Experimental ocular schistosomiasis

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Schistosomiasis is a widespread incapacitating disease, but ocular involvement is relatively rare.

The first of the few cases of ocular schistosomiasis recorded in the literature was that reported in Egypt by Sobhy Bey (1928). Others were recorded by Wilson (1934), Barsoum (1938), Ibrahim (1941), Kamel (1948), Naim (1948), Mohamed (1956), and Sparrow (1966). All these took the form of bilharzial granuloma in the conjunctiva, which revealed the presence of *Schistosoma haematobium* ova. Adult *Schistosoma* worms were reported for the first time by Badir (1946) in a branch of the superior ophthalmic vein. Newton, Kanchanaranya, and Previte (1968) extracted a specimen of *Schistosoma mansoni* from the anterior chamber.

Other ocular lesions in cases of generalized schistosomiasis, which were claimed to have responded to antischistosomal treatment, were albuminuric retinitis (Ahmad, 1924); embolus in the inferior nasal branch of the central retinal artery (Cecchetto, 1931); keratitis, iritis, chorio-retinitis, vitreous opacities (de Andrade, 1940); mydriasis, palpebral oedema (Meira, Behmer, and Bloise, 1951); chronic conjunctivitis, corneal dystrophy, exophthalmos, chronic uveitis, tortuosity of the retinal veins, errors of refraction especially mild myopia (Machado, 1956); and pre-retinal haemorrhage (Massa, Laurijs, and Wijns, 1964).

Bilharzial granulomata in the conjunctiva may appear in the form of yellowish-pink nodules, either single or multiple, in the bulbar conjunctiva. In the palpebral conjunctiva, they may resemble chalazia, or may appear as polypoid cauliflower masses, resembling trachomatous pannus, arising from the fornices and extending up to the corneal margin. Scattered nodules at the caruncle may resemble *Streptothrix* nodules (Kamel, 1948; Attiah, 1962).

Histologically, the bilharzial granuloma consists of a subepithelial mass made up of endothelial cells, lymphocytes, plasma cells, eosinophils, and giant cells. *Schistosoma* ova are found in the centre in various stages of disintegration, and are frequently surrounded by giant cells (Duke-Elder, 1965).

In the early period of generalized invasion by *Schistosoma*, ocular manifestations in the form of urticaria and oedema of the lids may be seen (Sorsby, 1963). In the terminal phase of eastern schistosomiasis (kayayama disease), the involvement of the meninges and cerebral cortex may give rise to visual disturbances and blindness (Somerset, 1962; Sorsby, 1963).

In an attempt to solve the aetiological problem of ectopic schistosomal lesions, such as those recorded in the skin or the eye, various theories have been postulated on the different routes by which the *Schistosoma* ova, or even the adult worms, can reach the systemic circulation. These include a patent foramen ovale; local infection (Diamantis, 1932);

migration of worms against the blood stream (Day, 1937; Black, 1945); dissemination via the vertebral venous system (Batson, 1940; Gama and Marques Sa, 1945; Faust, 1948), or via the pulmonary capillaries.

Present investigations

The aim of our experiments was to discover whether the local route of infection could cause ectopic schistosomal lesions, whether infection leading to generalized schistosomiasis could enter by way of the eye, or whether schistosomiasis could affect the ocular structures.

The penetration of the *cercariae* of *Schistosoma mansoni* into ocular structures (including the eyelids, conjunctiva, sclera, and cornea) was studied in hamsters and guinea-pigs both *in vivo* and *in vitro*, and also the effect of injecting *cercariae* subconjunctivally and into the anterior chamber.

Evidence of generalized schistosomiasis was sought in animals subjected to the instillation of *cercariae* on to the eyelids, on to the conjunctival sac, and subconjunctivally. The eyes of animals which had been injected with *cercariae* intraperitoneally were also examined.

Clinical cases of schistosomiasis (*Schistosoma haematobium*, *Schistosoma mansoni*, and infections mixed with other parasites) were examined for ocular lesions.

Methods

Animals

Twenty golden Syrian hamsters weighing 80 to 100 g. and 28 South American guinea-pigs weighing 320 to 350 g. were used. The *cercariae* of *Schistosoma mansoni* (Egyptian strain) were used in concentrations of 80, 150, and 250 ml. The anaesthetic used was Nembutal (60 mg./kg. body weight) intraperitoneally, and urethane was also used for some animals (1–2 g./kg. body weight).

