In vitro uptake of ivermectin by adult male *Onchocerca ochengi*

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SUMMARY

Onchocerca volvulus differs in its susceptibility to ivermectin in a stage dependent manner. Whilst the microfilariae are susceptible, ivermectin is not lethal to the adult worms nor those of *O. ochengi*, a cattle parasite closely related to *O. volvulus*. Ivermectin can penetrate the nodules in which the adults of these nematodes live and achieve high concentrations, but it is not known what levels enter worms. Adult male *O. ochengi*, from naturally infected cattle in Cameroon, were incubated in [^{3}H]ivermectin to measure uptake by the oral and transcuticular routes, and in [^{3}H]inulin to ascertain if oral ingestion occurs *in vitro*. After a 3 h incubation, only low levels of [^{3}H]inulin [4 disintegrations/min (dpm)] were detected in worms, indicating that the gut is probably not functional *in vitro*. However, there was a large uptake of [^{3}H]ivermectin (1040 dpm), with no significant difference between total and transcuticular uptake ($P > 0.05$), implying that uptake occurs predominantly by the transcuticular route. [^{3}H]ivermectin uptake by heat killed worms was reduced compared to living worms, but not significantly ($P > 0.05$), indicating that uptake of ivermectin probably occurred by both active transport and passive diffusion. Incubation in 100 μM ivermectin phosphate did not kill worms, although motility was reduced. In conclusion, ivermectin is able to accumulate in adult *O. ochengi* males at concentrations sufficient to kill non-filarial nematodes.

Key words: ivermectin, *Onchocerca ochengi*, adults, onchocerciasis, drug uptake, gut function.

INTRODUCTION

Ivermectin has recently been distributed in West Africa to control *Onchocerca volvulus*, the causative organism of human onchocerciasis (River Blindness). The Onchocerciasis Control Programme (OCP), which has largely utilized vector control but latterly introduced ivermectin usage, now nears completion and the future pan-African programme to eliminate onchocerciasis as a public health problem (APOC) will largely rely on ivermectin treatment (Molyneux, 1995; Remme, 1995). Interestingly, the activity of ivermectin against *Onchocerca* species is stage-restricted. A single, oral dose of 150 μg ivermectin/kg is extremely effective at reducing levels of skin microfilariae of *O. volvulus* (Albiez *et al.* 1988*a*) but does not kill the adult worms (Albiez *et al.* 1988*b*), although it interferes with embryogenesis (Duke, Zea-Flores & Muñoz, 1991). A single high dose of 800 μg/kg (Awadzi *et al.* 1995) and multiple doses of 150 μg/kg (Chavasse *et al.* 1992) are not much more effective. The reasons for ivermectin's inability to kill adult worms are unknown but, because of the crucial role of ivermectin in onchocerciasis control, a better understanding of the mechanisms of its stage-restricted efficacy is important. Specifically, it may enable the rational design of effective macrofilaricidal drug regimens and also may provide insights into potential mechanisms of acquired ivermectin resistance.

We are using the cattle parasite, *O. ochengi*, to investigate this problem. *O. ochengi* occurs endemically in Cameroon (Wahl *et al.* 1994), and, because of its close relationship to *O. volvulus* and intradermal nodular habitat, provides an excellent analogue for this worm (Trees, 1992). It has been adopted as the tertiary drug screen for potential anti-*Onchocerca* macrofilaricides. Ivermectin's effect against *O. ochengi* is

similar to that against *O. volvulus*, with a single 200 μg/kg dose rapidly reducing skin microfilariae levels without killing the adults (Renz *et al.* 1995), and it is not lethal to the adult worms even with seven monthly treatments at 500 μg/kg (B. M. Bronsvoort, A. Renz, A. J. Trees, unpublished observations). Significantly, we have recently shown that ivermectin has prophylactic activity against natural infection, presumably by activity against L_3 or L_4 stages (Wood *et al.* unpublished observations).

By excision of nodules from *in vivo* treated cattle it has recently been demonstrated that ivermectin is able to enter the nodules in concentrations that would be sufficient to kill non-filarial nematodes (Cross *et al.* 1997). However, since host material within the nodules was not separated from worm tissue, it was not known if the drug could permeate the actual worms, or, if so, at what concentrations. The aim of this study was to measure the amount of [^{3}H]ivermectin entering *Onchocerca* adult males *in vitro* by scintillation counting and to determine the relative contributions of oral and transcuticular uptake, since it is known that this drug is able to enter other nematodes via the cuticle (Ho *et al.* 1990; Richards, Behnke & Duce, 1995; Smith & Campbell, 1996). [^{3}H]inulin, a macromolecule (mol. wt. > 5000) which can be ingested orally but is too large to cross the cuticle of nematodes (Court *et al.* 1988; Ho *et al.* 1990), was used to evaluate oral ingestion. The effect of ivermectin phosphate, which has greater solubility in aqueous solutions than ivermectin, on motility and viability of adult males was also determined.

