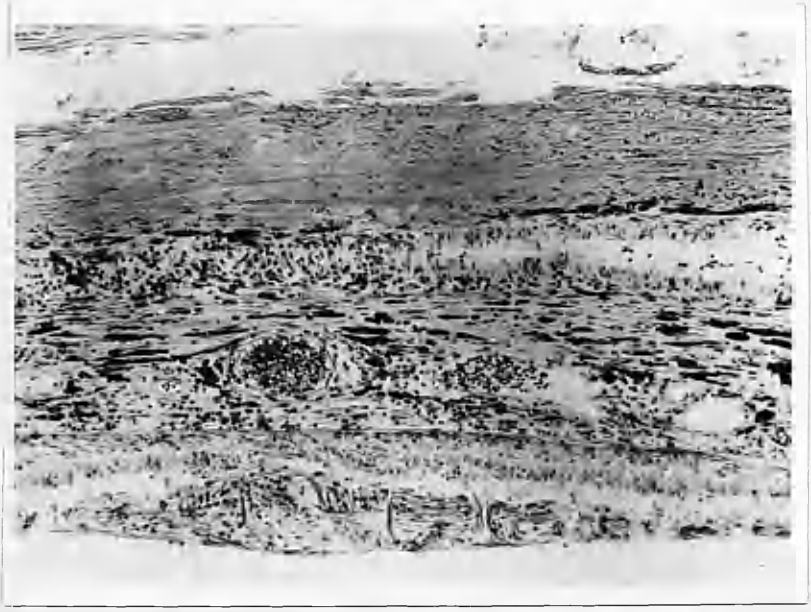


Fig. 74

Fig. 75

Fig. 76: Blessig-Iwanoff cysts and colloid bodies in old quiescent exudative uveitis where both the anterior part of the choroid and the anterior uvea were affected. H & E. X 110.

Fig. 77: Acute phase of posterior exudative uveitis with great oedema of the papilla. Mallory. X 220.

Fig. 78: Posterior exudative uveitis, now healed, exhibiting long area of chorioretinal destruction at the posterior pole. This is the stage which resembles the degenerative lesion most. Masson. X 30.

Fig. 76

Fig. 77

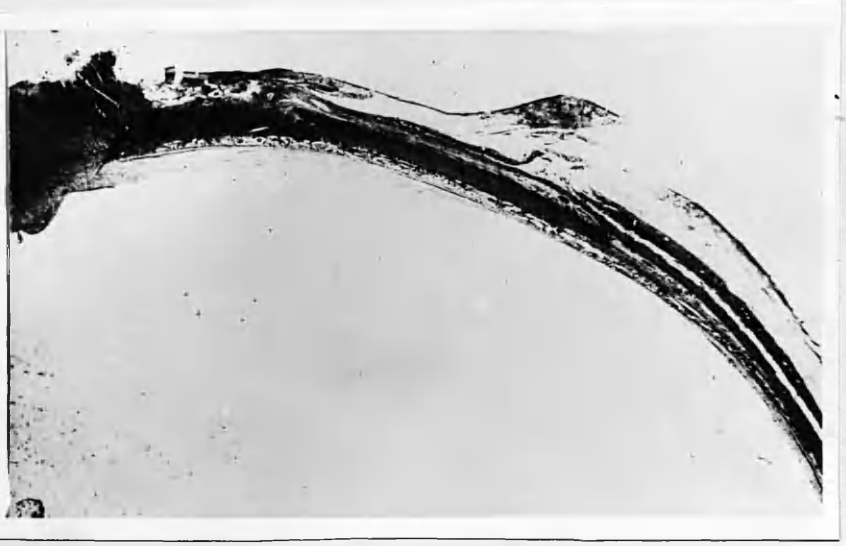


Fig. 78

Fig. 79: Old chronic onchocercal anterior uveitis with some involvement of the choroid as well, which has led to a secondary glaucoma. Deep cupping of disc. Masson. X 120.

Fig. 80: Normal appearance of retinal pigment granules. Phloxine tartrazine. X 1000.

Fig. 81: First change in the posterior degenerative lesion: retinal depigmentation, giving rise to term "freckling of the fundus". Mallory. X 500.

