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The effect of 5-fluorouracil and 5-fluorocytosine on the development of the filarial nematodes *Brugia pahangi* and *Dirofilaria immitis*

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Summary

5-fluorouracil and 5-fluorodeoxyuridine at 30 mg/kg body weight daily for four days inhibit microfilarial production in *Brugia pahangi* in the jird. Disruption of intrauterine embryogenesis was observed in treated female worms but the compounds were not macrofilaricidal or microfilaricidal under the conditions employed. 5-fluorocytosine possessed no filaricidal or embryostatic activity. The inhibition of microfilaria production by 5-fluorouracil was temporary and larval production was resumed within nine weeks. The compound also inhibited the development of *B. pahangi* and *Dirofilaria immitis* larvae in the mosquito *Aedes aegypti*, when administered to cages of mosquitoes as a 0.01 or 0.001% solution in a 10% aqueous sucrose solution on cotton wool wicks. The development of infective larvae of *B. pahangi* in the jird was inhibited by 5-fluorouracil.

Key words: Filaria; Brugia pahangi; Dirofilaria immitis; pyrimidine; 5-fluorouracil; 5-fluorocytosine; 5-fluorodeoxyuridine; *Aedes aegypti;* development; microfilaria.

Introduction

The dependence of the erythrocytic stages of the malaria parasite on exogenous preformed purines stimulated Trigg et al. (1971) to investigate the use of the purine analogue cordycepin as an antimalarial. An extensive study of purine and pyrimidine analogues in the experimental chemotherapy of *Plasmodium vinckei* infections subsequently revealed a particularly good plasmodio-

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static activity for purine-6-sulfonic acid-3-N-oxide (Heischkeil, 1974). Ochoa and Collado (1972) reported the cure of a case of cutaneous leishmaniasis with 5-fluorocytosine.

Hawking (1973) briefly described an effect against the intrauterine embryos of *Litomosoides carinii* with amethopterin, 6-mercaptopurine, cytoxan and 6-azauridine but no detailed study has been made of the effect of purine and pyrimidine analogues against filarial worms. Microfilariae of *Dirofilaria immitis* incorporate uracil, uridine, adenine and adenosine into RNA but insignificant or no thymidine, orotic acid, formate or glycine are incorporated into nucleic acids (Jaffe and Doremus, 1970). Chen and Howells (1981) subsequently showed that adult and larval *Brugia pahangi* incorporate adenosine, adenine, uridine and uracil but not thymine, thymidine or cytosine. In this paper we have pursued these observations and describe the effect of the fluorinated derivatives, 5-fluorouracil, 5-fluorocytosine and 5-fluorodeoxyuridine on *Brugia pahangi* and *Dirofilaria immitis.*

Materials and methods

The subperiodic strain of *Brugia pahangi* used in this study was originally imported from Malaysia in 1959 by Professor J. F. B. Edeson. Cats were employed for the routine maintenance of the strain and as a source of microfilariae for infecting mosquitoes. Mongolian jirds *(Meriones unguiculatus)* were used as experimental hosts for obtaining late larval and adult worms. The jirds were infected by the intraperitoneal (ip) injection of 100 infective larvae of *B. pahangi* harvested from *Aedes aegypti* of the SS or REFM stocks.

Mosquitoes were infected by the membrane-feeding technique of Wade (1976). Microfilaria-infected blood was withdrawn into a heparinised syringe (to give a final concentration of 10 units heparin per ml of blood) from the femoral vein of an anaesthetised cat. Aliquots of blood containing more than 400 microfilariae per 40 cmm were diluted with uninfected dog or cat blood to give a final larval density of 300–350 per 40 cmm. The blood was introduced to the reservoir of the membrane-feeding device and mosquito feeding was initiated after an appropriate interval to allow thermal equilibration of the stirred blood with the water jacket. Mosquitoes were maintained at $26 \pm 1°$ C and a relative humidity of 75–85%. All cages were supplied with cotton wool wicks soaked in 10% sucrose. Infective larvae were harvested from the mosquitoes 9–12 days after infection.

Infective larvae were harvested from mosquitoes by the method of Ash (1974). Chilled mosquitoes were crushed on a glass plate and washed into a gauze-lined Baermann funnel with Hanks' balanced salt solution (HBSS). The material was permitted to soak for 1 h after which infective larvae were drawn off, divided into groups of 100 and injected ip into jirds.

The strain of *Dirofilaria immitis* used here was received in 1969 from the National Institute for Medical Research, Mill Hill, London. The vector-borne stages of the parasite were employed in this study. Blood was withdrawn from the cephalic vein of an anaesthetised infected dog. Microfilaraemia was estimated from wet film preparations and adjusted to the required density by dilution with uninfected blood. Mosquitoes (*Aedes aegypti* of the SS or REFM stocks) were infected by the membrane feeding technique as described above for *B. pahangi.*

The effect of 5-fluorouracil on jirds infected with adult B. pahangi

Selection of dosage levels. Three groups of infected jirds, with 2 animals per group were dosed with 5-fluorouracil ip at 120, 60 and 30 mg/kg body weight, respectively, once daily for 4 days. A further pair of jirds served as undrugged controls. The animals which received the 120 mg/kg

dosage died on the seventh day after the commencement of drugging, those receiving 60 mg/kg died on the eighth day. Neither of the undrugged pair of jirds, nor those receiving 5-fluorouracil at 30 mg/kg died by the eighth day and 30 mg/kg was therefore selected as the dosage for subsequent employment in the experiments. The number of animals in this preliminary test was restricted by a limitation on the availability of jirds but no attempt to employ a higher dosage level was made subsequently, since even at this dosage some deaths occurred in experimental animals.

