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Effects of various concentrations of diethylcarbamazine citrate applied as eye drops in ocular onchocerciasis, and the possibilities of improved therapy from continuous non-pulsed delivery

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SUMMARY Diethylcarbamazine was given as eye drops in varying concentrations in a half-log dilution series from 1·0 to 0·0001% to patients with ocular onchocerciasis. Migration of microfilariae into the cornea, followed by their straightening and disintegration, was observed with delivery rates as low as 0·1 μg/hour. Dose-related adverse inflammatory reactions, including the development of globular limbal infiltrates with itching and redness, were seen with delivery rates as low as 0·6 μg/hour, but substantial inflammatory reactions, including severe vasculitis, were seen only with delivery rates of or above 1·0 μg/hour. This suggests that it should be possible to achieve beneficial clearing of the microfilarial load, without adverse reactions, by continuous non-pulsed delivery of the drug. Technology exists for such delivery, either directly into the eye or systemically by a transdermal system that could give 3 to 7 days' treatment from each application. The observations reported suggest that after preliminary clearing of the microfilarial load by carefully controlled delivery of DEC it may be possible to maintain therapy by less strictly controlled delivery in DEC-medicated salt, or to use treatment with suramin, without incurring substantial adverse reactions, such as a deterioration in vision in cases in which the optic nerve is already compromised. Continuous non-pulsed DEC delivery systems could have a place in the management of onchocercal sclerosing keratitis. The unique opportunities for using the ocular model to define the requirements for beneficial non-damaging therapy with DEC should be explored in further field trials.

Diethylcarbamazine citrate (DEC) is the most effective microfilaricide available for treating ocular or other forms of onchocerciasis. It is therefore potentially of great public health importance in the prevention of blindness from onchocerciasis and for treating certain other filarial diseases, but it is a very difficult drug to handle in cases of severe ocular onchocerciasis (Anderson *et al.*, 1976a). It has low toxicity for animals and man, but serious adverse systemic reactions can occur when it is given orally by ordinary therapeutic schedules to heavily parasitised patients. These reactions include itching, papular and exfoliative skin eruptions, arthralgia, vertigo, collapse (Fuglsang and Anderson, 1974; Duke *et al.*, 1976; Bryceson *et al.*, 1977), and even death (Oomen, 1969; Anderson *et al.*, 1976a).

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Ocular adverse reactions occur also with systemic administration of the drug. These comprise acute exacerbations of ocular inflammation with watering, photophobia, bulbar and circumcorneal hyperaemia, the formation of minute globular infiltrates at or near the limbus, and snowflake or fluffy punctate corneal opacities (Anderson *et al.*, 1976a). Twice-daily administration of 1 to 1·5 mg betamethasone may diminish but not prevent these ocular reactions. There is evidence that a 10- to 14-day oral course of DEC followed by a weekly maintenance dose may improve vision in onchocercal sclerosing keratitis or iritis, but it seems not to benefit the other ocular lesions and may even worsen the visual deficit from retinal or optic nerve disease (Anderson *et al.*, 1976a).

Interest has therefore returned to the idea of delivering DEC topically (Lazar *et al.*, 1968; Ben-Sira *et al.*, 1970; Lazar *et al.*, 1970; Aviel and David,

1972; Anderson and Fuglsang, 1973) to treat or prevent those onchocercal lesions for which DEC therapy has been shown to be beneficial (Anderson *et al.*, 1976a). However, the topical administration of 3% DEC drops 4 times daily, without steroid therapy, to patients with ocular onchocerciasis can lead to severe adverse ocular reactions including severe itching, redness, swelling of lids and conjunctiva, or severe iritis with hypopyon (Anderson and Fuglsang, 1973).

In retrospect it seems that the selection of the DEC dosage regimen of a 3% solution given 4 times a day was arbitrary, without adequate basis in prior clinical pharmacokinetic and pharmacological evaluation. A review of this matter is therefore required.

A single topical administration of 5% DEC to the rabbit eye rapidly gives a level of 10 μg/ml in the aqueous at half an hour, followed by a rapid fall of 5 μg at 1 hour and 1 μg/ml at 3 hours (Lazar *et al.*, 1968). The more severe adverse reactions seen clinically with 3% DEC eye drops may thus relate to repeated high peak levels in the ocular tissues. Weaker solutions would be expected to give lower peak levels, but the duration of an effective drug level would be shortened. To maintain an effective tissue drug level without peak levels giving adverse reactions in heavily parasitised eyes it might suffice to use very frequent applications of weaker solutions of DEC with the addition of agents to prolong the contact time. But the frequency of application required might well be prohibitive. The levels of drug that are respectively beneficial or damaging, the margin between these, and the rate of rise and fall of tissue drug levels following topical administration will determine whether beneficial therapy or prophylaxis could be achieved with DEC eye drops, or whether continuous controlled delivery of the drug would be needed to achieve that goal.

Technology has developed that enables drugs to be delivered to the eye continuously and at a controlled rate, thereby attaining steady intraocular levels within a narrow therapeutic range, with the avoidance of undesirable side-effects that result from higher peak levels (Yates *et al.*, 1975). Thus pilocarpine can be delivered continuously day and night from an ocular therapeutic system (Ocusert) at a rate that can give effective reduction of intraocular tension in glaucoma with less miosis and without the disabling fluctuations in induced myopia that occur in some patients with pulsed delivery of pilocarpine from eye drops. The delivery rate: 10, 20, 40, or 60 μg/hour can be chosen to fit the therapeutic requirement.

The rapid kinetics of rise and fall in intraocular drug level of DEC after topical administration closely resemble those of pilocarpine. It is therefore possible that continuous controlled delivery of DEC from similar devices could provide effective therapy of ocular onchocerciasis, especially the potentially blinding sclerosing keratitis, without the adverse reactions which at present preclude definitive trials of topical DEC therapy. In addition, continuous delivery of low levels of DEC may protect the eye from entry of further microfilariae (MFS), thus preventing blindness in heavily parasitised persons at high risk.