Fig. 79

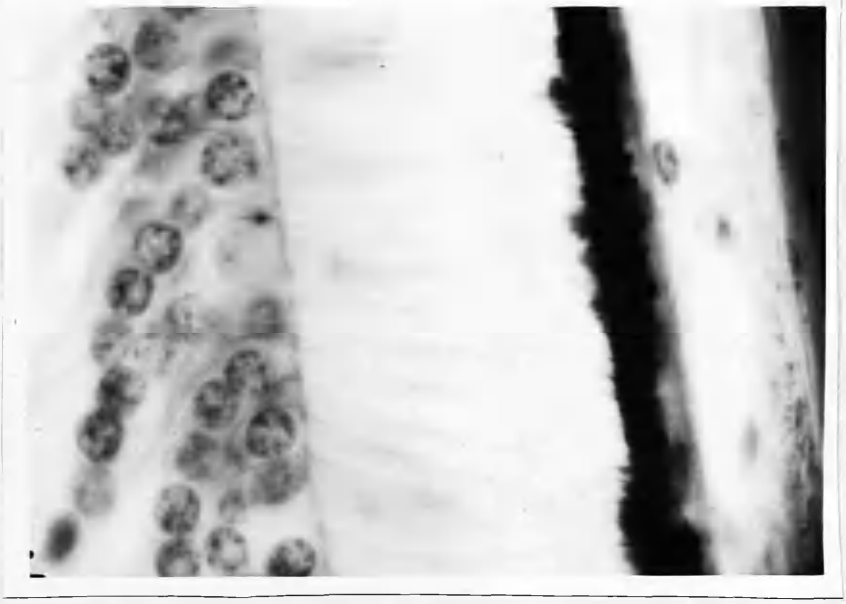


Fig. 80

Fig. 81

Fig. 82: Here the depigmentation of the retinal pigment epithelium is greater and the choroidal pigment has migrated inwards. Van Gieson - Weigert. X 500.

Fig. 83: A more advanced stage still of retinal depigmentation with still no sign of clumping. Masson. X 440.

Fig. 84: Compare with Fig. 83. This section shows a similar change in the retinal pigment epithelium in the eye of a monkey which has been made deficient in vitamin A. Unpublished work being completed in India by the author. Masson. X 440.

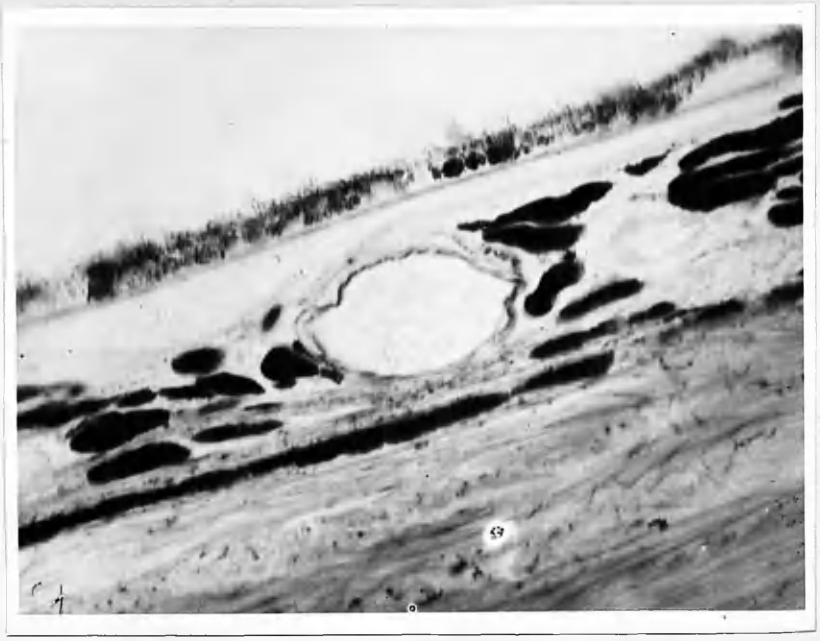


Fig. 82

Fig. 83

Fig. 84

Fig. 85: Healthy macula in early case of degenerative lesion. Masson. X 55.