Examination of the effect of 5-fluorouracil on B. pahangi adults. Infected jirds were dosed with 5-fluorouracil at 30 mg/kg body weight ip once daily for 4 days. On the eighth day after the commencement of drugging the jirds were killed and the peritoneal cavity exposed. Adult worms and peritoneal washings were removed under aseptic conditions. The peritoneal washings were examined for microfilariae and the adult worms were examined for evidence of motility. The worms were divided into groups which were processed as follows: one group of male and female worms were placed in a tissue culture flask in growth medium 199 (GM 199) composed of medium TC 199 containing 10% calf serum and 400 μg/ml crystamycin. The flasks were incubated at 37° C and microfilarial output by the female worms was observed during seven days. Following incubation the worms were processed for histological examination or transferred to the peritoneum of clean, uninfected jirds. A second group of worms, on removal from the peritoneum of the drugged jirds, was fixed in 10% neutral buffered formalin and then dehydrated and embedded in paraffin wax prior to sectioning. Sections were cut at 3 μm and stained with Ehrlich's haematoxylin and eosin. The third group of worms were processed for electron microscope examination as described by Chen and Howells (1979).

Attempts were also made to determine whether 5-fluorouracil exerted a long-term embryostatic effect on adult *B. pahangi.* 5-fluorouracil was administered to infected jirds once daily for 4 days at 30 mg/kg ip. Pairs of jirds were killed 17 days, 24 days and 65 days after the initiation of dosing. Worms removed from jirds killed at 17 and 24 days were transferred to Falcon tissue culture flasks containing GM199 and incubated at 37° C for 7 days prior to transfer to the peritoneum of clean recipient jirds. Worms recovered from the jirds killed 60 days after dosing were transferred to clean recipient animals after only 24-h incubation in vitro. In each instance the culture flasks were examined for microfilariae liberated from the female worms and at an appropriate interval after transfer, the recipient jirds were sacrificed and their worm burdens determined.

In a further experiment jirds were dosed with 5-fluorouracil ip at 30 mg/kg body weight once weekly for 4 weeks. Six weeks after the last dose was administered the jirds were killed by anaesthetisation and adult worms recovered. The worms were washed in TC199 and transferred to Falcon flasks containing GM199 for 7 days at 37° C. The peritoneum of the dosed jirds were washed with 2 ml of Hanks' BSS and examined for microfilariae. Control, undrugged jirds were also included in this test.

Examination of the effect of 5-fluorouracil on third stage larvae of B. pahangi in the jird. Jirds were infected with 100 infective larvae of *B. pahangi* by ip inoculation. Three jirds received 5-fluorouracil subcutaneously (sc) at 30 mg/kg body weight once daily for 5 days from the day of infection and three further animals received an equivalent volume of sterile water sc for 5 days. On the eleventh day after infection the animals were killed and the peritoneum was examined for larvae. Larvae recovered were counted, fixed and mounted as described by Wharton (1959) then measured with the aid of a camera lucida mounted on a Zeiss microscope. In a further experiment 18 jirds were infected with *B. pahangi* by the inoculation ip of 100 infective larvae. Six jirds were retained as undrugged controls and the remaining animals were given 5-fluorouracil sc at 30 mg/kg daily for 5 days from the day of infection. Drugged and undrugged control animals were killed on day 8, day 11 and day 22 after infection. As in the previous experiment the peritoneal cavity was examined for larvae. Larvae recovered from the jirds were counted, fixed, mounted and measured as described above.

Examination of the effect of 5-fluorouracil on the development of B. pahangi in Aedes aegypti. *Aedes aegypti* of the SS stock were infected with *B. pahangi* by direct feeding on an infected cat. 24 h after the infective blood meal 100 fed females were transferred to each of three experimental cages.

Solutions of 0.01% and 0.001% 5-fluorouracil were prepared in a 10% w/v aqueous solution of sucrose. Cotton wool wicks were soaked in the solutions and one solution was administered to each of two cages of mosquitoes, a further cage receiving a wick soaked in 10% sucrose only. The cotton wool wicks were replaced at 48-h intervals until the mosquitoes were removed. Mosquitoes were dissected under a standard binocular microscope and examined for the presence of larvae. Larvae were counted, then fixed and mounted as described by Wharton (1959) and examined microscopically.

The effect of treatment with 5-fluorouracil on glucose, adenosine, glycine and uracil incorporation by adult B. pahangi

Infected jirds were dosed ip for 4 consecutive days with 5-fluorouracil at 30 mg/kg body weight. Twenty-four hours after the last dose was given the animals were sacrificed and the adult worms removed from the peritoneum. Untreated infected jirds were also sacrificed and their worms harvested. Male and female worms were separated and individual worms were placed in tubes containing 1 ml of GM 199. The uptake and incorporation of D-[U-^{14}C] glucose (sp act 33 mCi/mmole), of [2-^{14}C] uracil (sp act 60 mCi/mmole), of [8-^{14}C] adenosine (sp act 58 mCi/mmole) and of [U-^{14}C] glycine (sp act 118 mCi/mmole) was determined for individual male and female worms incubated in 1 ml GM 199 containing D-[U-^{14}C] glucose at 2 μ Ci/ml, [2-^{14}C] uracil at 1.0 μCi/ml, [8-^{14}C] adenosine at 0.4 μCi/ml and [U-^{14}C] glycine at 1.0 μCi/ml, respectively. In each test male and female worms were employed in triplicate and incubated for 1 h at 37° C. Following incubation the worms were washed 4 times in 10 ml aliquots of GM 199, 5 min per wash and processed for scintillation counting as described by Chen and Howells (1981). Heat killed worms were employed in each test to serve as controls. Male and female worms from undrugged jirds and from jirds treated with 5-fluorouracil were killed by heating to 50° C in 1 ml GM 199 for 20 min. Radioactivity was measured in a Packard Model 3388 scintillation counter (Nuclear Chicago) and correction for quenching was performed by the sample channels ratio method, as described by Neame and Homewood (1974), with an efficiency of between 69% and 90% for ^{14}C. With all samples 0.1 ml of the final washing solution was also counted to confirm that the disintegrations per minute (dpm) of this solution was the same as that of the 'background' count.

Experiments employing 5-fluorocytosine and 5-fluorodeoxyuridine

All experiments involving 5-fluorocytosine and 5-fluorodeoxyuridine were performed in a manner identical to that described with 5-fluorouracil. The effect of 5-fluorocytosine on infective larvae of *B. pahangi* was not investigated.

Experiments with Dirofilaria immitis

The effects of 5-fluorouracil and of 5-fluorocytosine on the development of *D. immitis* microfilariae within *Aedes aegypti* were studied in parallel with the experiments on the mosquito-borne stages of *B. pahangi*. The procedures employed with each species were identical and have been described above.