Technology also exists for controlled, non-pulsed, continuous systemic delivery of DEC by the transdermal route (Yates *et al.*, 1975). This system has the great advantage of potentially being able to provide 3 to 7 days' controlled therapy of the whole body from a single application.

If experiments show unequivocally that carefully adjusted, continuous, non-pulsed delivery of DEC to the eye can result in beneficial topical therapy without serious adverse reactions, it could be deduced that a comparable controlled, non-pulsed, continuous systemic delivery of DEC should be able to yield the benefits to be had from gross reduction in microfilarial load without the potentially blinding, disabling, and disagreeable adverse effects that at present render DEC of little use in the therapy of ocular onchoceriasis, and which contraindicate its use in mass therapy of severely parasitised persons, especially those who already have an onchocercal visual deficit from retinal or optic nerve disease.

A fundamental requirement for continuous delivery to be practicable for mass therapy, either locally in the eye or systemically by the transdermal route, is that the chosen drug must have high intrinsic activity so that a practical duration of therapy could be provided by each insertion or application. DEC does not kill MFS directly in *in vitro* systems but rapidly brings about the destruction of MFS by host mechanisms (Hawking *et al.*, 1950). In the absence of an animal model of infection with *Onchocerca volvulus* the level of activity of DEC against this organism can be determined only in man.

The present preliminary investigation was undertaken to provide data on the minimum concentration and delivery rate of topically administered DEC that affects the microfilariae of *O. volvulus* in the cornea, and the delivery rates of DEC that produce undesirable inflammatory side effects.

Materials and methods

Fourteen patients from the rain forest village of Baduma in the United Republic of Cameroon volunteered to take part in the study. All were

moderately to heavily infected with onchocerciasis, and all showed MFS in the cornea or anterior chamber or both. Skin snips were taken near the outer canthus with a corneoscleral punch, and the MFS that emerged in saline were counted under the microscope (Fig. 1). The patients were randomly allocated to the different drop regimens.

Different concentrations of DEC drops were instilled at varying intervals and for different periods as described under each experiment. The anterior segments were examined with a Haag-Streit 900 slit lamp, and the numbers of MFS in the anterior chamber (MFAC) were counted or estimated before and after bending the head down for a minute. Microfilariae in the cornea (MFC) and reactions resulting from their presence were counted or estimated if there were more than 50 per cornea. An attempt was made at each examination to chart the position and appearance of individual larvae (e.g., whether living or dead) and opacities.

Further observations were made and photographs taken of typical ocular lesions in other patients infected with the savanna strain of *O. volvulus* at Toubourou in North Cameroon.

Photographs of the MFS and the corneal lesions were taken with a portable photographic system (Jones, Sheen, and Minassian, in preparation) using a reversed Canon FL 35 mm f2·8 automatic diaphragm lens (Fig. 2) and a preset Leitz UM/0·22 geological microscope lens (Fig. 3), in each case with an extension tube to give the magnification indicated. Diffuse oblique illumination was from a modified Vivitar 283 flash unit. Positive transparencies taken on Kodachrome 64 were enlarged on Cibachrome.

Results

Table 1 gives the age and sex of the patients taking part in experiments 1 to 3, indicates the number of MFS counted in the skin snip near the eye, and shows the concentration and dosage of the drops which each patient received.

Experiment 1: the effects of 9 different DEC concentrations given 3 times daily over 2 days
Patients *A–I* received 1 drop in the right eye only, at concentrations in a half-log dilution series ranging

Table 1 *Patients* A–N *taking part in experiments 1, 2, and 3: their age and sex, and indication of the number of microfilariae (MFS) in a skin snip near the outer canthus, the DEC concentration, the DEC content per drop (in μg), of the solution, the drop regimen, and the stage at which drug effect was observed*

Patient	*Age*	*Sex*	*MFS*	*DEC solution (%)*	*DEC per drop (μg)*	*DEC drop regimen*		*DEC effect after*
						Day 1	*Day 2*	
A	34	M	26	1·0	50	1×3	1×3	3 drops
B	46	M	16	0·3	15	1×3	1×3	3 drops
C	37	M	116	0·1	5	1×3	1×3	3 drops
D	40	F	9	0·03	1·5	1×3	1×3	3 drops
E	35	F	19	0·01	0·5	1×3	1×3	3 drops
F	32	M	6	0·003	0·15	1×3	1×3*	Nil
G	33	M	128	0·001	0·05	1×3	1×3*	Nil
H	45	M	2	0·0003	0·015	1×3	1×3*	Nil
I	27	F	13	0·0001	0·005	1×3	1×3*	Nil
J	40	F	84	0·003	0·15	1/15 min ×20	Nil	20 drops
K	45	M	50	0·001	0·05	1/15 min ×20	Nil	20 drops
L	9	M	122	0·0003 (right) 0·0001 (left)	0·015 0·005	1/15 min ×20 1/15 min ×20	1/5 min ×20 1/5 min ×20	Day 2 20 drops Nil
M	41	M	54	1·0	50	1×2	1×3†	2 drops
N	29	M	79	0·03	1·5	1×3	1×3†	3 drops

*An additional drop was given at 0900, 1000 and 1100 h on the second day. †see text—Experiment 3 for additional dosage

from 1·0 to 0·0001%, at 0800, 1200, and 1700 h on 2 consecutive days (Table 1). Patients *F–I* were given an additional drop at 0900, 1000, and 1100 h on the second day. Examinations were carried out just before the instillation of the first drop, and 24 and 48 hours later.