Fig. 86: Affected macula, pigmentary changes and oedema, in another early case of the degenerative lesion. H & E. X 110.

Fig. 87: High power view of the macular oedema in the previous case. H & E. X 500.

Fig. 85

Fig. 86

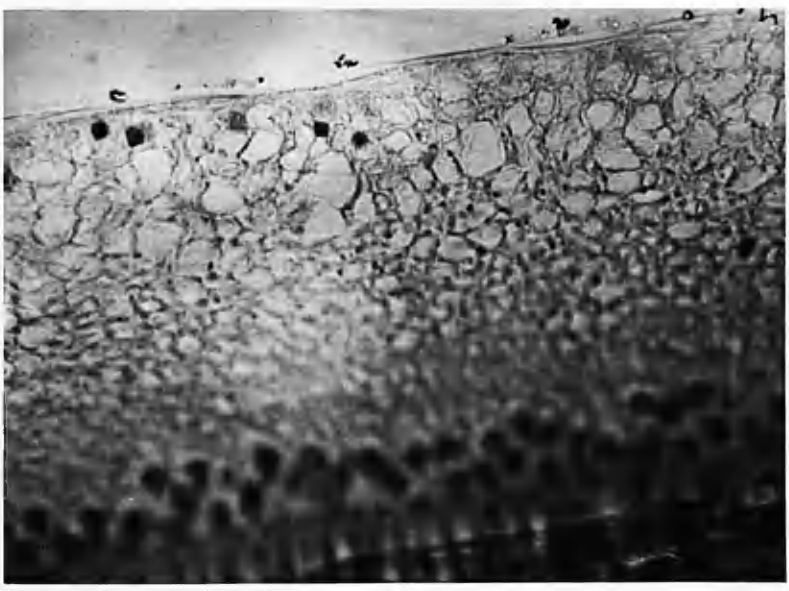


Fig. 87

Fig. 88: Edge of the degenerative lesion showing disappearance of rods and cones and their nuclei, loss of retinal pigment and very slight plasma cell infiltration only. Van Gieson. X 110.

Fig. 89: The same type of case showing aggregation of retinal pigment at punched-out edge of the degenerative area. Choroidal pigment has moved well inwards. There is sclerosis of the choroidal artery on the right, and very little infiltration considering the destruction. Van Gieson. X 110.

Fig. 90: High power view in middle of degenerate area showing few plasma cells, overgrowth of choroid with fibrous tissue, total disappearance of choriocapillaris and the arteries, and persistence of the membrane of Bruch. Masson. X 250.

Fig. 88

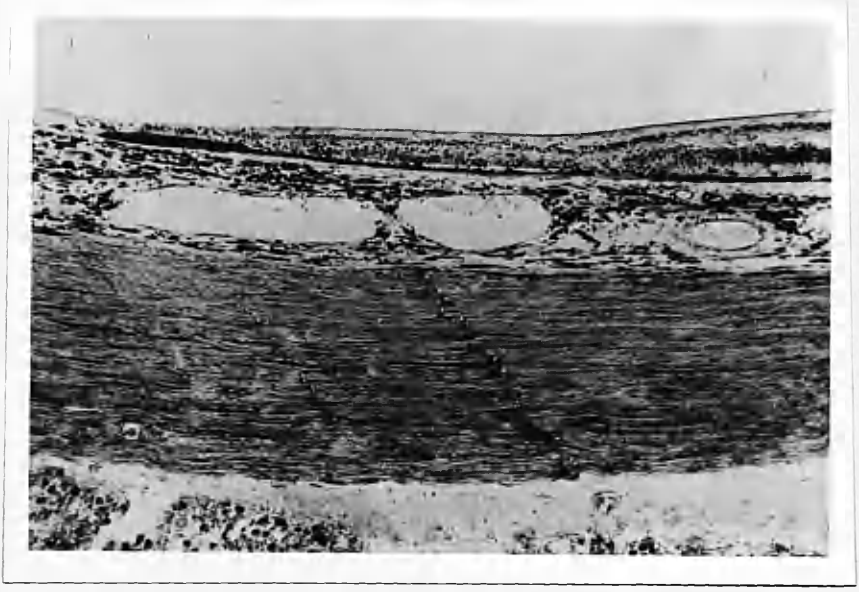


Fig. 89

Fig. 90

Fig. 91: Pigment mantle in a retinal artery in a posterior degenerative lesion exhibiting peripheral corpusculation. Weigert's elastin stain. X 110.