Results

5-fluorouracil: effects on adult B. pahangi

5-fluorouracil at 30 mg/kg daily for 4 days did not exert a macrofilaricidal effect on *B. pahangi* in the jird. The worms recovered on days 8, 17, 24 and 65

Fig. 1. a–d) Eggs of *Brugia pahangi* produced by worms in vitro after treatment with 5-fluorouracil in vivo. Worms were recovered from jirds on the day following the completion of 4 daily doses of 5-fluorouracil at 30 mg/kg (× 640). e) Fourth stage larva of *B. pahangi* recovered from a jird on day 11 post infection. The jirds were dosed with 5-fluorouracil at 30 mg/kg from day 1 to 4. Note remnant of third larval cuticle attached to the oral aperture of the larva (× 640).

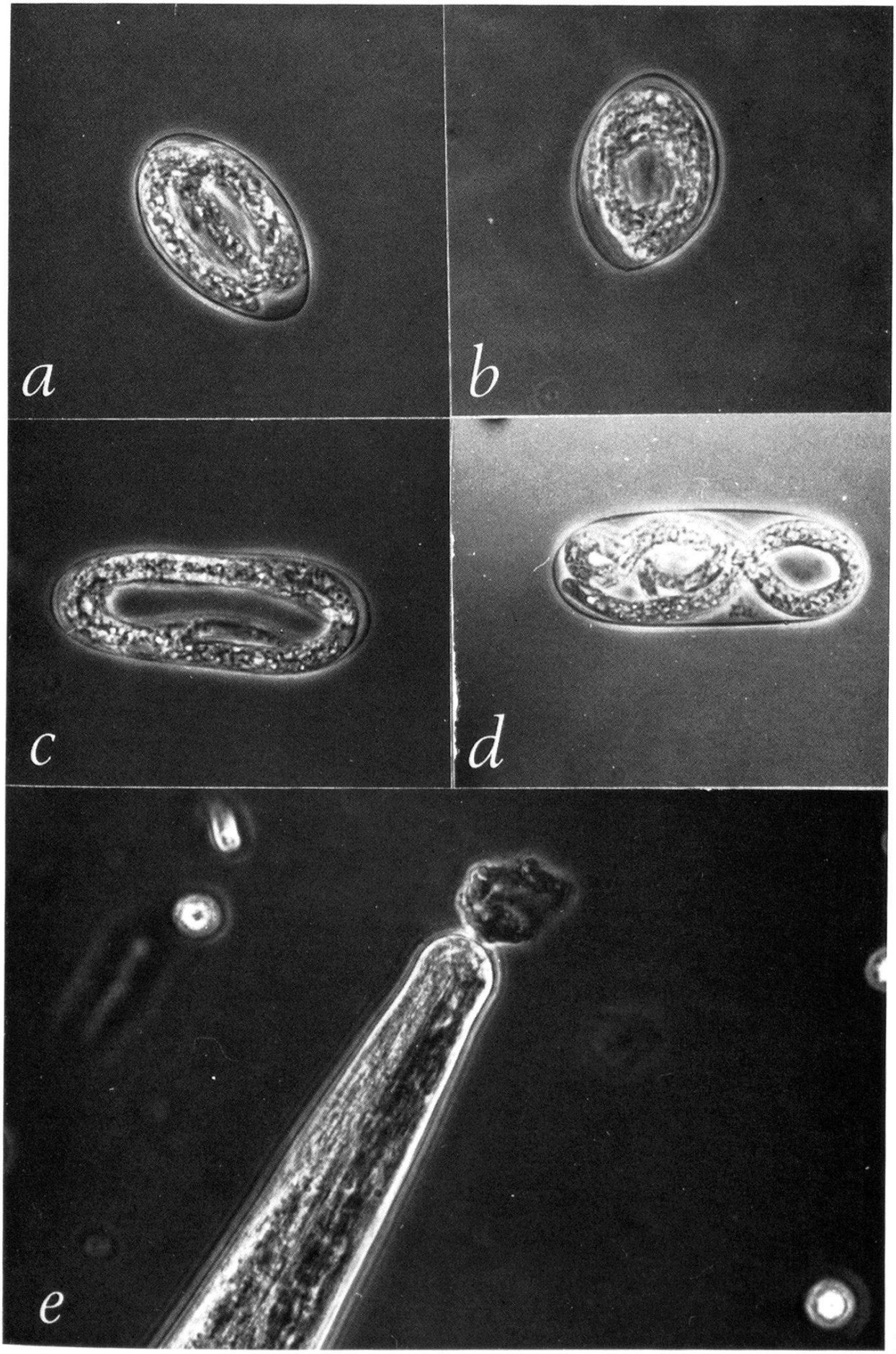


Fig. 1

were active and appeared morphologically normal. The peritoneal washings obtained from treated jirds on days 8, 17 and 65 contained large numbers of motile microfilariae but few microfilariae were recovered from the peritoneal washings of the treated jirds killed on day 21 even though these animals harboured many adult worms. No attempt was made to quantitate the microfilariae in various groups of treated or control jirds since there was a very great variation in the worm burdens of individual animals.

No microfilariae were liberated from the worms cultured in vitro which had been removed from the 5-fluorouracil-dosed jirds killed on days 8, 17, and 24. The female worms transferred to culture on day 65, and all worms from untreated control jirds however, extruded large numbers of active, normal microfilariae. In a single experiment in which worms were removed from the jirds on the day following the last of the four daily dosages of 5-fluorouracil the worms in culture released a few, apparently, normal microfilariae but numbers of eggs, in varying stages of development were also recovered from the incubation medium (Fig. 1a–d).

The administration of 5-fluorouracil at a once weekly dosage of 30 mg/kg ip for 4 weeks did not result in a macrofilaricidal action nor in an elimination of microfilariae from the peritoneal cavity of the jirds. Worms recovered from jirds sacrificed approximately 6 weeks after the termination of weekly drug administration were apparently normal and on transfer to culture in vitro, liberated large numbers of microfilariae into the culture medium.