Table 2 shows the numbers of MFS seen in the right anterior chamber and cornea at the 3 examinations. MFAC were present in all but patient *H* before treatment, and in this patient they were not seen at any examination. In the other patients there was little change in the numbers of MFAC throughout the observations. By contrast, there was an increase in the numbers seen in the right cornea in patients *A–E*. It was particularly striking in patient *B*, in whom 14 MFS had entered the previously clear cornea within 24 hours of starting treatment with 0·3% DEC drops. No increase in numbers was noted in patients *F–I*, who received additional drops on the second morning. In addition to the entry of MFS into the cornea there was an increase in the ratio of straight to curled-up MFS (Fig. 2) in patients *A–E*, and at 48 hours very few curled-up larvae were seen. This change was not observed in the control left corneas nor in either eye of patients *F–I*.

Table 2 shows the numbers of globular limbal 'infiltrates' observed in the right eye at 24 and 48 hours. They presented an appearance of very small (usually less than 0·1 mm diameter) grey-beige dots, which straddled the limbus and were more often just on its corneal side (Figs. 3, 4, 5, and 6). At first they were sharply defined, oval or circular, like a globule. The most superficial globules raised the epithelial surface like a blister (Fig. 3). Over the ensuing day or so the contours gradually became more diffuse (Figs. 4, 5, and 6). In patient *A* the majority were at the lower limbus and only 1 was seen above, while in patient *C* the distribution was exactly the reverse, and only 1 of his 14 infiltrates was not covered by the upper lid. In patients *A–D* infiltrates were already present at 24 hours, but in patient *E* the infiltrate was not seen until the examination at 48 hours. Infiltrates were not seen in patients *F–I*, nor in the control left eyes.

Slight itching of the right eye and lids was noticed on the first day of drop therapy by patients *A*, *C*, and *D*, and mild conjunctival injection developed in patients *B*, *C*, and *D*. Before treatment patient *C* showed a fine flare with circulating cells in the anterior chamber, which remained unchanged during treatment. No other patient developed signs of anterior uveal irritation. The average daytime DEC delivery rates for patients *A–I* are shown in Table 3.

Experiment 2: the effects of weak DEC solutions given repeatedly at short intervals

Patients *J*, *K*, and *L* received DEC drops quarter-hourly as indicated in Table 1, and the corneas were examined as above.

On the day following the drop therapy, the effect

Table 2 *The number of microfilariae seen before treatment, and at 24 and 48 hours after treatment, in the right anterior chamber and cornea, together with the number of infiltrates seen at the right limbus*

Patient	Number of microfilariae						Number of infiltrates		
	Anterior chamber*			Cornea					
	Before treatment	After treatment (hours)		Before treatment	After treatment (hours)		Before treatment	After treatment (hours)	
		24	48		24	48		24	48
A	2	3	4	33	44	50+	0	1	8
B	3	3	1	0	14	15	0	3	3
C	20+	20+	20+	13	30	30	0	12	14
D	1	3	5	10	21	17	0	4	4
E	2	1	4	16	22	22	0	0	1
F	10	15	15	50+	50+	50+	0	0	0
G	2	0	0	100+	100+	100+	0	0	0
H	0	0	0	4	5	5	0	0	0
I	5	10	10	4	4	4	0	0	0

*The highest number seen either before or after bending the head down for 1 minute

of DEC was seen in the corneas of patients *J* (0·003%) and *K* (0·001%). The average daytime DEC delivery rates are shown in Table 3. New MFS had entered the right corneas of both these patients in significant numbers. Globular limbal infiltrates developed in patient *J*, but they were not seen in patient *K*. Neither patient complained of itching, and the eyes remained white.

The quarter-hourly instillations in patient *L* gave rise to no detectable changes in either eye (0·0003% in the right eye, and 0·0001% in the left eye). On the day following the instillations at 5-minute intervals a new limbal infiltrate was present in his right eye, and scattered new MFS had entered this cornea. No changes were seen in his left eye. Both eyes remained white throughout the period of observations and he never complained of itching.

Experiment 3: the effect of 0·03% and 1·0% DEC drops given 3 times daily over 2 to 3 weeks

Patients *M* and *N* were given drops for longer periods. The former received a 1·0% solution in his right eye: 1 drop 3 times daily for 5 days, and then 2 drops 3 times daily for the succeeding 16 days, with additional drops on certain days as described below. Patient *N* was given a 0·03% solution in his right eye for 2 weeks: 1 drop 3 times daily for the first 6 days, and 2 drops 3 times daily for the other 8 days. Each patient was examined almost daily throughout the period of therapy. They were again examined 1 and 2 weeks after the end of therapy respectively. The average daytime DEC delivery rates are shown in Table 3.

After the first 2 drops conjunctival injection was so gross in patient *M* that the evening drop was not given. He also complained of itching of the eye. The next morning the injection had largely gone, but within 45 minutes of his next drop the eye was again very red. The midday drop was instilled and by the evening the eye was almost white again, and it remained so thereafter. However, this patient continued to complain of itching of the eye for a few more days, and also of intense itching of the whole head. Patient *N* experienced slight itching of his right eye during the first day only, but the eye was never injected.

The 2 patients had respectively 1 and 20+MFAC in their right eyes before treatment, and similar numbers were still present at their last examinations. In particular, there was no reduction in the large numbers of MFAC in patient N. Neither patient showed signs of active anterior uveal irritation throughout the observations, though in both there were 1 or 2 old keratic precipitates which did not change.

Globular limbal infiltrates, as described under experiment 1, were present in both patients 24 hours after the first instillation of drops. Patient *M* presented about 20, and patient *N* about 50, the large majority of which were at the lower limbus. They all faded gradually over the next few days, though 1 or 2 new ones occurred in patient *M* at the end of the first week, and a fresh globular infiltrate was seen in patient *N* on the last day of therapy.