MATERIAL AND METHODS

Ivermectin, [22,23-^{3}H]-labelled ivermectin B_{1a} (specific activity 43.5 μCi/μg) and

ivermectin phosphate were very kindly supplied by Merck Research Laboratories (Rahway, U.S.A.). Ivermectin and ivermectin phosphate were dissolved in dimethyl sulphoxide (DMSO) to give concentrations of 1.144mM and 10mM, respectively. [^{3}H]inulin (specific activity 0.25 mCi/mg) was purchased from Amersham Life Science (U.K.) and dissolved in DMSO. All were administered in a final DMSO concentration of 1% v/v.

Parasites

Skins were collected from cattle naturally infected with *O. ochengi* from an abattoir in Ngaoundere, northern Cameroon, an area in which this parasite is endemic. Nodules were excised from their intradermal site and male worms were removed from opened nodules and stored in Roswell Park Memorial Institute medium (RPMI 1640, Sigma, U.K.) containing 5% foetal calf serum (FCS, GibcoBRL, U.K.) and 200U/ml penicillin and streptomycin (BioWhittaker, U.K.). Male worms can easily be removed without damage from the female worm mass expressed from cut nodules. Worms were visually checked for lack of damage prior to use and were used within 6 hours of removal from the host; for all experiments, they were grouped in pairs in medium (as above) containing the appropriate reagents to give a final volume of 200 μl, and incubated at 37°C. The apparatus used for incubation of worms was a modification of that described by Howells & Chen (1981). Plastic eppendorf tube lids, stuck to glass slides, were used as containers for incubating the worms. When measuring cuticular-only uptake of radiolabel by the worms, the ends of the worms were held out of the medium with pieces of glass coverslip and placed in adjacent lids containing normal medium.

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Total (oral plus cuticular) uptake of [^{3}H]ivermectin

Worms (n=64) were incubated for 3 h in medium containing 0.07 μCi [^{3}H]ivermectin and 11.44 μM unlabelled ivermectin (the maximum soluble concentration of ivermectin in aqueous solutions) per 1 ml. To assess whether viability is necessary for ivermectin uptake, some worms (n=10) were killed by heating to 55°C for 30 min and the effectiveness of heat treatment confirmed by addition of MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide, Sigma, used at a final concentration of 0.5 mg/ml] (Comley *et al.* 1989). Non-living worms, as judged by the MTT reaction, were incubated in medium as described above. To provide background radioactivity levels, some live worms (n=21) were incubated in normal culture medium. After 3 h, all worms were washed 3 times in 10 ml of PBS, and placed on filter paper to dry. The length of each worm was measured, then they were individually wrapped in foil and transported to Liverpool for analysis.

Cuticular uptake of [^{3}H]ivermectin

Worms (n=55) were incubated as for total uptake of [^{3}H]ivermectin, but with the ends removed from the radiolabel-containing medium, as described previously. After drying, the parts of the worm that had not been exposed to radioactivity were removed and discarded; the remainder of each sample was then measured and wrapped as for evaluation of total uptake of [^{3}H]ivermectin.

Uptake of [^{3}H]inulin

Total (n=53) and cuticular (n=67) uptake of [^{3}H]inulin were determined as for

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[^{3}H]ivermectin, with the medium containing 0.16 μCi [^{3}H] label/ml. The cuticular uptake of inulin acted as a negative control to ensure that the apparatus used prevented oral uptake of solutes. Uptake of [^{3}H]inulin by heat killed worms (n=10) was also assessed, using the method already described. All incubations were for 3 h.

Uptake of [^{3}H]ivermectin over time

Worms were totally immersed in [^{3}H]ivermectin-containing medium and incubated for different time intervals, ranging from 5 min to 12 h. Uptake of ivermectin was assessed from 10 worms at each time point. Washing, drying and measurement of worms was conducted as described earlier.