Fig. 92: The posterior degenerative lesion of onchocerciasis. This low power view reveals the clearly-demarkated edge of the lesion on each side of the disc, and the uniformity of the destruction within it. Mallory. X 30.

Fig. 93: Early atrophy of the optic nerve in the degenerative lesion. Mallory. X 220.

Fig. 91

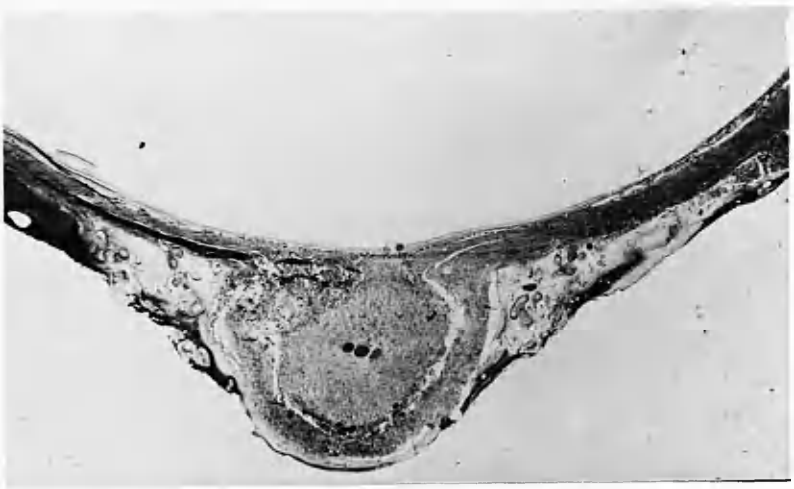


Fig. 92

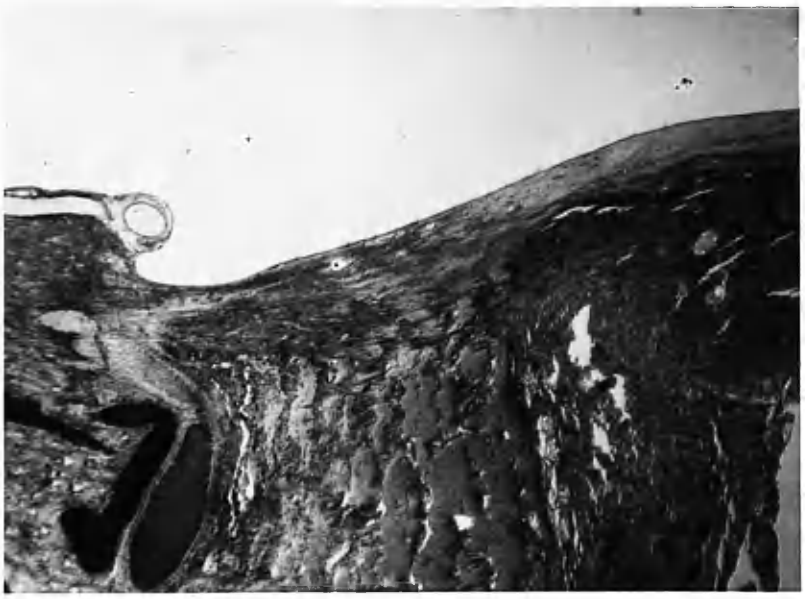


Fig. 93

Fig. 94: Old case of degenerative lesion with atrophic cupping of the disc. H & E. X 220.