Patient *M* was estimated to have between 50 and 100 MFC before treatment, most of which were living. Within a day or 2 after the start of treatment almost all the larvae were straight, and their numbers were now estimated in hundreds. Before treatment he showed 2 fluffy opacities, and 1 or 2 other opacities around MFS. By day 4 the periphery of the cornea was littered with straight larvae, and most of them were surrounded by some degree of reaction, producing an overall haziness. The entire limbus was slightly swollen on day 5, and on day 6 the individual reactions were maximal. Thereafter the

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Fig. 1 *Actively motile, wriggling* O. volvulus *MFS from a single snip, suspended in saline.* (a) *Viewed by oblique illumination against a dark background.* (b) *Viewed by retroillumination against light reflected from out-of-focus bubbles* (×40: primary magnification ×10, enlarged ×4)

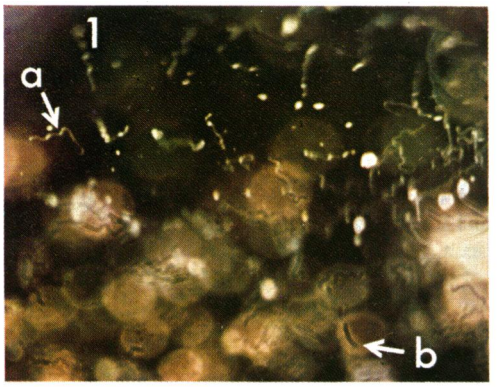
Fig. 2 O. volvulus *MFS in the cornea 24 hours after DEC eye drops.* (a) *Wriggling actively motile MFS viewed by retroillumination from the out-of-focus pupil border.* (b) *Straight non-motile MFS* (×40: primary magnification ×10, enlarged ×4)

Fig. 3 *Fresh, sharply defined globular infiltrates 24 hours after DEC eye drops.* (a) *Deep, apparently subepithelial globule in peripheral cornea.* (b) *Superficial, apparently intraepithelial globule raising the surface like a micro-blister or pustule at the corneoscleral junction.* (c) *Pigmented epithelial cells* (×64: primary magnification ×16, enlarged ×4)

Fig. 4 *Soft-edged dispersing globular limbal infiltrates 48 hours after DEC eye drops* (×18·8: primary magnification ×4·7, enlargement ×4)

Fig. 5 *Peripheral corneal vasculitis 24 hours after DEC eye drops.* (a) *Soft-edged patchy yellowish perivascular infiltration around both arterial and venous segments of the vascular loops.* (b) *Microhaemorrhage from terminal vascular loop with focal perivascular inflammatory cuffing* (×18·8: primary magnification ×4·7, enlargement ×4)

Fig. 6 *Intense peripheral corneal vasculitis 48 hours after DEC eye drops.* (a) *Dense yellowish perivascular sheathing.* (b) *Fading globular infiltrates* (×18·8: primary magnification ×4·7, enlargement ×4)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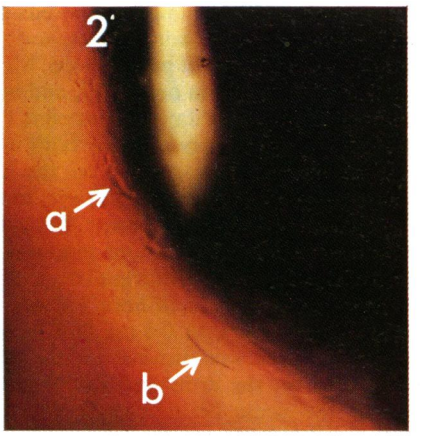
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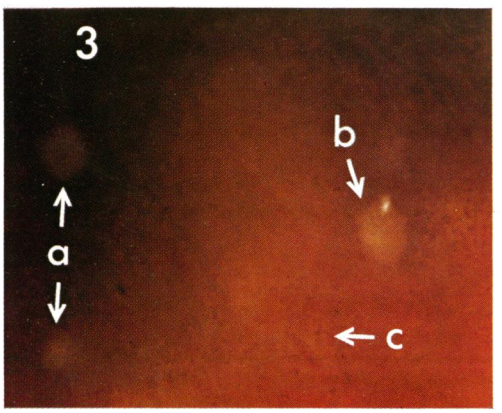
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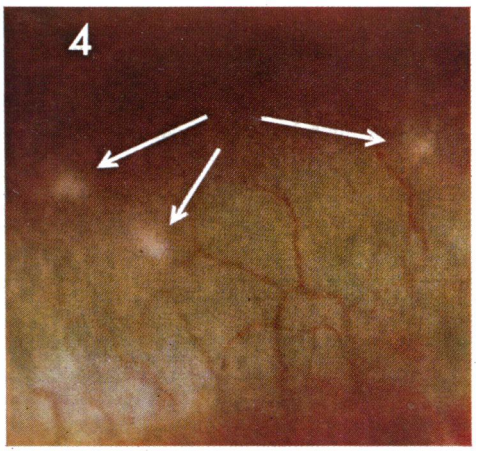
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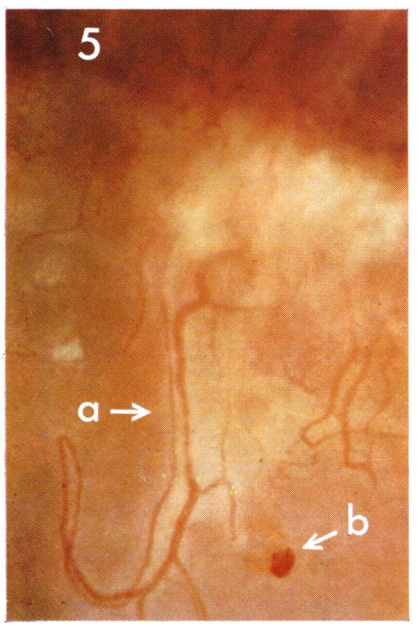
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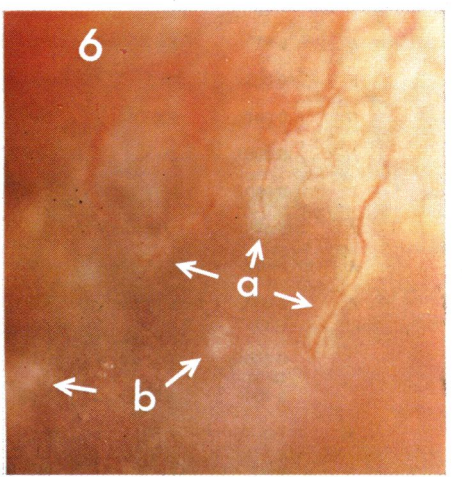
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cornea, though it still contained hundreds of visible straight larval bodies, remained quiet, and reactions around the larvae were not pronounced. Movements were seen in a microfilaria on day 12, and in another on day 14. On the 15th day of treatment 1 drop of 1% DEC was given every hour in both eyes for 4 hours during the morning, and on the 16th day every half hour for 4 hours (the left eye, in fact, had also received DEC drop treatment, but with additional steroid drops). This more intensive therapy did not lead to any irritation or conjunctival injection, but after the half-hourly drops (a total of 16 drops in 4 hours, or 0·8 mg DEC) the patient complained of renewed itching on the scalp and face and also on the arms and other parts of the body. On the arms he developed small papules resembling a Mazzotti reaction. One week after the end of therapy about 10 living MFS were observed in the right cornea.