Solubilization of worms and scintillation counting

Individual worms were placed in glass scintillation vials, to which was added 100 μl water. After rehydrating for 30 min, 30 μl acetic acid and 1 ml NCS II tissue solubilizer (Amersham, U.K.) were added and the vials were kept at 50°C, with frequent mixing, until the samples were completely solubilized. Following the addition of 10 ml OCS scintillant (Amersham), the disintegrations/minute (dpm) were counted 3 times on a Packard Tri-Carb 4640 beta counter for 10 min or until a 5% significance level was achieved. The 3 dpm counts were meaned, background counts were subtracted and the mean dpm per 10 mm length of worm exposed to radiolabel was calculated.

Motility of males incubated in ivermectin phosphate

To determine if motility was affected by high concentrations of ivermectin phosphate,

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groups of 10 worms were incubated in medium containing ivermectin phosphate at final concentrations ranging from 0.5 μM to 100 μM. Following a 3 h incubation, the motility of each worm was assessed visually and scored from 0 (no movement) to 2 (normal, full motility).

MTT measurement of viability

The effect of different treatments on viability of worms was assessed using the MTT reaction. The following groups were tested (n=10 in each group): heat killed negative controls; positive controls incubated in medium; males exposed to 11.44 μM ivermectin (total uptake); males exposed to 11.44 μM ivermectin (cuticular uptake); males exposed to either 40 μM or 100 μM ivermectin phosphate. All incubations were for 3 h. After being washed, each group of worms was transferred to 450 μl medium, to which was added 50 μl of MTT solution (5 mg/ml in PBS). Following incubation for 1 h, the worms were washed 3 times in 10 ml PBS and the length of each was measured. They were then placed in individual wells of a 96-well flat-bottomed microtitre plate, and the formazan was leached out by the addition of 100 μl DMSO to each well. Formazan formation was measured on an ELISA reader at 492 nm, and the optical densities obtained were adjusted for the length of worm. Data ?

Statistical analysis

As normal distribution could not be assumed, differences between 3 or more groups were analysed using a non-parametric analysis of variance test, the Kruskal-Wallis test, whilst differences between 2 groups were examined using the Mann-Whitney test

(Minitab, version 10, Minitab Inc., U.S.A.). P values ≤ 0.05 were taken as significant. Results are presented as mean [95% confidence interval (C.I.)].

geometric ?

RESULTS

Uptake of [^{3}H]ivermectin

High levels of radioactivity were detected in worms tested for both total and cuticular uptake of [^{3}H]ivermectin [1040 (873-1208) dpm and 984 (806-1162) dpm respectively, (Fig. 1)], with no significant difference between them. The total uptake represented 3.8% of the available radiolabel in 200 μl medium. Large amounts of the radiolabel were also counted from those samples that had been heat killed prior to incubation in the drug [522 (428-616) dpm]. Although the level of uptake by these worms was lower than that by the live worms, there was no significant difference between any of the groups ($P = 0.063$).

Uptake of [^{3}H]inulin

Dpm counts from worms exposed to [^{3}H]inulin were far lower than those from worms that had been incubated in [^{3}H]ivermectin (Fig.1). However, uptake by heat killed males [49.7 (32-68) dpm] was significantly greater than that measured for total uptake [4.1 (2.6-5.6) dpm; $P < 0.001$] or cuticular uptake [12.2 (9.0-15.3) dpm; $P < 0.001$] by live worms. The mean radioactivity count from each heat killed worm constituted < 0.001% of the available radiolabel in 200 μl medium.

Uptake of [^{3}H]ivermectin over time

Radiolabel was detectable in worms after only 5 min of incubation in medium [174 (96.8-185) dpm]. Uptake continued for up to 12 h (Fig. 2), at which time samples emitted 2450 (1101-3800) dpm; however, even with this level of uptake, there was no obvious loss in motility, although this was not scored. This degree of uptake per worm represented 8.6% of the available radiolabel in 200 μl medium.

Motility of males incubated in ivermectin phosphate and MTT measurement of viability

Motility of *O. ochengi* males was reduced by exposure for 3 h to concentrations of ivermectin phosphate greater than 20 μM (Fig. 3), with increasing concentrations resulting in greater reductions in motility. However, even at 100 μM, motility was not completely inhibited; although 3 worms were immotile, the remainder retained some motility.

Viability was not affected by incubation of worms in 11.44 μM ivermectin for 3 h (Fig. 4). Neither the apparatus used to prevent oral ingestion of solutes nor incubation in 40 μM ivermectin phosphate significantly altered viability compared to either untreated controls or those that had been totally immersed in ivermectin-containing medium. However, in those worms exposed to 100 μM ivermectin phosphate, the reduction of MTT to formazan was decreased, although not significantly compared to untreated controls ($P = 0.38$), and they had significantly greater optical densities than heat killed controls ($P = 0.001$). When they were compared to worms exposed only to the possibility of cuticular uptake of ivermectin, the difference was significant ($P = 0.007$).

