

## THE GOLDEN HAMSTER AS A DEFINITIVE HOST OF *TAENIA SOLIUM* AND *TAENIA SAGINATA*

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### ABSTRACT

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The susceptibility of golden hamsters to infestation with *Taenia solium* is increased by treatment with antilymphocytic serum (ALS) or whole body irradiation or chemical immunosuppressants, viz. methylprednisolone acetate and betamethazone disodium phosphate. Although the worms develop to sexual maturity they do not produce eggs.

Such treatment is less effective in increasing their susceptibility to *T. saginata*. When young hamsters were exposed to whole body irradiation and subsequently treated with ALS, 60 per cent of them became infested with *T. saginata*. As young animals do not survive immunosuppression for any length of time the further development of this tapeworm was not investigated.

### INTRODUCTION

Immunosuppressants have been extensively used to increase the susceptibility of various laboratory animals to several species of nematodes (Campbell, 1963; 1968; Campbell & Collette, 1962; Di Netta, Katz & Campbell, 1972; Miller, 1966; Mougeot & Lancastre, 1970; Parker, 1961; Ritterson, 1959; Wiest, 1972; Wakelin, 1970). Only a few of these compounds have been used to increase susceptibility to cestodes. Corticosteroids were used by: Verster (1971) to increase the susceptibility of golden hamsters to *Taenia solium* and *Taenia saginata*; by Ritterson (1971) in Chinese hamsters and by Moss (1972) in mice for *Hymenolepis microstoma* and by Hopkins, Subramanian & Stallard (1972) in mice for *Hymenolepis diminuta*. The latter authors found that mice treated with anti-lymphocytic serum (ALS) did not reject *H. diminuta* 9 to 12 days after infestation while Okamoto & Koizumi (1972) showed that anti-thymocytic serum (ATS), but not ALS, abolished the acquired immunity of mice to *Hymenolepis nana*. Although mice treated with the anti-metabolite methotrexate did not expel *H. diminuta*, these ceased to grow after 16 days (Hopkins *et al.*, 1972). In rats neither cortisone nor methotrexate prevented destruction of *H. microstoma* which usually occurs after nine days (Goodall, 1972).

Mice fed either an antihistamine drug or an anti-serotonin drug or one possessing both properties, had heavier burdens of *Trichinella spiralis* 17 and 21 days after infestation than untreated mice (Campbell, Hartman & Cuckler, 1963). When guinea pigs infested with *Trichostrongylus colubriformis* are treated with antihistamines the development of resistance is suppressed and the parasites are not rejected (Rothwell Dineen & Love, 1971).

Verster (1971) showed that golden hamsters treated with Depo-Medrol<sup>(1)</sup> are markedly more susceptible to infestation with *T. solium* than with *T. saginata*. Their susceptibility to *T. solium* is directly correlated with the amount of Depo-Medrol administered. The rate of differentiation of the genitalia of the tapeworms is primarily dependent on the amount of immunosuppressant used but is also influenced by the number of worms present in a host. Despite the fact that the tapeworms developed to sexual maturity they did not become patent. Immunosuppression, however, also increased the susceptibility of hamsters to various infections and this influenced the period they could survive such treatment.

The present paper reports on subsequent investigations on the effect of other immunosuppressant substances and an antihistamine on hamsters infested with *T. solium*. In addition short term experiments were carried out with some of these substances to determine their effect on the resistance of the hamster to infestation with *T. saginata*.

### MATERIALS AND METHODS

#### Infestation of hamsters

A total of 680 golden hamsters were force-fed three to five cysticerci of *T. solium*. A further 25 were fed five cysticerci of *T. saginata*.

Prior to infestation of the hamsters the viability of each batch of cysticerci was tested *in vitro*. From 10 to 20 cysticerci were placed in 1 per cent pepsin<sup>(1)</sup>/HCl solution (pH 1.6 to 1.8) in a waterbath at 38 °C for one hour. Thereafter the pepsin solution was decanted and replaced with a freshly prepared 1 per cent trypsin<sup>(2)</sup> 1 per cent bile salts<sup>(3)</sup> solution (pH 7.2 to 7.5) at the same temperature. After 30 minutes the cysticerci were examined and if less than 90 per cent had evaginated, that batch was not used.