Histological examination of female worms recovered on days 8, 17 and 24 from jirds dosed with 5-fluorouracil from days 1–4, showed a marked reduction in the size of the reproductive organs; with an apparent shrinkage of the uteri which contained scattered embryos in varying stages of development but no fully mature microfilariae. At the electron microscope level marked degenerative changes were visible in the nuclei of the morulas contained in the uteri (Fig. 2a); the appearance of these embryos contrasting markedly with the normal appearance of embryos in 5-fluorocytosine treated worms (Fig. 2b).

The uptake and incorporation of [2-^{14}C] uracil, D-[U-^{14}C] glucose, [U-^{14}C] glycine and [8-^{14}C] adenosine by worms from undrugged and 5-fluorouracil dosed jirds was performed as described in the Materials and methods. The worms were recovered from dosed jirds on the day following the cessation of the four daily doses. The results, illustrated in Table 1, show that there was no impairment of utilisation of any of the radio-labelled substrates by 5-fluorouracil treated worms.

5-fluorodeoxyuridine: effects on adult B. pahangi

5-fluorodeoxyuridine was administered to infected jirds at 30 mg/kg ip once daily for five days. On the eighth day after commencing dosing the jirds

Fig. 2. a) Uterine contents of *B. pahangi* following treatment with 5-fluorouracil (× 2,600). b) Uterine content of *B. pahangi* following treatment with 5-fluorocytosine (× 2,600).

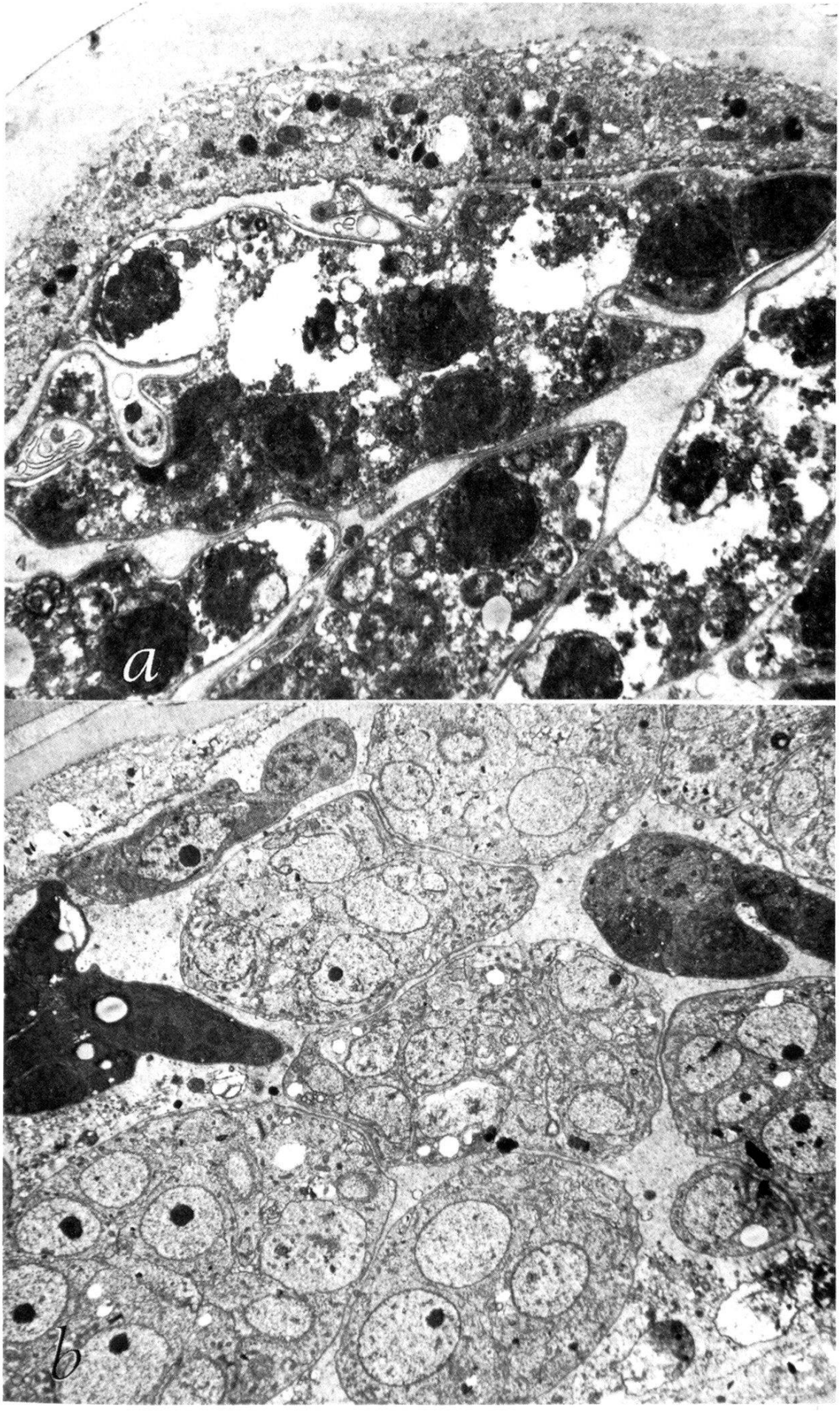


Fig. 2

Table 1. The influence of 5-fluorouracil treatment on the incorporation of [2-^{14}C] uracil, D-[U-^{14}C] glucose, [U-^{14}C] glycine and [8-^{14}C] adenosine by *Brugia pahangi* adults. Worms from jirds treated with 5-fluorouracil and from untreated jirds were incubated individually in 1.0 ml of TC 199 containing the radio-labelled substrate for 1 h at 37 ± 0.5° C. Each reading represents the mean ± S.D. obtained from at least three worms. Heat killed control worms were maintained at 50° C for 20 min in TC 199

Sex of worm and 'pretreatment'	Treated/ control*	[2-^{14}C] Uracil dpm/worm	D-[U-^{14}C] Glucose dpm/worm	[U-^{14}C] Glycine dpm/worm	[8-^{14}C] Adenosine dpm/worm
Male, live	treated	2461 ± 672	2963 ± 1192	856 ± 169	6721 ± 1389
Male, heat killed	treated	78 ± 7	105 ± 11	55 ± 28	721 ± 236
Male, live	control	2399 ± 418	4534 ± 1077	463 ± 198	6906 ± 974
Male, heat killed	control	82 ± 8	179 ± 41	86 ± 12	744 ± 212
Female, live	treated	23704 ± 474	16525 ± 2630	5766 ± 266	25969 ± 934
Female, heat killed	treated	206 ± 26	1156 ± 78	91 ± 7	572 ± 316
Female, live	control	21195 ± 835	20410 ± 1774	4348 ± 679	27429 ± 994
Female, heat killed	control	1305 ± 562	701 ± 28	89 ± 1	676 ± 473

* worms obtained from 5-fluorouracil treated or control jirds

were killed and the peritoneal content examined. Large numbers of microfilariae were present in the peritoneal fluid and the adult worms were apparently normal. On transfer to medium GM 199 as described in the section of Materials and methods, the dosed worms did not extrude microfilariae whereas larval production was normal in a control group of undrugged worms.