DISCUSSION

This study has demonstrated the ability of ivermectin to reach high levels in adult *O. ochengi*, and that uptake continues for up to 12 h without loss of motility of the worms. Since *O. ochengi* nodules can be penetrated by ivermectin (Cross *et al.* 1997), presumably the drug is also able to enter the worms *in vivo*. Ivermectin has also been shown to enter *O. volvulus* nodules (Baraka *et al.* 1996). Baraka *et al.* (1996) used a radioimmunoassay to measure the levels of ivermectin in collagenased *O. volvulus* adults removed from patients given a single 150 μg/kg dose. However, only 3 values were obtained, with a mean value of 61 ng/g. It is difficult to directly compare these results with our own, since we did not record the weights of worms, only the lengths.* The lack of inulin ingestion and the fact that concentrations of [^{3}H]ivermectin after total immersion were no different from cuticular exposure implies that transcuticular uptake is the major route of entry of ivermectin into the worms, with little, if any, oral ingestion occurring. It was decided to physically remove the ends of the worms from medium containing radiolabel in order to measure cuticular uptake rather then using ligatures because of the small size of the nematodes. It has also been established that concentrations of ivermectin phosphate of 100 μM do not kill adult male *O. ochengi*, although their motility was reduced with concentrations greater than 20 μM.

* This could be calculated $V = 300.000\ \mu m^3$

The low levels of radiolabel counted in those worms exposed to [^{3}H]inulin can most probably be accounted for by adherence of the chemical to the cuticle (Court *et al.* 1988) rather than ingestion by the parasites. This theory is supported by incubation of worms in up to 100 μM concentrations of ivermectin phosphate for 3 h followed by a

3 h incubation in [^{3}H]inulin. A mean of 10 dpm was counted from these males (data not presented), even though the pharynx should be effectively paralysed by this concentration of drug, since Geary *et al.* (1993) found that 0.1 nM ivermectin inhibited pharyngeal pumping in *Haemonchus contortus* and reduced by ≥ 95% the amount of solute ingested orally by the parasite.

The lack of inulin ingestion by male *O. ochengi* implies that the gut is not important for uptake of solutes *in vitro* in that sex. Histological studies on adult *O. volvulus* have revealed that the lumen is often reduced to little more than small clefts in male and female worms, including very young females (Franz, 1988), and is sometimes completely blocked (Schulz-Key, 1988). In worms which were alive at the moment of fixation, the intestinal epithelium of females (Franz & Büttner, 1983*b*) and males (Franz, 1982*b*; Franz & Büttner, 1983*b*; Schulz-Key, 1988) was completely disintegrated in some sections of worms. It has therefore been postulated that the gut has only a limited role in the nutrition of *O. volvulus* (Franz & Büttner, 1983*b*; Striebel, 1988), and considering their similarities, this is also likely to be the case with *O. ochengi*. Histochemical studies on *Onchocerca fasciata* have also confirmed that the intestine of adult *Onchocerca* is likely to be non-functional. When the tissue distributions of hydrolytic enzymes, glycolytic and related enzymes and succinate and NADH dehydrogenase were examined, little or no enzyme activities were detected in the intestine of these parasites (Omar & Raoof, 1994*a*, *b*, 1996), suggesting that the intestine has little role in the acquisition of nutrients. In contrast, high activities of dehydrogenase and glycolytic enzymes in the hypodermal tissue suggested that the cuticle represents the major route for nutrient absorption, with metabolism occurring in

the hypodermis (Omar & Raoof, 1994*b*, 1996).

Nematodes have a complex cuticle with a multilayered structure consisting of an outer epicuticle, cortical zone, median zone and inner basal zone (Bird, 1980). The cuticle of *O. volvulus* conforms to this general pattern (Franz, 1980), but with some differences from other filarial nematodes. The most notable distinction is the presence of a 'honey-comb' formation of the cuticle surface of both male and female *O. volvulus*, which is thought to increase the surface area (Deas, Aguilar & Miller, 1974; Franz, 1980, 1982*a*; Franz & Büttner, 1983*a*). This pattern has not been detected in other filariae (Franz, 1982*a*), but is likely to exist in *O. ochengi*. Deas *et al.* (1974) believed that certain structures within the basal layer and underlying hypodermis of *O. volvulus* were indicative of an active transport mechanism. It is now well established that transcuticular uptake of solutes by nematodes occurs. Work by Chen & Howells (1981) demonstrated that male and female *Dirofilaria immitis* were able to absorb D-glucose and adenosine by a transcuticular route. Similar results were obtained with adult female *Brugia pahangi* (Howells & Chen, 1981), with oral uptake of trypan blue occurring *in vivo* but not *in vitro*, whilst *H. contortus* appeared to depend on the trancuticular uptake of glucose in culture (Geary *et al.* 1993). Adult female *B. pahangi* were also able to take up L-glycine and L-arginine transcuticularly (Howells, Mendis & Bray, 1983), and *Onchocerca gutturosa* showed a marked transcuticular uptake of glycine, despite having an intestinal epithelium which morphologically appeared functional (Howells, 1980).