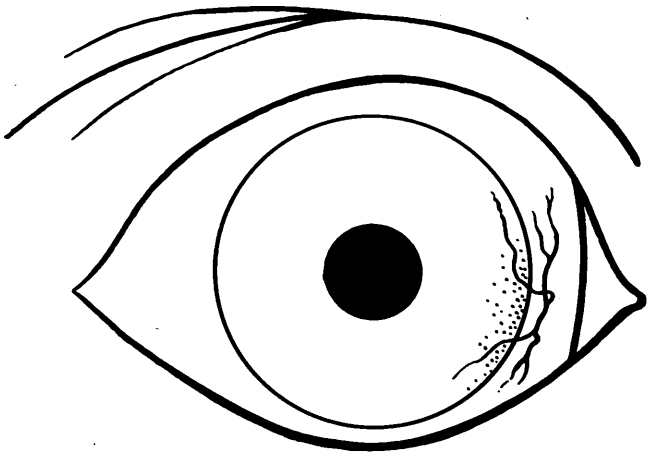
Fig. 94

APPENDIX B

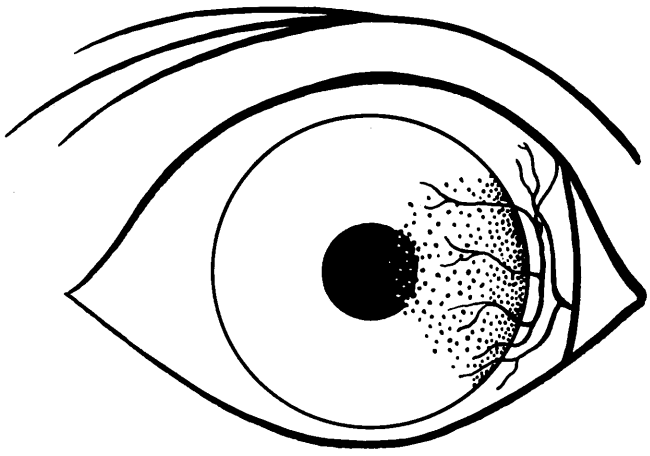
Line Drawings I to III

Drawing I.

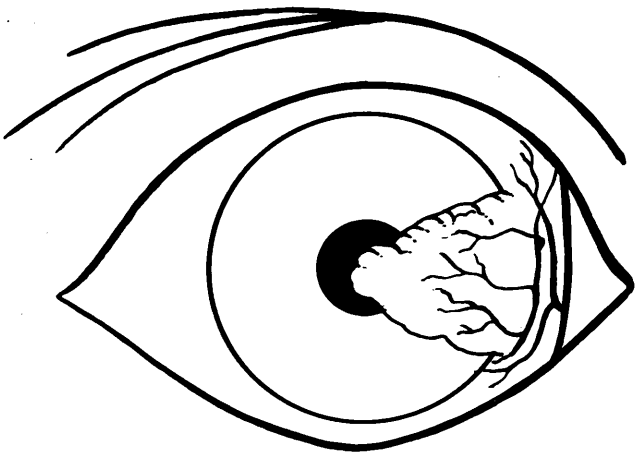
The development of a sclerosing keratitis from a limbitis in the rabbit eye.



a.



b.

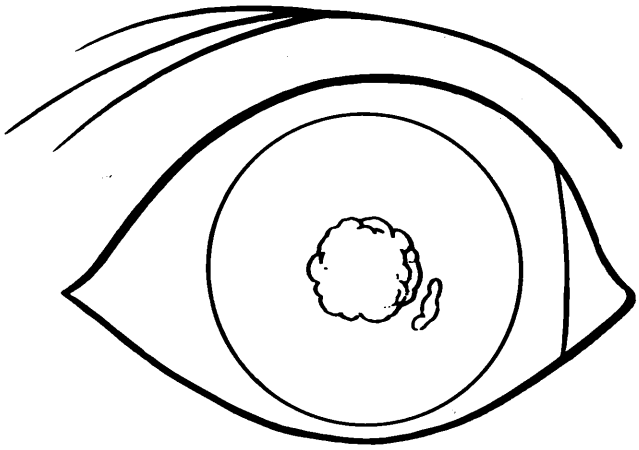


c.