5-fluorocytosine: effects on adult B. pahangi

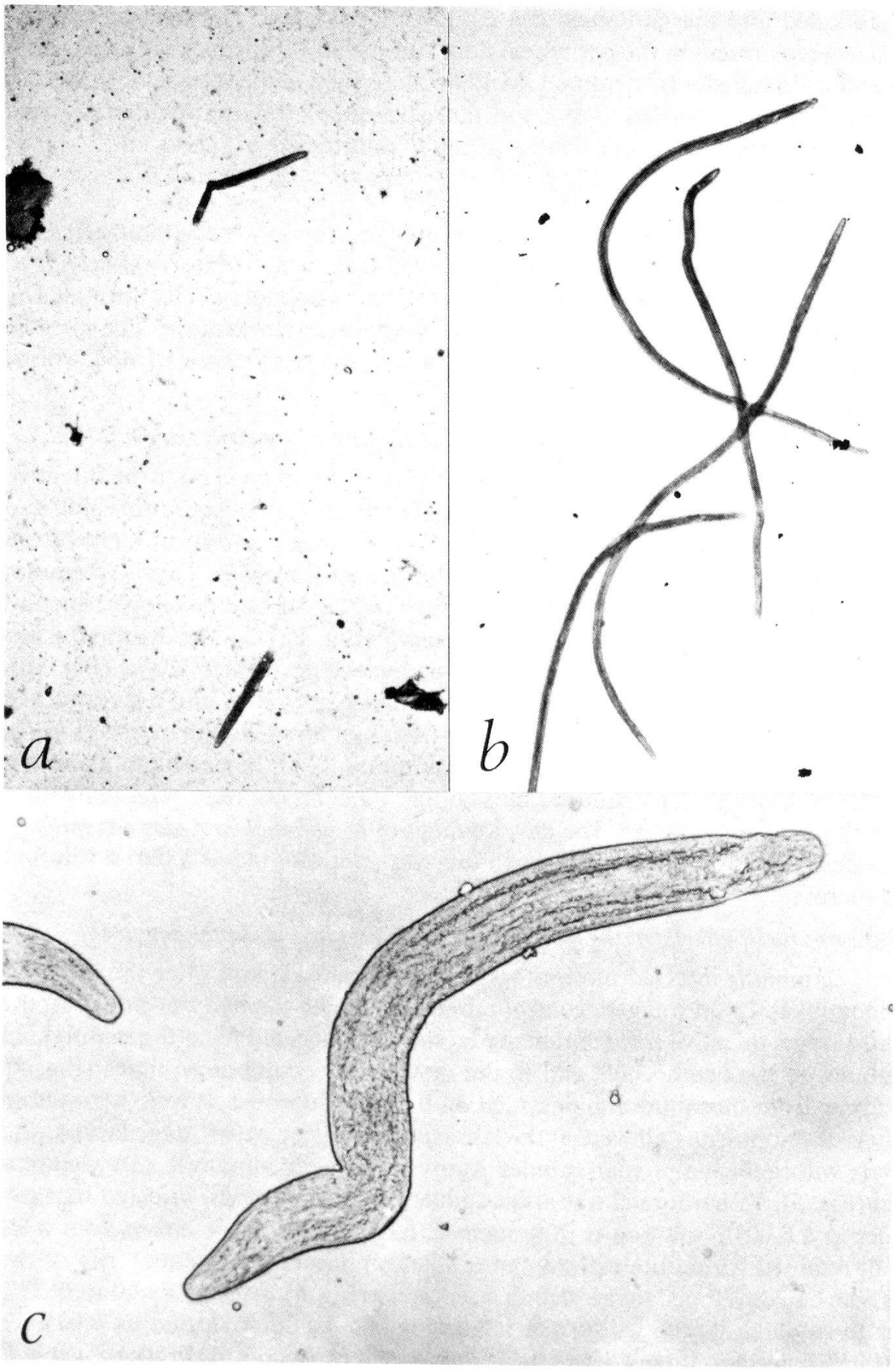
Four daily doses of 5-fluorocytosine at 30 mg/kg ip were without effect on *B. pahangi* in the jird. Adult worms removed from jirds at intervals similar to those used for 5-fluorouracil tests were active and morphologically normal. The female worms released large numbers of microfilariae in culture. The intrauterine larvae were morphologically normal in 5-fluorocytosine treated worms (Fig. 2b).

5-fluorouracil: effects on the development of B. pahangi in Aedes aegypti

B. pahangi infected mosquitoes were dissected ten days after the infective blood meal. Larval development was greatly retarded in mosquitoes which had been maintained on a 0.01% solution of 5-fluorouracil and most larvae from 'drugged' mosquitoes were still within the thoracic muscles. Larvae were not killed by exposure to 5-fluorouracil but very few larvae had developed beyond the late first stage, the 'sausage stage' larva (Fig. 3a, c). The worms in undrugged, control mosquitoes had developed to the third stage larvae (Fig. 3b). The difference in the development of *B. pahangi* in control and 5-fluorouracil dosed mosquitoes is reflected in the distribution of larvae in the insects (Fig. 4), larvae in control insects being found predominantly in the head and abdomen whereas in drugged mosquitoes the sausage stage larvae were retained within the thoracic musculature. The development of *B. pahangi* in *Aedes aegypti* was unaffected by 5-fluorocytosine when this was administered as a 0.01% solution in sucrose.