It is not only nutrients that are able to enter nematodes in this manner. Uptake of levamisole by *Ascaris suum* occurred almost exclusively via the transcuticular route (Verhoeven, Willemsens & Van den Bossche, 1976), and it was later demonstrated that

the same is true for many other drugs, including ivermectin (Ho *et al.* 1990, 1992). Other nematodes have demonstrated transcuticular uptake of ivermectin and other anthelmintics and non-electrolytes, including *H. contortus* (Ho *et al.* 1994; Sims *et al.* 1996), the filarial worms *B. pahangi* and *Dipetalonema viteae* (Court *et al.* 1988), *Caenorhabditis elegans* (Smith & Campbell, 1996) and hookworms (Richards *et al.* 1995). The rate determining step for absorption of drugs is thought to be their partitioning into the lipoidal hypocuticle tissue (Fetterer, 1986; Ho *et al.* 1990, 1992, 1994), and since ivermectin is highly lipophilic, it would be expected to readily cross the cuticle (Fetterer, 1986). However, this is not the only predictor of permeation, since molecular size and electrical charge of the solutes are also important (Ho *et al.* 1990, 1994). As ivermectin has a relatively large molecular mass (mol. wt. 874), the rate of absorption is slower than would be predicted from its partition coefficient (Ho *et al.* 1990; Thompson *et al.* 1993). This may explain why, in this study, equilibrium of ivermectin between worms and medium was slow to be achieved (uptake began to level off after 4 h but continued for up to 12 h), whereas in free-living nematodes [^{3}H]water reached equilibrium within 30 min (Marks, Thomason & Castro, 1968). Another possible explanation is that the worms are metabolising the drug (Thompson *et al.* 1993), although the capacity for drug metabolism by nematodes is thought to be low (Precious & Barrett, 1989*a*, *b*).

The levels of radiolabel we detected in worms after a 3 hour incubation were approximately ten-fold higher than those which immobilized *Ancylostoma ceylanicum* incubated for the same length of time in medium containing 0.89 μCi/ml [^{3}H]ivermectin (Richards *et al* 1995). These worms and male *O. ochengi* are roughly the same size, so

this would not account for the difference in levels of uptake. The relatively large surface area of *Onchocerca* may allow greater permeation of these worms by drugs (Deas *et al* 1974; Franz, 1980, 1982*a*; Franz & Büttner, 1983*a*). Ivermectin has been used as a chemical ligature in uptake assays, since it paralyses the pharynx at concentrations far lower than are required to inhibit motility (Avery & Horvitz, 1990; Geary *et al.* 1993; Sims *et al.* 1996), and this has been proposed as a mode of action of the drug (Geary *et al.* 1993). As *Onchocerca* does not appear to rely on oral ingestion of nutrients, this may explain ivermectin's lack of killing activity against these worms; even 100 μM ivermectin phosphate did not kill them. However, there are likely to be other factors involved, since the microfilariae, which are a non-feeding stage, are extremely susceptible to ivermectin *in vivo* (Albiez *et al.* 1988*a*).

Nematodes exhibit some selectivity in their transcuticular uptake of nutrients (Marks *et al.* 1968; Chen & Howells, 1981; Howells *et al.* 1983). This seems to be an energy-dependent process, with uptake being inhibited at 0°C (Howells *et al.* 1983) and selectivity being lost when the worms are killed (Marks *et al.* 1968). However, other more recent work suggests that permeation of the cuticle by compounds that are 'not structurally related to normal cellular components' occurs by passive diffusion, with the rate of transport being determined mainly by solute lipophilicity (Fetterer, 1986; Court *et al.* 1988; Ho *et al.* 1990). Results of the MTT assay in the present study indicate that incubation of worms in the apparatus used for cuticular uptake was not detrimental to viability of the worms, so that both active uptake and passive diffusion would be expected to occur normally. When the worms were heat killed prior to incubation in radiolabels, uptake of ivermectin still occurred at relatively high levels. In contrast,