#### Immunosuppression

1. *Chemical immunosuppressants*: Treatment with various immunosuppressive drugs commenced on the day of infestation. The effectiveness of each drug and its optimum dosage rate were determined in short term trials lasting 10 days.

Depo-Medrol (5 mg and 10 mg) and Endoxan<sup>(4)</sup> (10 mg) were administered by subcutaneous injection at weekly intervals. The following compounds were administered each day in the drinking water: Betnesol<sup>(5)</sup> (0.0085 mg; 0.0175 mg; 0.025 mg and 0.05 mg), Imuran<sup>(6)</sup> (0.1 mg; 0.25 mg; 1.0 mg; 2.5 mg and 5.0 mg) and Endoxan (0.5 mg; 1.5 mg and 3.0 mg).

2. *Antilymphocytic serum (ALS)*: This was rabbit-anti-hamster-thymocytic serum prepared as described by Levey & Medawar (1966) with the following modifications:

(a) The thymus glands were ground in a tissue grinder;

<sup>(1)</sup> Riedel de Haan

<sup>(2)</sup> Merck

<sup>(3)</sup> Bile Salts No. 3, Difco

<sup>(4)</sup> Cyclophosphamide, Noristan

<sup>(5)</sup> Betamethazone disodium phosphate, Glaxo-Allenbury

<sup>(6)</sup> Azathioprine, Burroughs Wellcome

<sup>(1)</sup> Methylprednisolone acetate, Upjohn

(b) immediately before the second injection of thymocytes the rabbits were injected intramuscularly with 0,5 ml 2,5 per cent Anthisan<sup>(1)</sup> solution followed by 0,5 ml adrenalin 1:10 000 and

(c) the rabbits were not exsanguinated but 30 to 50 ml of blood were withdrawn from the heart at fortnightly or monthly intervals.

Hamsters were injected subcutaneously with 0,5 ml ALS on Day +2, i.e. two days after infestation, and with 0,25 ml ALS on Day +5, Day +8 and Day +11 respectively.

3. *Irradiation*: Hamsters were exposed to 600 rad whole body irradiation from a <sup>60</sup>Co source on Day -1, i.e. one day before infestation (Day 0)

*Treatment with antihistamine*

Anthisan was added to the drinking water at a rate of 20 mg per animal for 15 hamsters and 40 mg per animal for 10 hamsters.

*Maintenance of the hamsters*

The animals were housed singly in mouse cages which were changed, washed and autoclaved twice weekly. The bottles used for drinking water were washed and the pipettes washed and sterilized by

boiling each day. The feed consisted of commercial rat cubes supplemented with mealies and sunflower seed.

Except those that were treated with Anthisan only, all the animals were treated in rotation for three days at a time with Meds 2000<sup>(2)</sup> (20,8 mg per 100 ml), Terramycin soluble powder with vitamins<sup>(3)</sup> (20 mg per 100 ml) and Tribissen<sup>(4)</sup> (1,4 g per 100 ml) in their drinking water.

In the short term experiments to determine the immunosuppressive action and dosage rate of the various drugs, the animals were examined 10 days after infestation. In the long term experiments the animals were examined as and when they died, or the experiments were terminated 59 or more days after infestation.

RESULTS

*A. T. solium*

Data on the effect of the various chemical immunosuppressant drugs alone and in combination with ALS on the susceptibility of hamsters to infestation with *T. solium* are summarized in Tables 1 and 2, while Table 3 summarizes data on the survival time of hamsters to these treatments.

TABLE 1 Effect of immunosuppressive drugs on the susceptibility of the golden hamster to *T. solium*

Drug	Administration	Dosage (mg)	Duration of experiment			
			Short term (<10 days)		Long term (>10 days)	
			No. of hamsters	Positive %	No. of hamsters	Positive %
Depo-Medrol.....	s.c., weekly.....	10,0	20	80,0	—	—
		5,0	30	80,0	80	76,2
Betnesol.....	<i>per os</i> ; daily.....	0,05	10	60,0	10	90,0
		0,025	10	90,0	20	75,0
		0,0175	10	80,0	—	—
		0,0085	10	90,0	—	—
Imuran.....	<i>per os</i> ; daily.....	5,0	20	80,0	10	30,0
		2,5	10	90,0	10	50,0
		1,0	10	80,0	10	40,0
		0,25	10	90,0	—	—
		0,10	10	100,0	—	—
Endoxan.....	<i>per os</i> ; daily.....	10,0	10	100,0	70	2,8
		3,0	10	60,0	50	8,0
		1,5	10	100,0	20	20,0
		0,5	10	80,0	20	0

