STUDIES ON THE USE OF SETARIA CERVI FOR IN VITRO ANTIFILARIAL SCREENING

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Abstract—The present study demonstrates the optimum conditions required for survival of the adult *Setaria cervi* in-vitro and the effect of some drugs on these worms. Continuous replacement of the perfusion fluid favoured survival of the worms. Aeration or oxygenation of the fluid had no effect. The optimum concentration of the glucose in Ringer's solution ensuring longest survival period was found to be 0.25 g/l. In such a modified Ringer's solution all the worms were living at the end of 72 hr. Addition of $MgCl_2$ and NaH_2PO_4 into the fluid adversely affected the worms. Coal tar dyes, methyl violet, gentian violet and mercurochrome were found to be lethal to the adult worm in very low concentrations. Thiabendazole, diethylcarbamazine and acetylarsan were next in the descending order of potency. Piperazine hexahydrate, chlorpromazine and promethazine produced paralysis of the worms which regained normal motility after transference to the fresh solution. Sulphathiazole, streptomycin, penicillin and metronidazole had no effect.

The need for effective chemotherapeutic agents against filariasis has stimulated the search for a rapid and reliable test for screening potential antifilarial agents in the laboratory. The methods available at present possess some inherent disadvantages such as the need for an intermediate orthopod vector, a long incubation period (1, 2, 3) (50-240 days) and lack of specificity. These factors are largely responsible for the slow pace of development of new drugs for filariasis.

The method reported by the authors (4, 5) using *Setaria cervi* as a test organism in rats eliminates the use of an intermediate orthopod vector. The incubation period is short (10 ± 3 days). Microfilaremia persists for a duration (54 ± 6 days), sufficiently long for the performance of the test. Using this method microfilaria disappeared from the peripheral circulation due to treatment with diethylcarbamazine (15 days) and 3-acetamido-4-hydroxy phenyl arsonic acid (7 days). It has been suggested that the effect of these agents is exerted on the microfilarial forms and not on the adult worms because the live worms were recovered, at autopsy, from the peritoneal cavity, after treatment with these drugs at a stage when the microfilaria had been persistently absent from the peripheral blood for three consecutive days.

The present study concerns the effect of drugs on the adult *Setaria cervi* in-vitro, with a view to elucidating the devitalising effect, if any, of the drugs, and the possibility of such in vitro effect being used as a screening method.

MATERIALS AND METHODS

Adult worms of *Setaria cervi* were collected from the peritoneal cavity of the freshly slaughtered cattle. The worms were brought to the laboratory in modified Ringer's solution (as described below). In the laboratory, worms were given repeated washings with the same solution to free them from extraneous material.

In a preliminary study, an attempt was made to determine optimum conditions which would ensure a fairly long period of survival of the worms.

Adult worms were placed in 100 ml of Ringer's solution in a beaker and observed. Over 90% of the worms were found dead at the end of 24 hr. Aeration of the fluid was then attempted and it was observed that slow and continuous streaming either of air or oxygen through the fluid did not favourably affect the survival of the worms. It was then concluded that a continuous replenishment of the nutrients and removal of the metabolites may have a favourable effect. The following experiment was, therefore, devised.

Ten adult worms were placed in 100 ml of Ringer's solution in a conical flask. The glucose concentration of the fluid was varied. Fluid was gradually replaced by fresh solution flowing into the conical flask from a 10 liter aspirator bottle at a rate of 40 drops per min. The volume of the fluid in flask was maintained constant by allowing the excess fluid to overflow through an outlet. The arrangement is shown in Fig. 1.

FIG. 1

The worms were examined at intervals of 24, 48, 72, 96 and 120 hr and the number of motile and consequently surviving worms in each group was recorded. Table 1 shows the effect of alteration in glucose concentration in the perfusion fluid and Table 2, the effect of addition of NaH_2PO_4 and MgCl_2 to the perfusion fluid containing 0.25 g glucose/l

TABLE 1. Effect of different concentrations of glucose in Ringer's solution on the survival of adult *Setaria cervi* in vitro.

Concentration of glucose Gm/Liter	No. of worms	Survivors				
		24 hr	48 hr	72 hr	96 hr	120 hr
1*	50	46 $P>.10$	25 $P<.01$	0	0	0
0.5	50	50	40 $P>.10$	16 $P<.001$	0	0
0.25	50	50	50	50	43	20
0.125	50	50	46 $P>.10$	31 $P<.01$	5 $P<.001$	0

* Concentration of glucose in original Ringer's solution.

P=Probability calculated with respect to the corresponding figure observed with 0.25% glucose.

on the survival of the worms at different periods. The probability value (P) for the significance of difference between the number of worms surviving at the end of different periods was calculated using the X^2 test.

It is observed from Table 1 that 0.25 g/l is the optimum concentration of glucose in the perfusion fluid enabling all the worms to survive for a period of 72 hr. With 1 g/l concentration of glucose some worms were found dead at the end of 24 hr but the survival was significantly lowered compared to the 0.25 g/l concentration only at the end of 48 hr ($P<0.01$). All the worms survived a period of 24 hr with 0.25 and 0.125 g/l of glucose. Some in each group were found dead at the end of 48 hr. Adverse effect on survival became statistically significant at the end of a 72 hr period. The probability that survival is due to chance is $<.001$ for 0.5 g/l and $<.01$ for 0.125 g/l.