uptake of inulin was significantly increased, suggesting that killing increased permeability of the cuticle. This may be a result of the method of killing, or there may be rapid degradation of the cuticle after death, although this is not likely (Franz, 1988). Alternatively, the lipoidal tissue of the hypodermis may be damaged by heating or may degrade rapidly. This result offers two possible explanations for the uptake of ivermectin by *O. ochengi*. Firstly, active uptake may be the most important method, which is prevented following killing, but increased permeability of the cuticle allows passive diffusion to occur. Secondly, and most likely, uptake consists of both active transport and passive diffusion - killing the worms prevents active uptake, but passive diffusion continues. Further work would be required to confirm this.

In conclusion, ivermectin entered male *O. ochengi* in amounts sufficient to kill susceptible nematodes. Therefore, lack of uptake does not explain its inability to kill this parasite. Entry of the drug occurred by the transcuticular route, with no evidence of oral ingestion of solutes. High concentrations of ivermectin phosphate were able to reduce motility of worms, but did not kill them.

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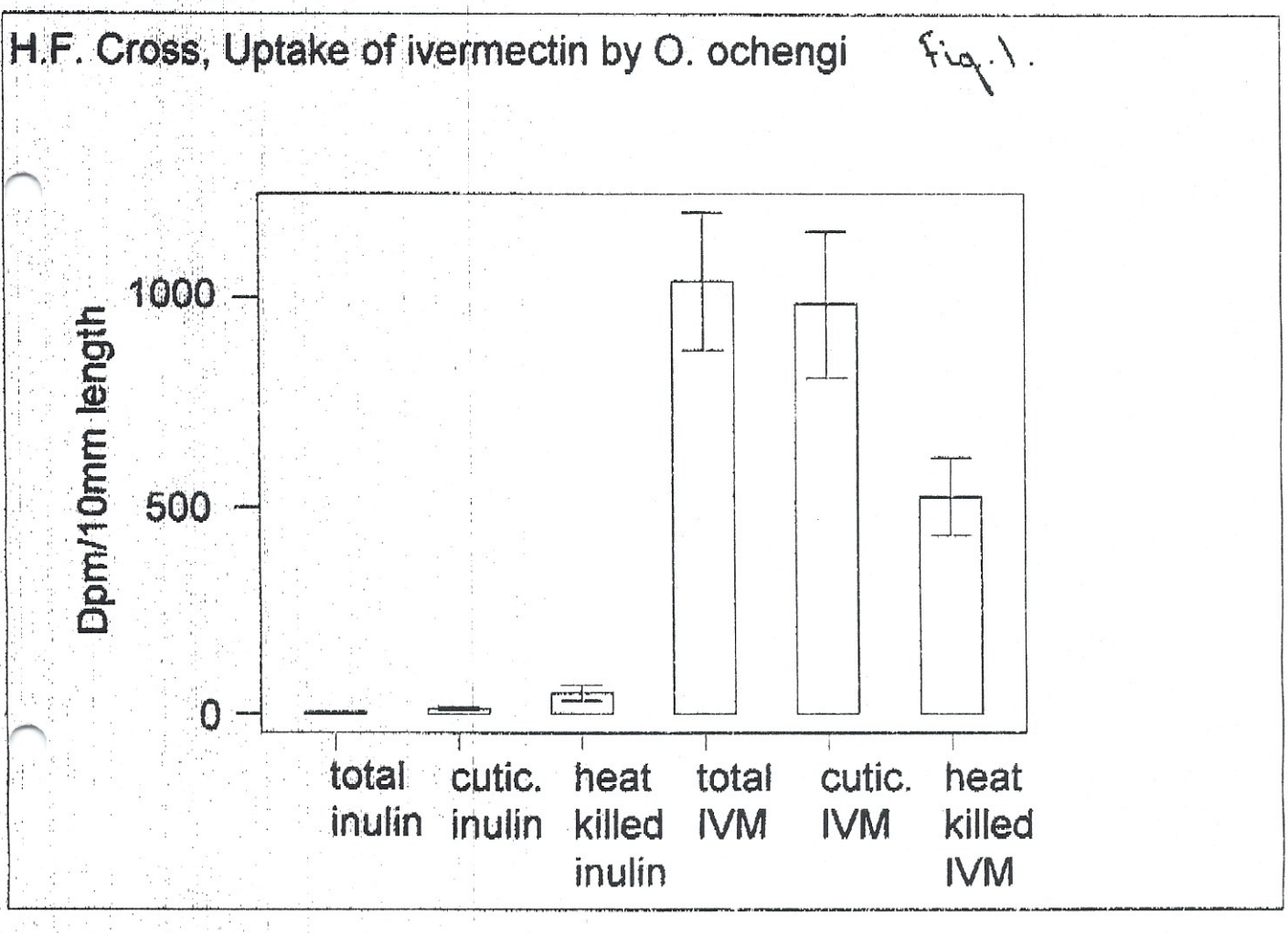
Ivermectin uptake by *Onchocerca ochengi*