(<sup>1</sup>) Mepyramine, Maybaker

(<sup>2</sup>) Proterocycline R, Meds Laboratories

(<sup>3</sup>) Oxytetracycline, Pfizer

(<sup>4</sup>) Trimethaprim and Sulphadiazine, Wellcome

TABLE 2 Effect of ALS combined with other immunosuppressants on the susceptibility of hamsters to *T. solium*

Group	Treatment: ALS plus							Hamsters		
	Irradiation	Depo-Medrol		Imuran		Betnesol	Anthisan	No.	Positive	
		600 r	2 mg	5 mg	2,5 mg	5,0 mg	0,025 mg		40 mg	No.
1.....	+	+	-	-	-	-	-	7	4	57,1
2.....	+	-	+	-	-	-	-	5	4	80,0
3.....	-	-	+	-	-	-	-	39	34	87,2
4.....	-	-	+	-	-	-	+	10	9	90,0
5.....	+	-	-	+	-	-	-	7	1	14,3
6.....	-	-	-	-	+	-	+	9	4	44,4
7.....	+	-	-	-	-	+	-	7	5	71,4
8.....	-	-	-	-	-	+	-	66	51	77,3
9.....	-	-	-	-	-	-	+	9	0	0
10.....	+	-	-	-	-	-	-	6	2	33,3

TABLE 3 Survival of hamsters treated with immunosuppressants

Treatment	Dosage	30 Days		60 Days	
		No. of Survivors	Survivors %	No. of Survivors	Survivors %
Depo-Medrol.....	10 mg	4/30	13,3	0	0
	5 mg	27/70	38,6	0	0
Betnesol.....	0,05 mg	7/10	70,0	—*	—*
	0,025 mg	9/10	90,0	5/10	50,0
Imuran.....	5,0 mg	6/10	60,0	4/10	40,0
	2,5 mg	5/10	50,0	2/10	20,0
	1,0 mg	9/10	90,0	7/10	70,0
ALS + Depo-Medrol.....	5 mg	33/65	50,8	7/45	15,6
ALS + Depo-Medrol.....	2 mg	7/7	100,0	7/7	100,0
ALS + Betnesol.....	0,025 mg	42/57	73,7	5/7	71,4
ALS + Imuran.....	2,5 mg	6/7	85,7	5/7	71,4

\* No data available

*Depo-Medrol*: Sexually mature cestodes were recovered from hamsters treated with 5 mg Depo-Medrol only (Table 1) or in combination with ALS (Table 2, Groups 2, 3 and 4). At this dosage rate the hamsters were susceptible to various infections and in the earlier trials few hamsters survived for 30 and none for 60 days. In the later trials, when this drug was combined with ALS, 50 per cent of the hamsters survived for 30 and 16 per cent for 60 days. One animal in the latter experiment survived as long as 87 days.

The majority of the hamsters treated with irradiation, ALS and 2 mg Depo-Medrol survived until the experiment was terminated at 87 days. Differentiation of the genitalia was retarded in the worms recovered from these animals and development had not proceeded beyond differentiation of the primordia of the genital ducts.

*Betnesol*: The susceptibility of hamsters treated with this drug is comparable with those treated with 5 mg Depo-Medrol (Table 1), but they were less susceptible to infections and survived longer. Although some of these animals survived until the trials were terminated at 94 days, the cestodes recovered from them were sexually mature but not patent.

*Imuran*: When this substance was used alone it gave good results in short term experiments but in long term experiments relatively few hamsters were posi-

tive. The cestodes recovered from those hamsters that retained the infestation until the experiment was terminated at 94 days were not patent. This substance also gave poor results when it was used as a long term immunosuppressant after treatment with ALS.

*Endoxan*: In short term experiments this drug gave excellent results both when injected and when added to the drinking water. In long term experiments, however, the results were disappointing. In the first long term trial a different formulation was used from Day 30 onwards; *T. solium* was recovered from animals that died during the first 28 days but all those that were examined later were negative. In the second trial the substance was injected and also added to the drinking water of some animals but the results were essentially the same as in the previous experiment.