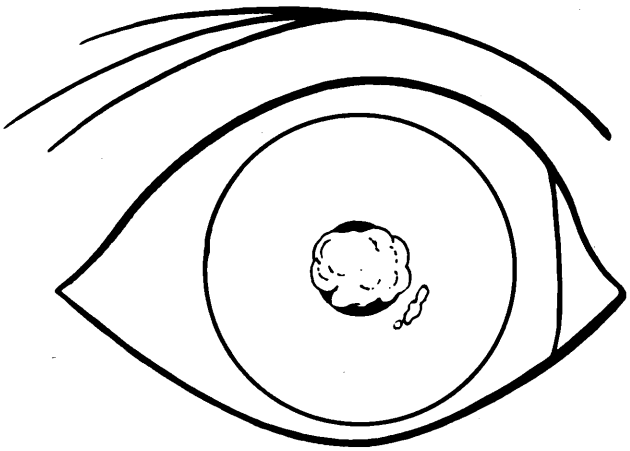
I

Drawing II.

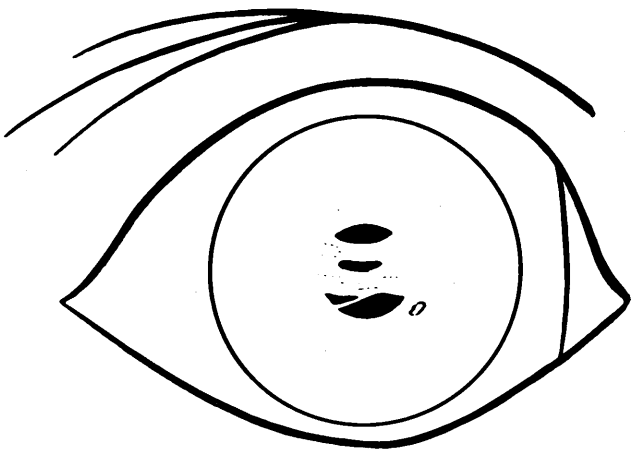
Resolution of the fibrinous exudate covering the pupil in the rabbit eye, until it is fully organised and reveals splits in the membrane.



a.



b.



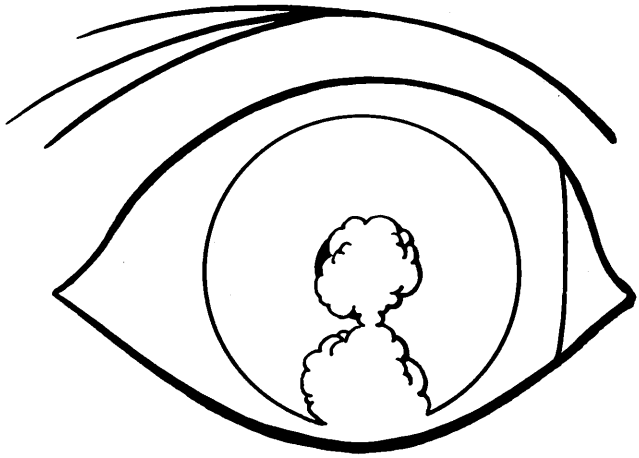
c.

II

Drawing III.

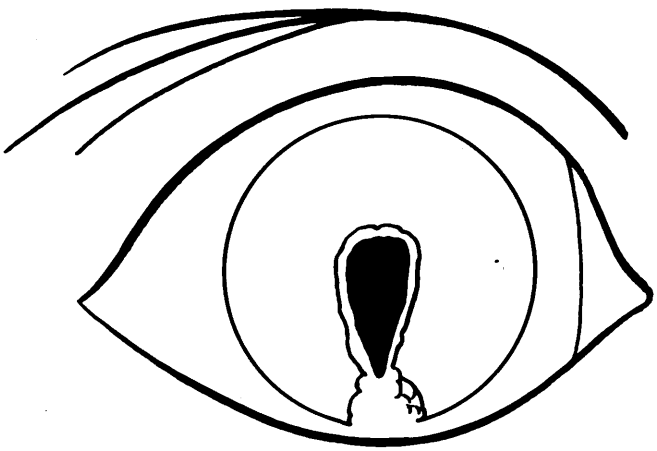
The same as Drawing II, only this time there was a greater amount of exudate, some spilling over into the anterior chamber. As a result after partial resolution the pupil was deformed adopting the pyriform appearance characteristic of the lesion in man.