Figure 1. Uptake of radiolabel by male *Onchocerca ochengi* incubated for 3 h in medium containing 0.07 μCi [^{3}H]ivermectin or 0.16 μCi [^{3}H]inulin. Bars illustrated are means, each based on ≥ 10 counts of dpm/10 mm length of worm. Vertical lines indicate 95% C.I. Cutic.= cuticular, IVM= ivermectin.

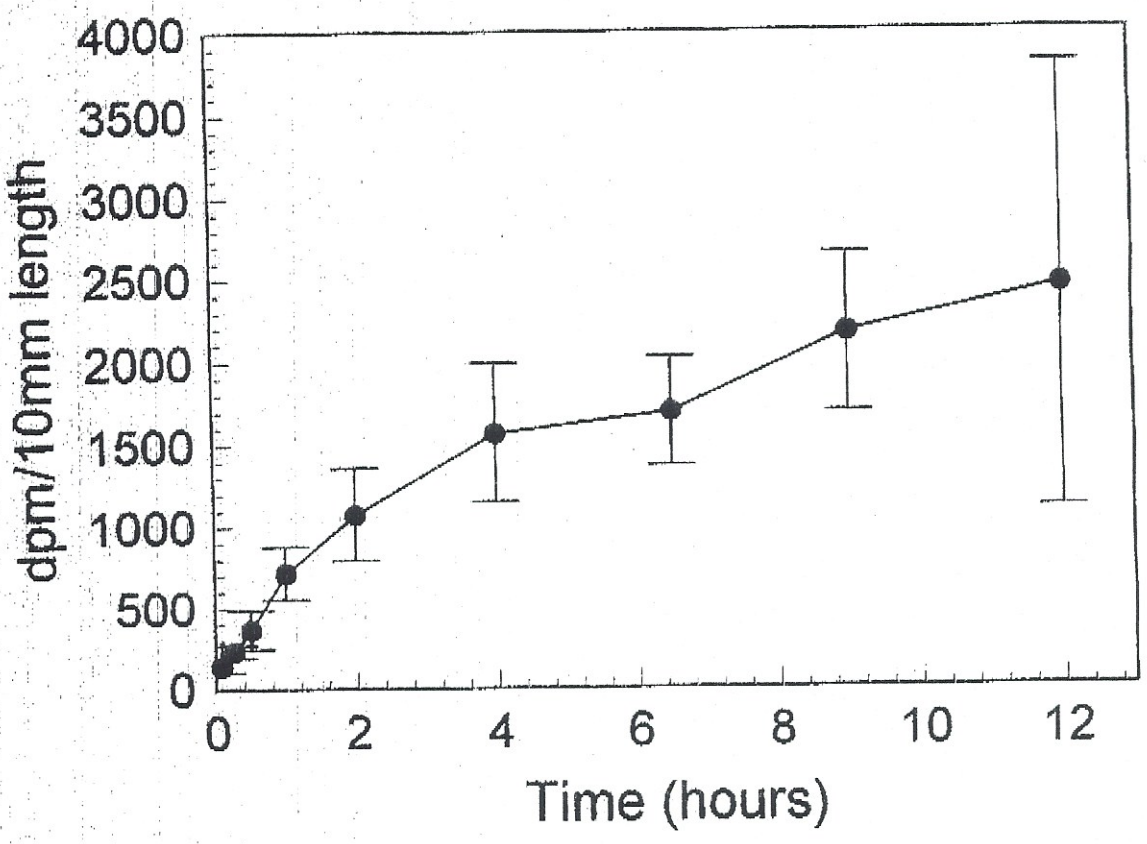
Figure 2. Uptake of radiolabel by male *Onchocerca ochengi* incubated for different periods of time in medium containing 0.07 μCi [^{3}H]ivermectin. Bars illustrated are means, each based on counts of 10 worms of dpm/10 mm length of worm. Vertical lines indicate 95% C.I.