5-fluorouracil: effects on the development of D. immitis in Aedes aegypti

D. immitis infected mosquitoes were dissected 12 days after the infective blood meal. In undrugged, control moquitoes the larvae had developed to the third stage, infective form and many larvae had migrated from the malpighian tubules to the haemocoele and to the mouthparts of the mosquitoes (Fig. 4). Larvae from mosquitoes maintained on 0.01% 5-fluorouracil were arrested in their development – all were at the late sausage stage, i. e. first stage larvae, and were within the malpighian tubules. Many larvae were immobile and vacuolated (Fig. 5). 5-fluorouracil was also administered to *D. immitis* infected mosquitoes as a 0.001% solution in 10% sucrose. Larvae from these mosquitoes were still retained within the malphigian tubules on day 12 but the majority of the larvae had developed to the second larval stage (Fig. 5) with approximately 23% as third stage larvae within the head (Fig. 4). 5-fluorocytosine as 0.01% or 0.001% solutions did not affect the development of *D. immitis* in *Aedes aegypti.*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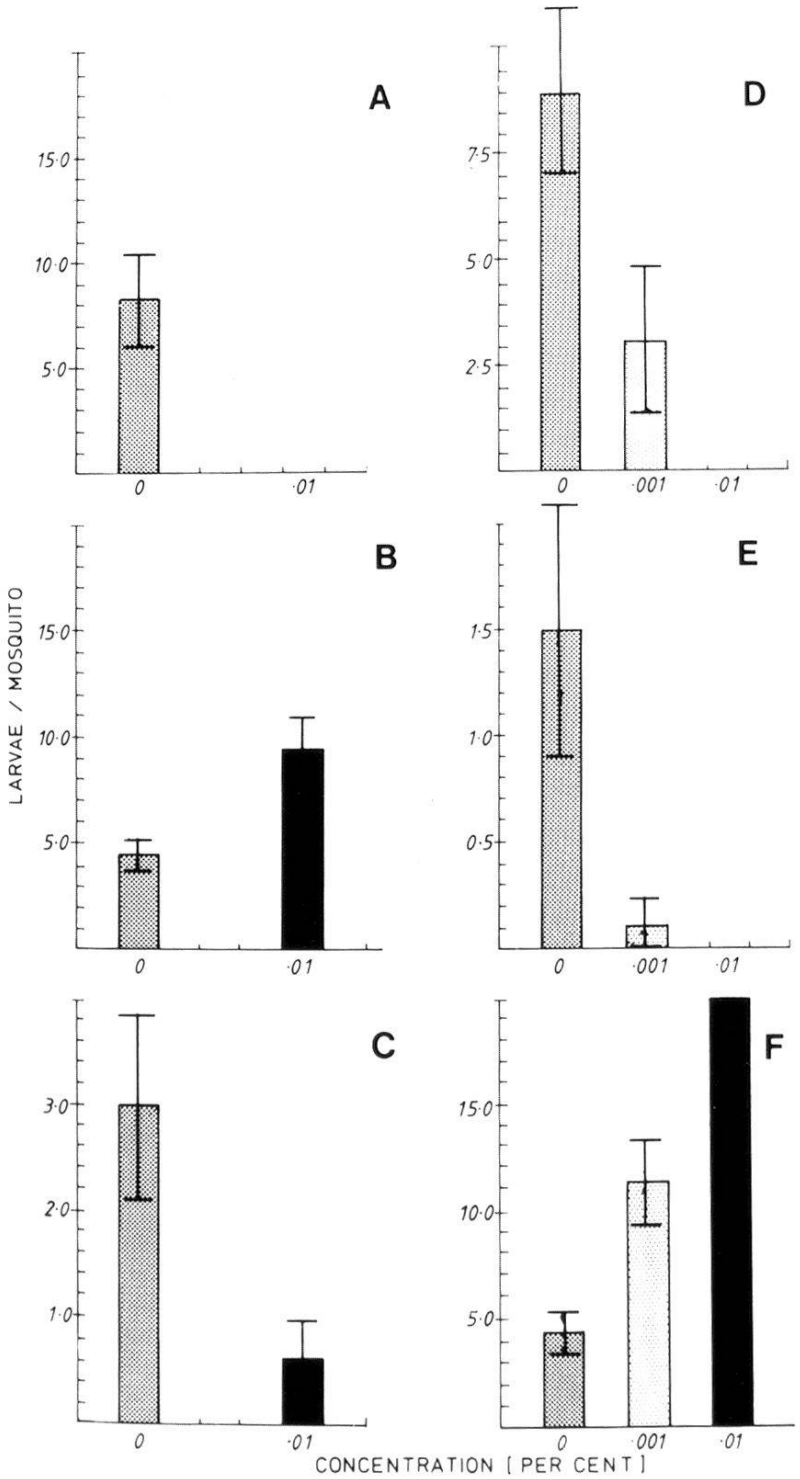
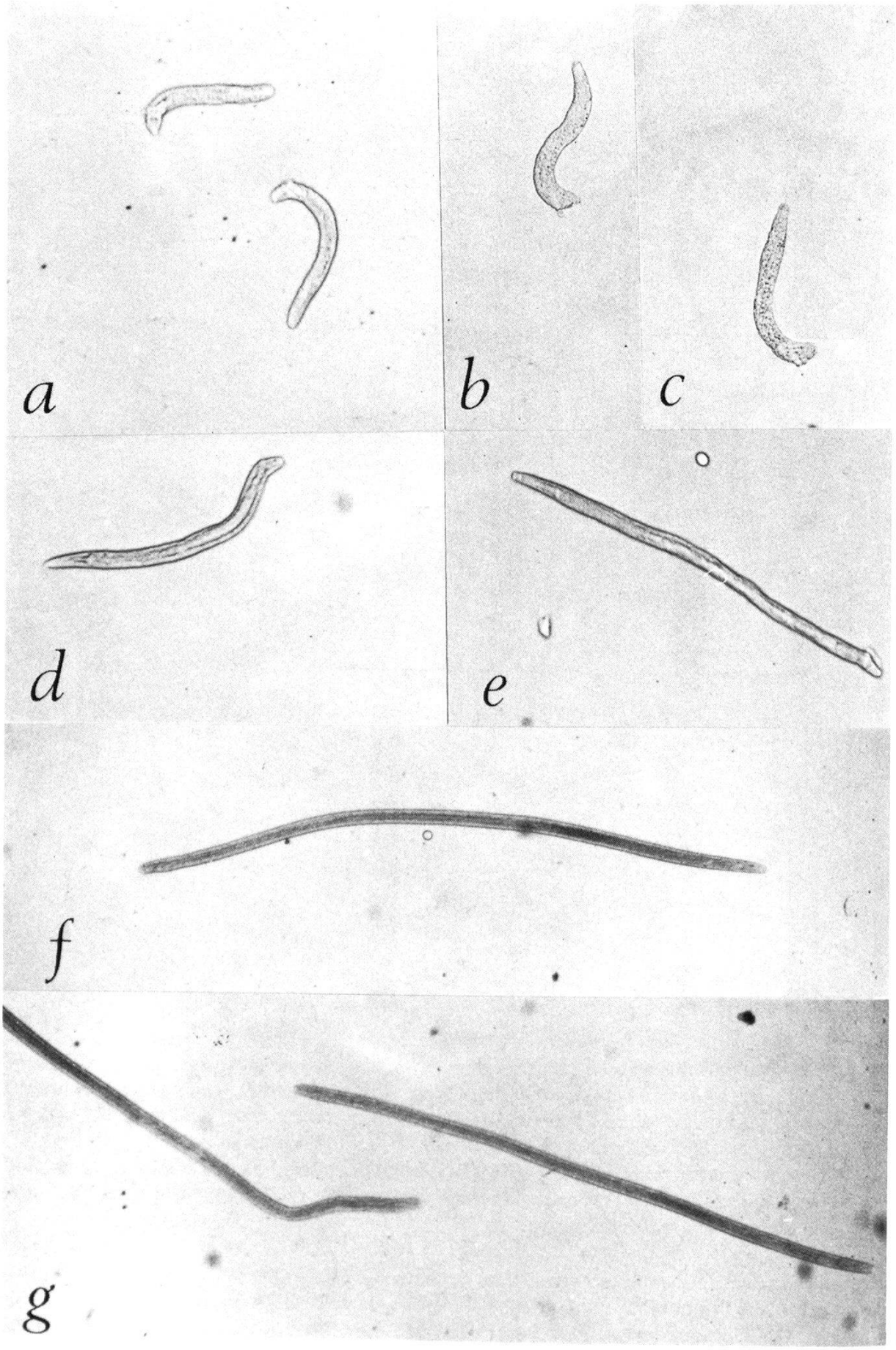