Before treatment, the right cornea of patient *N* presented 1 living microfilaria, 1 lying straight without reaction, and 1 in the anterior stroma with an early reaction around the middle of its length, plus 2 peripheral opacities in which microfilarial bodies were no longer visible. At 24 hours, after only 3 drops of the 0·03% DEC solution (about 4·5 μg had been instilled), this cornea contained no fewer than 40 larvae, of which about 10 were straight, while the remainder were still moving actively. At 48 hours only 30 larvae were counted, and almost all were now straight. By the next day reactions could be seen around many of the larvae. On the 12th day of therapy 2 actively moving MFS were seen, and a total of 41 MFS were counted. Most of the MFS showed reactions, but many were lying without detectable reaction, though their level in the cornea could be observed in the direct beam of the slit lamp. At the examination 2 weeks after the end of therapy 3 living MFS were seen in the patient's right cornea, and there were still many other larvae with reactions around them.

Discussion

PHENOMENA SEEN AFTER TOPICAL ADMINISTRATION OF DEC

The time sequence of the phenomena seen in this study and in a previous investigation of the effect

Table 3 *The average daytime DEC delivery rate (μg/h), the effects on microfilariae in the cornea (MFC), and the inflammatory sequelae in experiments 1, 2, and 3*

Experiment number	*Patient*	*DEC solution (%)*	*DEC drop regimen per day*	*Average daytime delivery rate (μg/h)*	*DEC effects at 24 hours or earlier*		*Inflammatory sequelae*		
					Effects on MFC				
					Migration	*Straightening*	*Itching*	*Globules*	*Redness*
1	*A*	1·0	1×3	10·0	+	+	+	+	−
1	*B*	0·3	1×3	3·0	+	+	−	+	+
1	*C*	0·1	1×3	1·0	+	+	+	+	+
1	*D*	0·03	1×3	0·3	+	+	+	+	+
1	*E*	0·01	1×3	0·1	+	+	−		−
1	*F*	0·003	1×3	0·03		−	−	−	−
1	*G*	0·001	1×3	0·01	−	−	−	−	−
1	*H*	0·0003	1×3	0·003	−	−	−	−	−
1	*I*	0·0001	1×3	0·001	−	−	−	−	−
2	*J*	0·003	1/15 min×20	0·6	+	+	+	+	+
2	*K*	0·001	1/15 min×20	0·2	+	+	−	−	−
2	*L*	0·0003 (right)	1/15 min×20	0·06	−	−	−	−	−
			1/5 min×20	0·18	+	+	−	+	−
		0·0001 (left)	1/15 min×20	0·02	−	−	−	−	−
		0·0001	1/5 min×20	0·06	−	−	−	−	−
3	*M*	1·0	1×2	10·0	+	+	+	+	+
3	*N*	0·03	1×3	0·3	+	+	+	+	+

of topical DEC in ocular onchocerciasis (Anderson and Fuglsang, 1973) is summarised in Table 4. There is considerable but variable overlapping due to the variable time span of the various features, not all of which appear in every case. In general, the more severe the reaction the more of the 11 phenomena are seen, the more severely and rapidly they develop, and the longer they tend to persist. Conversely, in the milder reactions fewer of the phenomena are seen, they are less intense, appear more slowly, and resolve more rapidly. The effects of DEC fall into 2 groups: those that directly reflect alterations in the MFS and those that are due to host reactions of an inflammatory nature.

We postulate that the increased migration of MFS into the cornea is brought about by the MFS moving down a gradient of DEC concentration. If this is true, the migration may be taken to indicate the presence of a drug gradient, but not necessarily to indicate the presence of a microfilaricidal level of drug. In the case of oral dosage (Anderson *et al.* 1976a) it is reasonable to assume that the drug reaches higher concentrations in the vascularised conjunctival and limbal tissues earlier and in higher concentration than in the avascular corneal tissue. This would provide a gradient that would drive the MFS into the cornea.