*ALS*: Initially a short term trial showed that either ALS alone or in combination with whole body irradiation or whole body irradiation alone was as effective as 10 mg Depo-Medrol. In long term experiments the effect of ALS combined with other immunosuppressants was comparable with the effect when the other substances were used alone. The immunosuppressive effect of ALS combined with irradiation was not sustained for any length of time because *T. solium* was only recovered from animals which died within 14 days of infestation (Table 2, Group 10).

*Anthisan*: When 20 mg Anthisan was added to the drinking water of 15 hamsters 11 of them were infested at 25 days. Since the worms recovered from these hamsters varied in length from 1,5 to 351 mm the dosage was increased to 40 mg in the subsequent trial with 10 hamsters, but these were negative when they were examined at 56 days. Moreover, this substance was ineffective both when it was used after ALS treatment alone and when it was combined with ALS and Imuran (Table 2, Groups 6 and 9). The satisfactory

DISCUSSION

Treatment with immunosuppressant substances causes a marked increase in the susceptibility of hamsters to infestation with *T. solium* but is decidedly less effective in increasing their susceptibility to *T. saginata*. Young animals are slightly more susceptible than adults to infestation with *T. saginata* but they do not survive immunosuppression for any length of time. In the case of *T. solium*, however, neither age nor sex

TABLE 4 Comparative susceptibility of male and female hamsters to infestation with *T. solium*

Treatment	Dosage (mg)	Males		Females	
		No. infested	No. positive (%)	No. infested	No. positive (%)
Imuran.....	5,0	5	40,0	5	20,0
Imuran.....	2,5	5	60,0	5	40,0
ALS + Depo-Medrol.....	5,0	19	89,5	29	89,7
ALS + Betnesol.....	0,025	8	62,5	10	40,0
ALS + Imuran.....	5,0	4	50,0	5	40,0
Total.....	—	41	70,7	54	64,8

results obtained when it was combined with ALS and 5 mg Depo-Medrol were undoubtedly due to the immunosuppressive effect of the latter two substances.

*Susceptibility of male and female hamsters*

In all except two of the trials the hamsters were all of the same sex. In two trials, however, the susceptibility of male and female hamsters was compared (Table 4).

Males and females treated with ALS and Depo-Medrol (5 mg) were equally susceptible to infestation. Although the male hamsters in the other four groups showed a slightly greater predisposition to infestation, the numbers of animals involved were small and the differences are probably not significant.

appears to have any effect on the susceptibility of the animals.

The immunosuppressant to be used, as well as its dosage rate, is dependent on the duration of the experiment. Depo-Medrol consistently increased the susceptibility of hamsters to *T. solium* but the dosage used must be selected with care. At 10 mg per week from 74 to 80 per cent of the hamsters become infested but the animals start dying from the eighth day onwards and no animal has survived more than 35 days. This dosage is therefore suitable only for short term trials of 10 days when maximum immunosuppression is required, e.g. when the susceptibility of the golden hamster is compared with that of the white-tailed rat, *Mystromys albicaudatus*, or in assessing the effect

TABLE 5 Effect of immunosuppression on the susceptibility of hamsters to infestation with *T. saginata*

Drug	Administration	Dosage	Hamsters <sup>(2)</sup>		
			No. infested	Positive	
				No.	%
Depo-Medrol.....	s.c. weekly.....	10 mg	5	2	40,0
Endoxan.....	s.c. weekly.....	10 mg	10	0	0
Irradiation + ALS <sup>(1)</sup> .....	—	—	10	6	60,0

<sup>(1)</sup> See Materials and Methods for administration and dosage

<sup>(2)</sup> All animals autopsied 10 days after infestation

*B. T. saginata*

Treatment with immunosuppressant substances did not significantly increase the susceptibility of hamsters to infestation with *T. saginata* (Table 5).