Cercarial penetration was studied with the dissecting microscope, the slit lamp, and serial histological sections. The right eyes of the animals were used throughout the experiments, leaving the left eyes as controls.

The specimens removed from the animals, which included the various ocular structures, enucleated globes, and internal organs such as the liver and spleen, were all fixed in 10 per cent. formalin solution and embedded in paraffin. Serial histological 6 to 8 μ sections were stained with haematoxylin and eosin.

The scheme on which our study was based is set out in Tables I to V.

INSTILLATION IN VIVO (Table I)

The hair on the eyelids was carefully shaved before the instillation, without injuring the skin, to avoid obstructing the penetration of *cercariae*. The instillation on to the eyelids and conjunctival

Table I *Instillation of cercariae in vivo*

<i>Animals</i>	<i>Site of instillation</i>	<i>Concentration/ml.</i>	<i>Animals killed after</i>
(a) 3 Hamsters	Eyelids	80	5, 10, 15 min.
(b) 3 Hamsters 3 Guinea-pigs	Conjunctival sac	80	5, 10, 15 min.
(c) 5 Guinea-pigs	Cornea	80–250	$\frac{1}{2}$, 1, 2, 6, 24 hrs

sac was made at a rate of one drop every 5 minutes for 15 minutes. For corneal instillation, a glass tube equal to the corneal diameter was placed vertically over the limbus for 10 minutes to allow as many *cercariae* as possible to come into contact with the cornea.

INSTILLATION IN VITRO (Table II)

The suspension was placed on the isolated cornea after rupturing the basal membrane. An artificial corneal ulcer was induced with the slit lamp where the corneal epithelium was scraped by a von Graefe knife and the basal membrane was ruptured. This was to permit the observation of the penetration of *cercariae* into the substantia propria, once the barrier of the basal membrane had been removed. The same procedure was then repeated with the anterior segment of the globe to detect whether the *cercariae* could penetrate Descemet's membrane and reach the anterior chamber.

Table II *Instillation of cercariae in vitro*

<i>Animals</i>	<i>Part instilled</i>	<i>Concentration/ml.</i>	<i>Duration of instillation (hrs)</i>
(a) 2 Hamsters 2 Guinea-pigs	Enucleated globes	250	1
(b) 1 Hamster 1 Guinea-pig	Isolated specimens from lids, conjunctiva, sclera, trephined cornea	250	2
(c) 2 Guinea-pigs	Isolated cornea (after rupturing basal membrane)	250	3

INJECTION IN VIVO (Table III)

The *cercariae* were injected subconjunctivally and into the anterior chamber, and the same amount of aqueous was previously aspirated from the anterior chamber so as not to alter the ocular tension.

Table III *Injection of cercariae in vivo*

<i>Animals</i>	<i>Site of injection</i>	<i>Amount (ml.)</i>	<i>Concentration/ml.</i>	<i>Animals killed after</i>
(a) 3 Hamsters	Subconjunctival space	0.2	250	3, 4, 5 mths
3 Guinea-pigs	Subconjunctival space	0.4	250	4, 5, 6 mths
(b) 3 Guinea-pigs	Anterior chamber	0.1	250	30, 40, 50 days

DETECTION OF GENERALIZED SCHISTOSOMIASIS (Table IV)

The animals were kept for 45 days to allow time for adult *Schistosoma* worms to develop, and perfusion was then performed to collect the organisms from the liver and mesenteric vessels. The animals were killed by cervical dislocation, as the use of an anaesthetic causes the worms to shift into the liver. Before the perfusion heparin was injected intracardially (0.2 ml. of 5,000 units) to facilitate flushing.

DETECTION OF OCULAR LESIONS (Table V)

The eyes were inspected by repeated slit-lamp and fundus examinations, and also by histological sections.