The phenomenon of migration of *O. volvulus* MFS observed after topical application of DEC was identical with that seen after oral administration. We therefore postulate that the drug passes through the conjunctival epithelial barrier more readily than the corneal epithelial barrier, thereby giving DEC gradients between conjunctiva, limbus, and cornea similar to those presumably given by oral administration. This would mean that transconjunctival penetration offers an important route of entry for DEC and other drugs such as pilocarpine that appear to have similar ocular pharmacokinetic behaviour.

Straightening of MFS under the influence of DEC is associated with immobility and impaired function of the parasite (Fig. 2). It probably indicates the initiation of a lethal process. The opacification which follows is even more likely to correlate with death of the parasite, and focal fluffy infiltration with granular disintegration of the microfilaria leaves no doubt about destruction of the parasite. Migration and straightening of the microfilariae thus offer to a skilled observer a convenient, rapid, sensitive, and practical index of antimicrofilarial action from DEC.

The appearance of peripheral corneal and limbal globular infiltrates is generally associated with itching and watering, and it preceeds the onset of limbitis; microhaemorrhages and gross vasculitis may follow. The speed and intensity of development of these infiltrates are readily quantifiable and appear to correlate with the development and intensity of ensuing inflammatory disease, although vasculitis appears to occur especially, and be most intense, in eyes that already have peripheral corneal vascularisation from previous disease. The sharply

Table 4 *Time sequence of effects of topical DEC on* O. volvulus *microfilariae (MFS) in the cornea, and host responses*

	Alterations in MFS	*Host responses*	*Time observed*
1.	Increased entry of MFS into the cornea		1½ hours to a few days
2.	Increasing ratio of straight to curled MFS (Fig. 2)		A few hours to a few days
3.		Itching and watering	2 hours to a few days
4.		Peripheral corneal and limbal globular infiltrates (Figs. 3–6)	2 hours to a few days
5.		Diffuse or patchy limbal, conjunctival or episcleral vascular congestion	2 hours to a few days
6.		Soreness and pain	4 to 48 hours
7.		Limbitis: vascular congestion, oedema and cellular infiltration causing visible swelling of the limbal zone	4 to 48 hours
8.	Opacification of straightened MFS		2 to 3 days
9.		9*a*. Faint focal infiltration around MFS	24 to 48 hours
	9*b*. Granular disintegration of MFS amidst increasing fluffy focal infiltration		A few days
		9*c*. Fluffy punctate opacities; when intense and confluent may give patchy stromal haze, especially in the periphery	48 hours to a few weeks
10.		Gross vasculitis of corneal vessels—engorgement, perivascular oedema and infiltration giving yellowish sheathing (Fig. 6), sometimes with microhaemorrhages from terminal vascular loops (Fig. 5). Neovascularisation may follow	36 hours to a few days
11.		Acute anterior uveitis, flare, cells, and even hypopyon	24 hours to a few days

defined borders and the homogeneous beige-grey or yellowish colour of the globules, when they first appear in the peripheral cornea or limbus, are very distinctive, as is the blister-like elevation of the epithelial surface that occurs with most superficial globules (Fig. 3). The borders become diffused during the ensuing day or 2 (Figs. 4, 5, and 6) so that these globular infiltrates are easy to recognise and to date. They can easily be distinguished from other lesions such as Trantas's spots or the marginal corneal infiltrates that commonly occur with staphylococcal disease. They seem to occur only in the presence of MFS in and around the eye. They have also been described as following treatment with systemic DEC (Anderson *et al.*, 1976a), suramin (Anderson *et al.*, 1976b), metrifonate (Fuglsang and Anderson, 1977), and topical administration of levamisole to the eye (Jones *et al.*, 1978). Histological observations on these globular infiltrates will be published elsewhere.

It is proposed that quantification of the globular infiltrate formation (and perhaps other signs) could offer a convenient, rapid, sensitive, and practical index of the ensuing potentially damaging sequelae to a particular profile of delivery of an antimicrofilarial drug.

EFFECTS ON MICROFILARIAE OF VARIOUS TOPICAL DELIVERY RATES OF DEC

In experiment 1, as judged by the entry of MFS into the cornea, the straightening of previously curled-up MFS, and the formation of globular limbal infiltrates, the weakest effective DEC solution was 0·01% given at the rate of 1 drop 3 times daily to a total of 1·5 μg. When this dosage is averaged over the 4- to 5-hourly administration it gives an average daytime delivery rate of 0·1 μg/hour (Table 3).

In experiment 2 DEC effects were seen in patient *K* after 1 drop of 0·001% every 15 minutes for 5 hours, or a total of 1·0 μg, giving an average daytime delivery rate of 0·2 μg/hour, and in patient *L* after 1 drop of 0·0003% DEC every 5 minutes, or a total of 0·36 μg, giving an average delivery rate of 0·18 μg/hour.

In this study, as in the previous one (Anderson and Fuglsang, 1973), topical administration of DEC grossly reduced the number of moving or curled and apparently viable MFS in the cornea, but in the short term did not eliminate them entirely even with higher dosage. The explanation of this subtotal clearance of viable MFS is not clear, although age of MFS might be a factor.

From these results it is clear that DEC has a very high level of intrinsic activity against the MFS of *O. volvulus* in the human cornea. This lies in the same range as the anti-inflammatory activity of prednisolone in the human cornea: minimal anti-inflammatory action with 0·05 to 0·15 μg/hour (Williams *et al.*, 1977). DEC would therefore meet the fundamental requirement for practical usage in mass chemotherapy by continuous non-pulsed delivery either to the eye or systemically by the transdermal route.

The results of experiment 3 indicate that locally administered DEC could keep the cornea more or less clear of MFS, and the small numbers that might enter would be of relatively little importance. One of the major causes of blindness from onchocerciasis in savanna regions of West Africa is sclerosing keratitis, which usually results from heavy parasitisation of the cornea, and the prevention of this complication by local therapy would be of great benefit. However, in highly endemic regions this parasitisation frequently begins before the age of 10, so it would be necessary to institute therapy at an early age.