Figure 3. Motility of male *Onchocerca ochengi* incubated in ivermectin phosphate for 3 h. Each point represents the mean score of 10 worms. Vertical lines indicate minimum and maximum values.

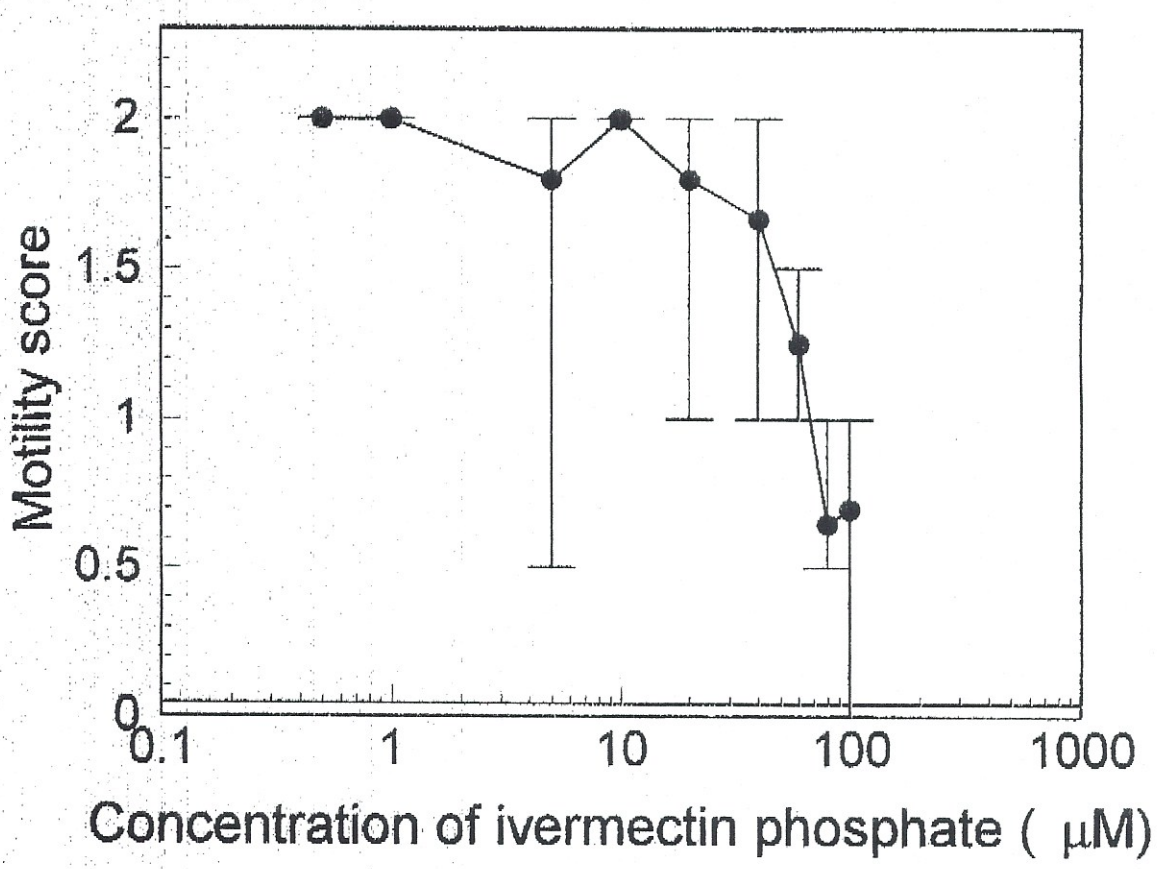
Figure 4. Viability of male *Onchocerca ochengi*, as measured using the MTT reaction, following 3 h of exposure to ivermectin or ivermectin phosphate under different incubation conditions. Each bar is the mean from 10 optical densities/10 mm length. Vertical lines indicate S.E. Cutic.=cuticular, IVM= ivermectin, IVM-PO4= ivermectin phosphate.


H.F. Cross, Uptake of ivermectin by O. ochengi
fig. 1.
Dpm/10mm length
1000
500
0
total inulin
cutic. inulin
heat killed inulin
total IVM
cutic. IVM
heat killed IVM

H.F. Cross, Uptake of ivermectin by
O. ochengi, Fig. 2



H.F. Cross, Uptake of ivermectin by
O. ochengi, Fig. 3



H.F. Cross, Uptake of ivermectin by O. ochengi, Fig. 4.