Fig. 4. The effect of 5-fluorouracil concentration on the distribution of *B. pahangi* and *D. immitis* larvae in the head, thorax and abdomen of *Ae. aegypti. B. pahangi* and *D. immitis* infected mosquitoes were dissected on the 9th and 12th day of infection, respectively. Each determination of mean parasite number is based on the dissection of at least 10 mosquitoes. Bars represent S. E. Figs. a, b and c represent the mean number of *B. pahangi* larvae in the head, thorax and abdomen, respectively of *Ae. aegypti.* Figs. d, e and f represent the mean number of *D. immitis* larvae in the head, thorax and abdomen, respectively of *Ae. aegypti.*

Fig. 3. *Brugia pahangi* larvae recovered from *Aedes aegypti* on day 10 post infection. a) Larvae (arrows) recovered from mosquitoes given 0.01% 5-fluorouracil (×50). b) Larvae recovered from control, undosed mosquitoes (×50). c) Larva recovered from mosquitoes given 0.01% 5-fluorouracil (×400).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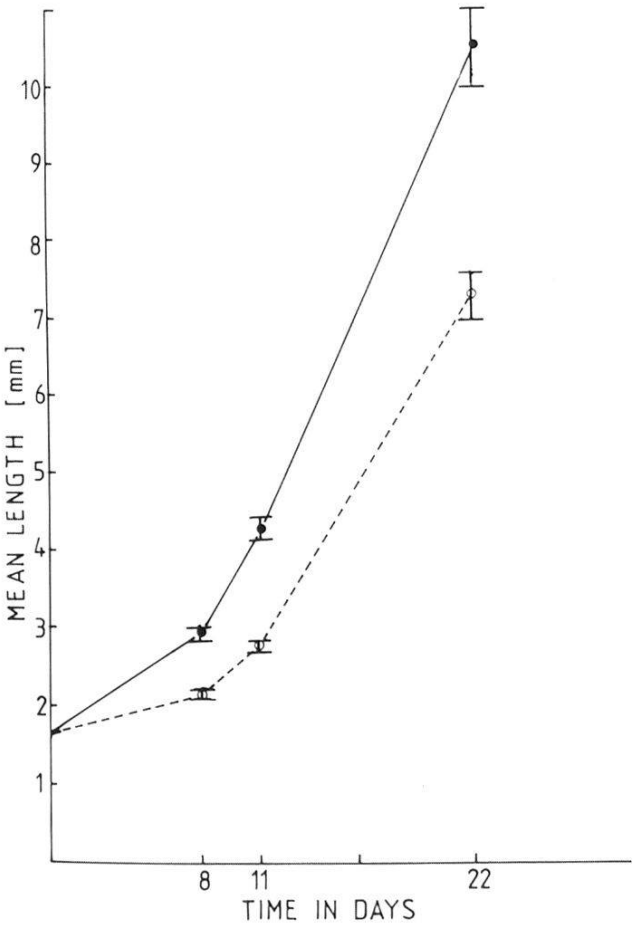

Fig. 6. The effect of 5-fluorouracil treatment on the growth of *Brugia pahangi* in the jird. · —— · —— = larvae from untreated control jirds, ---------- = larvae from 5-fluorouracil treated jirds. Bars represent S. E., n = 25.

5-fluorouracil: effects on the development of B. pahangi infective larvae

Jirds infected with 100 infective larvae of *B. pahangi* were dosed with 5-fluorouracil sc at 30 mg/kg for 5 days from the day of infection (day 1). The dosed jirds and untreated control jirds were killed on days 8, 11 and 22. The larvae were recovered from the peritoneal cavity of jirds, counted, fixed, mounted and measured as described in Materials and methods.