So far as MFS in the anterior chamber are concerned, it is known that they may be little affected by systemic DEC (Anderson and Fuglsang, 1973; Anderson *et al.*, 1976a; Duke, 1975). In a previous experiment 2 drops of 3% DEC instilled 4 times daily for 9 days, equivalent to 1·2 mg/day or a daytime average delivery rate of 92 μg/hour, did not completely clear the anterior chamber of MFS, although there was a reduction in the numbers in some patients (Anderson and Fuglsang, 1973). The lesser concentrations of DEC used topically in the present study were therefore not expected to clear the AC of MFS. In fact, after 15 days of treatment patients *M* and *N*, receiving 10 and 0·3 μg/hour during the day, had the same number of MFAC as before treatment. It is known that the destruction of MFS is brought about by host cells (Hawking *et al.*, 1950), so the rather poor and variable effect of DEC against MFS in the anterior chamber may possibly be ascribed to the low cellular content of the normal aqueous. It is of interest that DEC can, however, benefit onchocercal iritis, in which the cellular content of the aqueous is greatly elevated (Anderson *et al.*, 1976a).

INFLAMMATORY HOST RESPONSES FOLLOWING VARIOUS TOPICAL DELIVERY RATES OF DEC

The nature and the intensity of the inflammatory reactions that follow administration of DEC presumably depend on the interaction of 4 major potential factors: (*a*) The number and the density of living MFS in the ocular tissue concerned; (*b*) the immunological status in respect of *O. volvulus* and the reactivity of the individual patient; (*c*) the rate and duration of delivery of DEC which determine the drug levels reached and their time-related

profile of concentration in the target tissue; and (*d*) possibly the strain of *O. volvulus*, or other factors affecting the severity of adverse responses to oral DEC therapy.

The difference in number and density of living MFS between patients *A* and *M* may explain the different intensity of inflammatory responses. Patient *A* had 26 MFS in the skin snip near his outer canthus and 33 in his cornea. Patient *M* had 54 in his skin snip and 50–100 in his cornea. Each was allocated to treatment with 1% DEC drops 3 times daily. The reaction in patient *A* was very mild. At the time of the third drop he had no reaction, at 24 hours he had only 1 limbal globular infiltrate, very little injection, and no itching; thereafter the reaction subsided. In contrast, patient *M* had such intense itching, lid swelling, and injection of the eye after the second drop that the third drop was withheld. At 24 hours, after only 2 drops, he had 20 limbal globular infiltrates with limbitis. The thrice daily administration of drops was continued despite the moderately severe reactions, which were maximal on the sixth day. Thereafter they diminished, though the cornea was littered with straight opacified MFS. On the 15th day he received 1% every hour for 4 hours and on the 16th day every half hour for 4 hours without return of ocular irritation or signs of inflammation, though he developed a generalised Mazzotti reaction at that time.

There are very few data on the immune responses in onchocerciasis, largely because of the difficulty in preparation and supply of pure antigens of *O. volvulus*, and no immunological correlations have been reported with the nature or intensity of the reactions following administration of DEC. However, these probably important immunological variations between patients can be eliminated by comparing the responses of right and left eyes of individual patients to differing delivery rates of DEC, as was done with patient *L* in experiment 2.

From Table 3 it is apparent that although average daytime delivery rates of 0·1 μg/hour *(E)*, 0·18 μg/hour *(L)*, and 0·2 μg/hour *(K)* induced lethal effects in the visible MFS, it generally required delivery rates of 0·3 μg/hour *(D)* to 0·6 μg/hour *(J)* or higher to give inflammatory sequelae. The inflammatory reactions were rarely severe with delivery rates below 1 to 3 μg/hour. The topical doses in the eye that led to generalised reactions of the Mazzotti type were higher still: 1·2 mg over 24 hours (50 μg/hour) over 4 days totalling 4·8 mg in 1 patient (Anderson and Fuglsang, 1973) and 0·8 mg over 4 hours (200 000 μg/hour), following half this dosage schedule on the previous day, in patient *M*.

Corticosteroids can diminish the adverse inflammatory reactions to DEC therapy of onchocerciasis, but at present there are too few data relating timing and dosage of steroid with anti-inflammatory effect on which to plan and test optimal dosage schedules. Oral betamethasone 1 to 1·5 mg given twice daily for 1 day before and during the first 4 to 6 days of oral DEC therapy reduced, but did not abolish, the adverse effects. Nor did it prevent the further visual deterioration following DEC therapy in persons who already had a visual deficit from posterior segment or optic nerve disease at the time of treatment (Anderson *et al.*, 1976a).

There are suggestions that the effects of corticosteroids on cellular host defence mechanisms may substantially interfere with the killing of *Wuchereria bancrofti* MFS under the action of DEC (Schofield and Rowley, 1961). Under oral therapy with DEC alone *O. volvulus* MFS generally stop entering the cornea after a few days. In contrast, new MFS have been seen to enter the cornea more or less daily (or nightly) for 8 weeks in 2 patients under combined therapy with oral DEC and oral and topical prednisolone (unpublished data, B. R. Jones and A. Patterson).

This additional interaction may offer complications in working out optimal schedules of delivery of each drug in combined therapy. The already complex situations, with multiple and largely uncontrolled variables that exist in systemic chemotherapy experiments, are compounded by adding an arbitrary regimen of additional drugs that appear to act to a potentially variable degree by blocking the microfilaricidal effects of DEC. Attempts to disentangle these effects in such studies with ill defined and fluctuating levels of DEC would seem unlikely to yield solid data within a reasonable time. The opportunities are obvious for obtaining the essential data for this purpose by using right and left eye comparisons with graduated topical steroid therapy (Williams *et al.*, 1977) against a background of graduated continuous non-pulsed ocular delivery of DEC.