Table IV *Detection of generalized schistosomiasis*

<i>Animals</i>	<i>Site of instillation or injection</i>	<i>Concentration/ml.</i>	<i>Period before perfusion (days)</i>
(a) 1 Hamster 1 Guinea-pig	Eyelids	250	45
(b) 2 Hamsters 2 Guinea-pigs	Conjunctival sac	250	45
(c) 1 Hamster 1 Guinea-pig	Subconjunctival space	250	45
(d) 2 Guinea-pigs	Anterior chamber	250	45

Table V *Detection of ocular lesions*

<i>Animals</i>	<i>Injection</i>	<i>Concentration/ml.</i>	<i>Animals killed after</i>
(a) 2 Hamsters	Intraperitoneal	250	2, 4 mths
(b) 2 Guinea-pigs	Intraperitoneal	250	3, 5 mths

Humans

The eyes of 150 patients suffering from schistosomiasis, including *S. haematobium*, *S. mansoni*, and combined infestation (haematobium + mansoni), and also from mixed parasites (*Ascaris* and *Ankylostoma*) were also examined for ocular lesions.

Results

Animals

SKIN AND CONJUNCTIVAL INSTILLATION

The *cercariae* penetrated the skin of the eyelids at an average rate of 4.6 min. *in vivo* and 5.5 min. *in vitro* (Fig. 1).

FIG. 1 *Cross-section of a cercaria after penetration of the eyelids of a hamster. × 800*

The average time for penetration of *cercariae* through the conjunctiva was 4.2 min. in hamsters and 4.4 min. in guinea-pigs *in vivo*, and 5.4 min. *in vitro* (Fig. 2).

FIG. 2 Cross-sections of three cercariae, showing penetration of one through the conjunctiva of a hamster. $\times 800$

Penetration occurred mostly at the fornices and medial and lateral canthi. In the bulbar conjunctiva of enucleated globes of guinea-pigs, the average time of penetration was 5.4 min., and in isolated bulbar conjunctiva it was 5.1 min. Neither the sclera nor the episclera was penetrated by the *cercariae*.

CORNEAL INSTILLATION

When *cercariae* were instilled on to the cornea of guinea-pigs, they penetrated the epithelial layer only. An average of 13.1 min. elapsed before the *cercariae* reached the basal membrane *in vivo*, and an average of 14.7 min. *in vitro*. Three stages of epithelial penetration were noticed:

- (1) The *cercariae* were held to the corneal epithelium by their oral suckers, and penetrated the flattened epithelium in a perpendicular direction.
- (2) They coursed obliquely through the polyhedral cells to the basal membrane.
- (3) They were seen between the basal epithelial cells attempting to penetrate the basal membrane.

Histological sections of the corneae revealed filling defects at the site of penetration of the *cercariae* into the epithelium (Fig. 3), compared with the control corneae.

FIG. 3 Filling defect at site of penetration of a cercaria through the corneal epithelium of a guinea-pig, reaching the basal membrane.

$\times 3,000$

The *cercariae* penetrated the substantia propria of the trephined cornea only when the barrier of the basal membrane was artificially removed. The average time which elapsed for the *cercariae* to reach Descemet's membrane was 6.4 min. (Fig. 4). They did not penetrate Descemet's membrane. When the same procedure was repeated with the anterior segment of the guinea-pig eye, the *cercariae* took an average of 6.2 min. to reach Descemet's membrane, which was also not penetrated.

FIG. 4 (a) Filling defect at site of penetration of a cercaria through the corneal epithelium of a guinea-pig. (b) Cross-section of cercaria penetrating the corneal substantia propria after induction of an experimental corneal ulcer. $\times 550$

SUBCONJUNCTIVAL INJECTION

The subconjunctival injection of *cercariae* into animals which were kept for from 3 to 6 months led to no ocular lesions, either early or late, as seen clinically and by serial histological sections; but the animals developed generalized schistosomiasis with enlargement of liver and spleen, and ascites was observed, especially in hamsters. The presence of lateral-spined *Schistosoma* ova were detected in thick liver smears, and were also seen by the dissecting microscope and in histological sections of the liver.

PRODUCTION OF SCHISTOSOMIASIS

Generalized schistosomiasis was also produced by the instillation of *cercariae* on to the conjunctival sac and eyelids. Adult *Schistosoma mansoni* worms were collected by perfusion of the liver and mesenteric vessels 45 days after instillation. Histological sections in part of the liver removed before perfusion revealed cross-sections of male and female *Schistosoma* worms, with the female lying in the gynaecophoric canal of the male (Fig. 5).

FIG. 5 Histopathological section, showing adult male and female *Schistosoma mansoni* worms in liver of hamster, after instillation of cercariae into the conjunctival sac. $\times 400$

Serial histological sections of the eyelids and globes revealed no pathological changes.