(These drawings were all done by the author directly from experimental eyes.)



a.

b.



c.

III

APPENDIX C

Plates 1 and 2

Plate I: Early appearance of exudate in rabbit eye after injection of dead mf. into the iris.

Plate II: Healed quiescent anterior uveitis after two injections of dead mf. into the rabbit iris with a persistent inflammatory membrane.

(This is a photograph of the eye a section of which is shown in Fig. 9.)

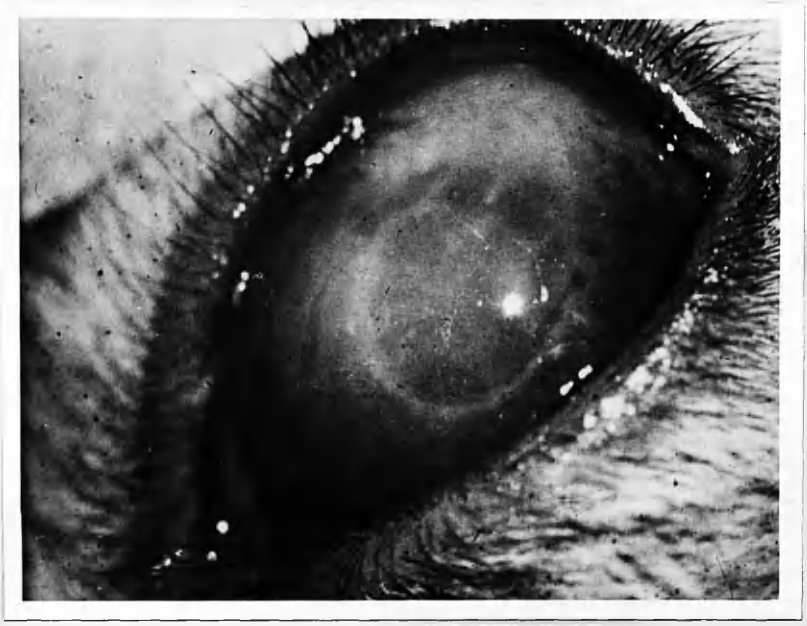


PLATE I

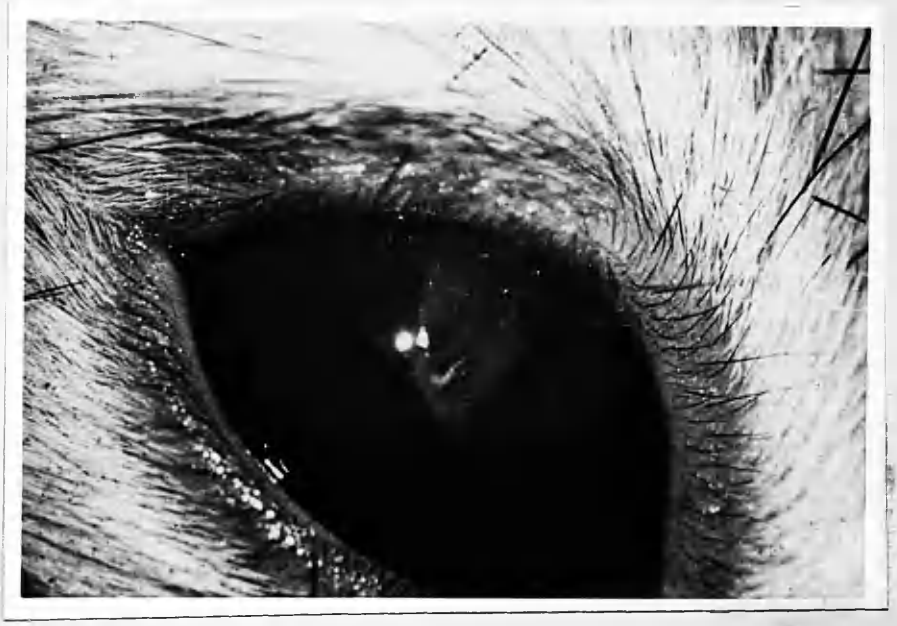


PLATE II

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BIBLIOGRAPHY

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