TABLE 2. Effect of addition of $MgCl_2$ and NaH_2PO_4 to Ringer's solution* on the survival of *Setaria cervi* in vitro.

Concentration mg/Liter	No. of worms	Survivors				
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Control	50	50	50	50	43	20
NaH_2PO_4						
200	56	55	55	52 $P>0.1$	32 $P<0.001$	8 $P>0.001$
100	56	56	52	50 $P>0.1$	37 $P>0.02$	13 $P>0.02$
$MgCl_2$						
200	100	100	98	95 $P>0.1$	74 $P>0.1$	33 $P>0.1$
100	56	53	53	50 $P>0.1$	41 $P>0.1$	18 $P>0.1$

* Concentration of glucose was 0.25 Gm/Liter in all experiments

P=Probability calculated with respect to the figure obtained in the control.

Table 2 shows that addition of NaH_2PO_4 or $MgCl_2$, normal constituents of Tyrode's solution to this perfusion fluid containing 0.25 g glucose per liter adversely affects the survival of the worms, however it is only with NaH_2PO_4 and at 96 hr period, that the decrease in surviving worms becomes statistically significant.

On the basis of the above result, Ringer's solution containing .25 g glucose/l, which

ensured the longest survival, was therefore employed in this study for evaluating the drug effect on adult worms and is referred as 'Modified Ringer's Solution'.

The effect of drugs on the worms, in the set up described above was studied by adding the drugs to fluid in varying concentrations. Each concentration of a drug was allowed to act for 24 hr. Thereafter, the worms were transferred to the fresh solution and were further observed for two hr.

On the basis of the effect on the worms, the drugs were grouped in the following categories;—

1. Inactive, when the worms remained visibly unaffected.
2. Paralyrant, when the worms became immobile but regained their motility within 2 hr of their transference to normal solution.
3. Vermicidal, when the motility did not return within 2 hr following their transference to the normal solution.

RESULTS

The above mentioned effects of the drugs on the survival period of *Setaria cervi* are summarized in Table 3. As is apparent from the Table 3, methyl violet, mercurochrome and gentian violet showed the greatest vermicidal activity, the former two being active in 2 $\mu\text{g/ml}$ while the last in 4.25 $\mu\text{g/ml}$ concentration. Thiabendazole and diethylcarbamazine come next in that order of potency being active in 300 and 750 $\mu\text{g/ml}$ concentration respectively. Acetylarsan required very high concentrations to show a vermicidal effect.

Among the paralytics, chlorpromazine and promethazine were equally potent and more so in 50 $\mu\text{g/ml}$ concentration. Piperazine, active against intestinal helminths, required a high concentration (1000 $\mu\text{g/ml}$) to produce an in vitro paralyrant effect.

Sulphathiazole, streptomycin, penicillin and metronidazole were ineffective in even quite high concentrations.

TABLE 3. In vitro activity of test compounds against adult worms of *Setaria cervi* after 24 hr.

Drug	No. of worms	Concentration $\mu\text{g/ml}$	Effect
Methyl violet	40	2	100% Mortality
Mercurochrome	30	2	"
Gentian violet	40	4.25	"
Thiabendazole	40	300	"
Diethyl carbamazine	50	750	"
Acetylarsan	40	3500	"
Chlorpromazine	30	50	Paralyrant
Promethazine HCl	30	50	"
Piperazine hexahydrate]	30	1000	"
Sulphathiazole	40	1600	No effect
Streptomycin	30	2000	"
Penicillin	30	2400 units/ml	"
Metronidazole	40	1600	"

DISCUSSION

The method described here appears suitable for examining the direct effect of drugs on the adult *Setaria cervi*. The worms can be kept alive and actively motile for over 72 hr, a period sufficient to assess the effect of a drug while in direct contact with the worm.

The well-established antifilarial agent, diethylcarbamazine, required a very high concentration to kill the worms in-vitro. Such high concentrations are not attained in therapeutic doses.

The present finding supports an earlier suggestion of the investigators (5) that the observed beneficial effect of diethylcarbamazine in filaria is exerted on the reproductive potential of the adult worm and/or directly on the circulating microfilaria and not on the adult worms. They recovered live adult worms from the peritoneal cavity of rats in which microfilaria had been persistently absent from the peripheral circulation for at least three consecutive days following treatment with diethylcarbamazine. The same is true of another antifilarial agent, acetylarson.

Metronidazol, sulphathiazole, and the antibiotics penicillin and streptomycin, as representatives of the antiprotozoal and antibacterial drugs had no effect.

The paralyzant effect of the phenothiazines chlorpromazine and promethazine and of piperazine can probably be attributed to their local anaesthetic and/or neuromuscular blocking effect.

The Coal tar dyes, methylviolet, mercurochrome and gentian violet are highly potent vermucidal in vitro. They have never been used therapeutically due to the general protoplasmic toxicity.

Thiabendazole is an effective broad spectrum anthelmintic (6). In addition it is absorbed after oral administration. It has been shown to be effective in the treatment of tropical pulmonary eosinophilia (7) a condition generally attributed to animal filariasis. This drug, therefore, appears promising for in vivo evaluation against *Setaria cervi*.

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