OCULAR LESIONS

The injection of *cercariae* solution into the anterior chamber of guinea-pigs caused aqueous flare, keratic precipitates, and hypopyon formation. Histological section also revealed keratic precipitates and a mild leucocytic infiltration of the corneal lamellae. The animals showed no evidence of generalized schistosomiasis.

The eyes of animals injected with *Schistosoma mansoni cercariae* by the intraperitoneal route showed no lesions, either clinically or in serial histological sections.

Humans

The eyes of 150 patients suffering from schistosomiasis (Table VI) showed subconjunctival haemorrhages (12), and flame-shaped retinal haemorrhages (3).

Table VI Ocular lesions in cases of *Schistosoma* infection

Type of <i>Schistosoma</i> infection	No. of cases examined	Subconjunctival haemorrhage	Retinal haemorrhage
<i>S. haematobium</i>	67	5	—
<i>S. mansoni</i>	8	2	—
Combined (H and M)	11	1	—
Mixed (<i>Ascaris</i> and <i>Ankylostoma</i>)	64	4	3

Discussion

Hitchcock (1949) noticed that *cercariae* penetrated the skin in 2 to 15 min. and the ear in 2.5 to 8 min. Lewert and Lee (1954) stated that the basement membrane and ground substance acted as barriers to larvae, and demonstrated hyaluronidase-like activity from extracts of *cercariae* of *S. mansoni* which might aid in penetration. Hutchison (1928) stressed that the *cercariae* penetrated the subcutaneous lymphatic vessels, saying that within 6 weeks adult worms could be found in the liver and portal vein. Girges (1934) also stated that, as soon as the *Schistosoma cercariae* came into contact with the skin or mucous membrane, to which they are attracted by warmth, they pierced their way through to the lymphatics.

We found that the *cercariae* easily penetrated the skin of the eyelids and conjunctiva, the latter at a quicker rate. Their penetration *in vivo* was also quicker than *in vitro*, probably because they are attracted by the warmth of the living tissues. They did not penetrate the sclera probably because of its dense fibrous structure and avascularity.

They penetrated only the epithelial layer of the cornea, being presumably held back by the basal membrane, as Bowman's membrane is absent in mammals such as the rat (Duke-Elder and Wybar, 1961) and rabbit (Paton, 1955). However, they did penetrate the substantia propria, when the basal membrane was artificially removed. Similarly, they were held back by Descemet's membrane. Although the *cercariae* were clearly seen by the dissecting microscope and slit-lamp to penetrate the epithelium, their presence in histological sections was difficult to demonstrate; since their penetration is only superficial, they may drop out when the sections are prepared. The site of penetration was clearly seen histologically in the form of filling defects. We did not use fixatives such as Bouin's solution, which enable the *cercariae* to be fixed and adhere to the tissues (Standen, 1953), as we aimed at working under natural conditions.

De Queiroz (1961) found that *cercariae* penetrated the corneo-scleral limbus and the superficial corneal epithelium. He observed a *cercariae* passing through the basal membrane of the cornea in experimental animals after 40 min. instillation. He also noted that the *cercariae* could not penetrate the sclera.

We kept certain animals under observation until their general condition deteriorated. No ocular lesions were evident in any of them, but they developed generalized schistosomiasis. The result was the same when the *cercariae* were instilled on to the eyelid as when they were injected into the ocular tissues.

Thus, the local route of infection theory which was suggested as a cause for the development of ectopic schistosomal lesions, and favoured by Badir (1946), Kamel (1948), and Naim (1948), was not confirmed by our experiments.

The injection of a suspension of *cercariae* into the anterior chamber of guinea-pigs produced aqueous flare, keratic precipitates, and hypopyon formation, but failed to cause generalized schistosomiasis. De Queiroz (1961), on the other hand, found adult flukes in the liver after the injection of *cercariae* into the anterior chamber of the rabbit, but failed to detect them after subconjunctival injection.

The eyes of animals previously infected with *cercariae* of *S. mansoni* through the intra-peritoneal route were unaffected, although they were kept for a long period until their general condition deteriorated from schistosomiasis. De Queiroz (1961) noticed lens changes in white mice, in the form of thickening of the anterior capsule with a granular deposit on the zonular lamella. He thought these might result from an increase in capsular permeability, perhaps through a toxic-allergic factor, from products elaborated by the adult worms.