On each day of examination larvae from drugged jirds were smaller than those from normal, undrugged animals. The growth of larvae in the normal animals was similar to that recorded by Chen and Howells (1979) and by Howells and Blainey (in preparation), but the larvae removed from 5-fluorouracil dosed jirds on days 8, 11 and 22 were equivalent in size to day 3–6, day 8 and day 15 larvae, respectively for control jirds (Fig. 6). The day 8 larvae in the drugged jirds were still third stage larvae but those obtained from jirds on days 11 and 22 had moulted to the fourth stage. Remains of a third stage cuticle were found attached to the mouth of some larvae collected from 5-fluorouracil treated jirds on day 11 (Fig. 1e).

Fig. 5. *Dirofilaria immitis* larvae recovered from *Aedes aegypti* on day 12 post infection. a–c) Larvae from mosquitoes given 0.01% 5-fluorouracil, d–e) larvae from mosquitoes given 0.001% 5-fluorouracil, f–g) larvae from control, undosed mosquitoes (× 80).

Discussion

Adult female *Brugia pahangi* incorporated greater amounts of ^{14}C-uracil than did male worms (Chen and Howells, 1981) and autoradiographs demonstrated that the pyrimidine was localised within the reproductive tissue of the female worm. The most striking effect of 5-fluorouracil on adult worms observed in this study was also associated with the reproductive system of the female worms; it has been shown that the female worms are sterilised by this compound although this effect was shown to be reversible. 5-fluorodeoxyuridine also inhibited microfilaria production by *B. pahangi* at 30 mg/kg for 4 daily doses although daily doses of 150 mg/kg azauridine were required to inhibit microfilaria production in *L. carinii* (Hawking, 1973).

The inability of 5-fluorocytosine to inhibit the development of either *B. pahangi* or *D. immitis* confirms the in vitro observation of a non-utilisation of cytosine by *B. pahangi* and demonstrates that the in vitro observations were not a reflection of cuticular impermeability to this pyrimidine. It is also evident that these filarial worms lack the deaminase necessary for the conversion of 5-fluorocytosine to 5-fluorouracil.

The administration of 5-fluorouracil to *B. pahangi* infected jirds has demonstrated the rapidity with which microfilaria production occurs in this species. The uterine larvae presumably mature in approximately one week, with no significant retention of a 'stock' of mature larvae, for five days after the commencement of dosing with 5-fluorouracil the female worms were extruding immature eggs, and larval production was completely inhibited by the eighth day. The very marked reduction in the number of microfilariae in peritoneal fluids of jirds on day 24 can best be explained by assuming that the microfilariae of *B. pahangi* have a short life span within this host. It cannot be considered that an immune response to microfilariae is stimulated following 5-fluorouracil treatment for normal larval levels were observed in the peritoneum of animals examined at 65 days. The interactions between *B. pahangi* microfilariae and the jird merit further study for the infection is unaffected by diethylcarbamazine (Denham and McGreevy, 1977) and no explanation has been proposed to explain the absence of a microfilaraemia in animals which possess millions of larvae within their peritoneal fluid. It would be of interest to determine whether microfilariae introduced intravenously to jirds continue to circulate in the blood stream for extended periods of time.

Jaffe and Doremus (1970) demonstrated that uridine was incorporated into RNA of *D. immitis*. An inhibition of RNA synthesis might equally explain the inhibition of both microfilarial and infective larval development observed here. The remarkable developmental stasis observed in mosquito-borne stages of *D. immitis* and *B. pahangi* following the administration of 0.01% 5-fluorouracil to the cotton wool wicks demonstrates the requirement of larvae for this pyrimidine. 5-fluorodeoxyuridine was also shown to inhibit the development of

B. pahangi microfilariae. It has been suggested (Howells, 1980) that the ability of microfilariae to develop to the sausage stage but not undergo ecdysis to the second larval stage following 5-fluorouracil treatment indicates that the transformation to the sausage stage larva does not require RNA synthesis. Sufficient quantities of nucleic acid precursors may have been derived from the insect and the blood meal to permit transformation to the sausage stage, before an inhibitory concentration of the fluorinated compound has been reached within the insect tissues. *D. immitis* did attain the second larval stage in mosquitoes maintained on 0.001% 5-fluorouracil.

Himmelhoch and Zuckerman (1978) employed 5-fluorodeoxyuridine as an inhibitor of acid mucopolysaccharide synthesis to determine if mucopolysaccharide turnover occurs in the surface of mature nematodes. Philipp et al. (1980) have shown antigenic proteins on the surface of *B. pahangi, L. carinii* and *Trichinella spiralis* and have shown that the surface proteins of *T. spiralis* are shed during culture in vitro. Vetter and Klaver-Wesseling (1978) have also suggested a rapid turnover of surface antigens on the cuticle of *Ancylostoma caninum* infective larvae, the rate of turnover controlling the binding of antibody to the surface of the worms. If the surface protein coat of *B. pahangi* is also continuously shed and is involved in the evasion of the host immune response by the worm, the apparent failure to observe a host immunological response to 5-fluorouracil or 5-fluorodeoxyuridine treated worms suggests that one or more of the following situations prevailed:

a) there is no inhibition of surface coat production following treatment,
b) there is no turnover of the surface coat elements,
c) there is inhibition of surface coat production but this does not result in an altered antibody-binding characteristic of the surface,
d) there is inhibition of surface coat production but no host immune response is observed since antibody production also is inhibited by the uracil or uridine analogues.

The toxicity of 5-fluorouracil precludes the possibility of its employment in the chemotherapy of filarial infections. It has however been shown that pyrimidine analogues can inhibit both development and embryogenesis in vivo and the use of such compounds as metabolic inhibitors can provide much information on the biology and host parasite interaction of filarial infections. Compounds which might inhibit microfilaria production would, in the absence of effective macrofilaricides, be of value in the treatment and control of *Onchocerca volvulus* infections. It would be of interest to study the effect of other purine and pyrimidine analogues to attempt the selection of one with effect against filarial worms but not vertebrate cells.

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