When young male hamsters (42 to 56 days old) were treated with Depo-Medrol (10 mg) two of five animals were positive when they were examined after 10 days. Comparable animals that were irradiated and treated with ALS were slightly more susceptible in that six of 10 animals became infested.

of various treatments on the infectivity of cysticerci. At 5 mg per week the hamsters are equally susceptible to infestation with *T. solium* and the animals survive longer. Although as many as 51 per cent may survive this treatment for 30 days and 16 per cent for 60 days, this is only possible when the utmost care is taken to protect the animals from various infections. At lower dosage rates, e.g. 2 mg per week, there is some increase in the susceptibility of the hamsters and they survive for longer periods, but the development of the worms is retarded.

Betnesol and Imuran gave good results because they increased the susceptibility of hamsters to infestation with *T. solium* but the animals were less susceptible to the various infections than those treated with Depo-Medrol. Thus 50 per cent of the animals treated with Betnesol (0,025 mg per day) and from 20 to 70 per cent of those treated with Imuran (2,5 and 1,0 mg per day) survived for 60 or more days. The animals treated with Imuran, however, expelled their tapeworms so that 30 to 50 per cent only were positive in long term trials.

Poor results were noted in Groups 5 and 6 (Table 2). This was not due to decreased infectivity of the cysticerci, however, because other groups infested with the same batches of cysticerci were positive when examined. Thus Groups 1, 2 and 7 can be compared with Group 5 and Group 4 with Group 6. Because hamsters do not drink the same volume of medicated water every day, the lower efficacy was probably due to erratic intake of the compound.

Irradiation was discontinued after the first long term trial because animals treated with ALS alone were as susceptible to infestation as those which were pretreated with whole body irradiation. When ALS is combined with relatively high doses of Depo-Medrol (5 mg) it has a marked immunosuppressive effect (Table 2, Groups 2, 3 and 4). The rate of differentiation of genitalia in the worms was rapid, but unfortunately only half of the hamsters survived for 30 days. When ALS was combined with Depo-Medrol at 2 mg, all the hamsters lived for 60 days but the worms did not develop beyond differentiation of the primordia of the genital ducts.

When mice are infested with *T. spiralis* the majority of the worms are expelled during the second week of infestation. Di Netta *et al.* (1972) found that such a loss of worms did not occur if treatment with ALS commenced two days before infestation. If treatment started one day after infestation, however, the worms were expelled and on Day 20 the mice had retained worm burdens comparable with those of the untreated controls.

In these trials treatment was initiated either by irradiation on Day -1 or with administration of ALS on Day +2. These animals were found to be equally susceptible to infestation. It is unlikely that the delay in administration of ALS in those groups that were not irradiated caused the rejection of worms in the long term trials as this also occurred when treatment was initiated with irradiation on Day -1 (Table 2, Groups 5 and 6).

When ALS was used alone or when its administration was preceded by irradiation, mortalities occurred within the first 12 days of the trial, i.e. before the animals were placed on chemical immunosuppressants for long term therapy. When these trials were started there were at least 10 animals in each group, but due to heavy mortalities as few as five animals survived the requisite 12 days before they were placed on chemical immunosuppressants. These mortalities were mainly due to a haemorrhagic diarrhoea. In the irradiated animals this diarrhoea is caused by denuding of the intestinal surface resulting in bleeding, infection and fluid loss (Evans, 1970). When diarrhoea occurred these animals were treated with Guaninmycin<sup>(1)</sup>, but the response was not as good as in animals which were treated for diarrhoea resulting from treatment with chemical immunosuppressants.

Mougeot & Lancastre (1970) found that mice treated with cyclophosphamide were susceptible to infestation with *Strongyloides stercoralis* and they retained the infestation for 20 days. In these trials this drug gave excellent results in short term experiments but in long term experiments the tape worms were expelled after 28 days. The long term experiments consisted of two trials. During the first trial the formulation was changed after 30 days and it was believed that this caused the poor results; the experiment was therefore repeated, but in this second trial the results were essentially the same.

The trial with mepyramine confirmed the findings of Campbell *et al.* (1963) and Rothwell *et al.* (1971), viz. that antihistamines suppress the development of resistance in the host and thus the expulsion of the helminths. This suppression is, however, only effective for a limited period.

#### CONCLUSION

If golden hamsters are treated with immunosuppressants their susceptibility to infestation with cysticerci of *T. solium* is greatly increased, but such treatment is considerably less effective in increasing their susceptibility to *T. saginata*. Hamsters may therefore be used to assess the infectivity *in vivo* of the cysticerci of *T. solium*.

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