Although subconjunctival and retinal haemorrhages were detected in few of the human patients examined, we still cannot be justified in saying that they were due to schistosomiasis as they were also seen in patients with mixed infections. No such lesions were evident in the experimental animals. El Tobgy and Wilson (1935) mentioned that, of 203 cases of schistosomiasis, not one showed "any abnormality in the eye". De Queiroz (1916) examined the eyes of 121 patients suffering from *S. mansoni* associated with other intestinal parasites, and although he found such lesions as chronic conjunctivitis, subconjunctival haemorrhages, keratitis, iritis, lenticular opacities, and retinal oedema, he stated that "it would be difficult to make *S. mansoni* specifically responsible for the ocular changes encountered". Massa and others (1965) observed pre-retinal haemorrhage, which they thought to be due to "vascular rupture caused by a fertilized egg of *S. mansoni*".

Various theories have been postulated as to the different routes by which the *Schistosoma* ova, or even the adult worms, can reach the systemic circulation and thereafter lodge in ectopic sites such as the eye. The presence of a patent foramen ovale was suggested, but lesions have been reported in patients in whom the foramen ovale was closed: e.g. *S. haematobium* in a branch of the left coronary artery (El Gazayerli, 1939); schistosomal myelitis (Bayoumi, 1939); schistosomal granulomata in the conjunctiva (Kamel, 1948).

Diamantis (1932) pointed out that *cercariae* develop to maturity and lay their eggs in the veins directly under the skin or mucous membrane through which they have penetrated, if the part is richly vascularized. Bilharz (1852) himself reported a lesion in the dorsal skin of a water carrier.

Day (1937), to explain the presence of these organisms in the lungs, stated that the gradual obstruction of the main portal channels in the liver by bilharzial cirrhosis, led to the opening up of various anastomotic channels, through which they might reach the lungs. Black (1945) suggested that the worms might pass against the blood stream, and that from

the iliac veins and vena cava, they might ascend the scrotal, gluteal, and lumbar veins. Gama and Marques Sa (1945) explained the deposition of *Schistosoma mansoni* ova in the lower level of the spinal cord by the negative pressure in the epidural space. Faust (1948) described the vertebral venous system as a valveless plexus of vessels which intercommunicate with the vesical and haemorrhoidal veins, which are the normal habitat of adult *Schistosoma* worms. The ova in the vertebral venous system can thus spread to distant sites, without undergoing hepatic and pulmonary capillary filtration. Patson (1940) also stated that most cases of aberrant malignant metastasis, aberrant pyogenic metastasis, and aberrant embolism were spread through the vertebral venous system.

It has been found that the ova and adult *Schistosoma* worms can pass through the pulmonary capillaries under certain conditions in which there is no obstruction of the alveolar capillaries resulting in arterio-venous anastomotic channels. It has been proved experimentally that beads of 500 μ diameter, when injected into the pulmonary artery, can pass into the pulmonary vein and hence to the left side of the heart, after passing through the arterio-venous anastomotic channels in the lung (El Mofti and El Zawahry, 1962). This finding provides strong evidence that *Schistosoma* ova are able to pass from the right side of the heart to the left through the pulmonary capillaries, and helps to explain the aetiological problem of ectopic schistosomal lesions.

Summary

The injection of suspensions of *cercariae* subconjunctivally in animals did not cause ocular lesions, but produced generalized schistosomiasis. The instillation of *cercariae* on to the eyelids and conjunctiva also produced generalized schistosomiasis. Injection of *cercariae* into the anterior chamber of guinea-pigs resulted in an aqueous flare, keratic precipitates, and hypopyon, but generalized schistosomiasis did not develop.

The eyes of animals previously infected with *cercariae* of *S. mansoni* through the intra-peritoneal route revealed no ocular lesions. The eyes of 150 patients suffering from schistosomiasis showed subconjunctival haemorrhages in twelve and flame-shaped retinal haemorrhages in three.

It is concluded from the experimental results that the theory of the local route of infection through the eye cannot account for ectopic schistosomal ocular lesions. Generalized schistosomiasis can be produced by cercarial penetration through either the eyelids or the conjunctiva. It is also concluded that schistosomiasis does not affect the ocular structures, except in the form of rare ectopic lesions.

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