The anterior segment of the eye offers the unique feasibility of controlled clinical trials with stratification of patients on the basis of quantification of the following: (1) Microfilarial loading in the cornea and adjacent skin; (2) previous vascular damage and neovascularisation; (3) various types of ocular lesion that may reflect various modes of immunological reactivity to *O. volvulus*, as well as the general factors; (4) age and sex of patient, and strain of *O. volvulus* (Braun-Munzinger and Southgate, 1977). Furthermore: (5) Right-left eye comparisons of responses enable many of the variables to be eliminated; (6) continuous, non-pulsed ocular delivery systems for both DEC and corticosteroid

could become available; (7) ethical acceptability results from the following considerations: *(a)* suitable patients with ocular onchocerciasis are at risk of blindness, and need treatment; *(b)* any adverse reactions that may occur take place in the relatively non-critical tissues of the anterior segment of the eye, rather than in critical tissues such as the optic nerve which may already be in a precarious state of compromised blood supply; *(c)* adverse reactions are immediately accessible for clinical microscopic observations to guide alterations in management; *(d)* no patient need be placed at risk of visual damage or substantial discomfort; *(e)* the precision possible in such studies yields clear-cut results with minimal numbers of observations on a few patients receiving only small quantities of drug for short periods. Finally (8) such studies can be expected to determine the single or combined drug delivery rates that cause: *(a)* death of microfilariae; *(b)* trivial inflammatory sequelae; and *(c)* potentially blinding adverse effects.

These data will determine the therapeutic index and guide optimal local and systemic therapy of onchocerciasis with DEC.

IMPLICATIONS FOR IMPROVED THERAPY OF OCULAR ONCHOCERCIASIS FROM CONTINUOUS, NON-PULSED OCULAR AND SYSTEMIC DELIVERY OF DEC

These results indicate that the intensity of adverse inflammatory sequelae to DEC therapy relates to the time-related profile of concentration of DEC reaching the target tissues, as well as a combination of other factors that may vary from patient to patient. These adverse effects may include an intense vasculitis (Figs. 5 and 6). Such changes could explain the worsening of visual defect from optic nerve damage following oral DEC therapy of onchocerciasis. DEC has sufficiently high intrinsic activity to offer practicable mass chemotherapy of ocular onchocerciasis by continuous, non-pulsed delivery either locally in the eye or systemically by the transdermal system.

The margin between the delivery rates causing death of MFS and those causing untoward inflammatory sequelae, that is to say, the therapeutic index, is probably sufficient to offer acceptable, continuous, non-pulsed systemic delivery of DEC that would lead to gross reduction in the microfilarial load without endangering the optic nerve and other critical tissues, especially in the central nervous system (Fuglsang and Anderson, 1974; Duke *et al.*, 1976).

The development of the Supratarsal Delivery Platform, designed initially for mass chemotherapy of trachoma and now found to have many advantages over the original Ocusert design for routine therapy of glaucoma, greatly improves the ease of insertion, the stability of retention, the ease of adaptation to wearing, and the duration of effective drug delivery (B. R. Jones and others, unpublished data). This should very greatly facilitate the conduct of the field trials of topical ocular therapy. The development of supratarsal DEC delivery systems should provide practicable, effective, and safe therapy of onchocercal sclerosing keratitis and iritis. They may well provide effective prophylaxis of other ocular lesions in persons at high risk. The data to be obtained with these continuous non-pulsed ocular DEC delivery systems should guide the development of continuous non-pulsed transdermal delivery of DEC.

The development of continuous non-pulsed transdermal DEC delivery systems would be likely to provide a safe and effective mass chemotherapy method of grossly reducing the whole body microfilarial load, hopefully without further endangering the optic nerve.

The results in the present study indicate that, after the initial systemic clearance, it should be safe to continue DEC therapy with higher doses under much less closely regulated conditions, provided the dosage is maintained more or less continuously. This might be provided safely by DEC delivered in tablets, or even in medicated cooking salt. Mass chemotherapy with DEC in salt has been tried fairly extensively in bancroftian filariasis (Hawking and Marques, 1967; Davis and Bailey, 1969; Basu *et al.*, 1971; Sen *et al.*, 1974; Fan *et al.*, 1975). It would appear to offer an effective and practical method of control that can rapidly and substantially reduce transmission (Fan *et al.*, 1975). However, no form of mass chemotherapy of onchocerciasis has so far been acceptable because of the adverse reactions.

It is reported in an accompanying paper (Anderson and Fuglsang, 1978) that suramin has been successfully used to treat ocular onchocerciasis without severe toxicity when used to kill the adult worms after preliminary DEC treatment to reduce the microfilarial load. This is in contrast with the severe toxicity and fatalities seen after treatment with suramin alone (Anderson *et al.*, 1976b). Continuous, non-pulsed transdermal systemic delivery of DEC would therefore be likely to provide a safe, acceptable, and eminently practical method of administering DEC to reduce the microfilarial load in preparation for suramin therapy to kill the adult worms.

These predictions need now to be confirmed or refuted by carefully conducted field trials. The cost of development, safety testing, and supply of the ocular and transdermal controlled DEC delivery

devices and the ensuing field trials cannot be trivial, but neither is the challenge. Unless acceptable non-damaging and effective therapy for severe ocular onchocerciasis is developed and applied in time, hundreds of thousands of already heavily infected persons will inevitably progress to total blindness. This will include those living in the Volta River basin where the Onchocerciasis Control Programme has succeeded in markedly reducing transmission, because each adult female worm may still go on discharging myriads of MFS within the host's body for up to 15 years.

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